CITY OF CORONADO

1825 STRAND WAY
CORONADO, CA 92118

OFFICE OF THE CITY MANAGER
(619) 522-7335
FAX (619) 522-7846

October 7, 2010

David W. Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego CA 92123

RE: Tentative Order No. R9-2010-0124, General Permit No. CAG999002
General Waste Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson:

The City of Coronado respectfully requests an extension of the October 18, 2010, public comment period and the November 10, 2010, Board hearing relating to Tentative Order R9-2010-0124 and General Permit CAG999002, which pertains to the public display of fireworks.

These are new regulations and there are many significant issues associated with this Tentative Order; the short public comment period precludes the maximum public participation. As such, a public workshop with all the different stakeholders and sufficient time to consider the staff's proposal would allow us to provide meaningful input on this important issue.

Thank you for your consideration of this request.

Sincerely,

Blair King
City Manager

BK/mlc
cc: City Council
Michelle Mata, Regional Water Quality Control Board

By Electronic Mail and U.S. Mail

600 West Broadway, Suite 1800
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LATHAM & WATKINS LLP

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October 7, 2010

VIA ELECTRONIC MAIL & U.S. MAIL

David W. Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Re: Tentative Order No. R9-2010-0124, General Permit No. CAG999002 General Waste Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson:

On behalf of the La Jolla Community Fireworks Foundation, we respectfully request an extension of the October 18, 2010 public comment period and the November 2010 Board hearing relating to Tentative Order R9-2010-0124 and General Permit CAG999002, which pertains to the public display of fireworks. The Tentative Order and General Permit were first circulated for public comment on September 23, 2010, only three weeks before the current deadline for public comments. The legal, scientific, economic and civic issues presented by the Tentative Order and General Permit are highly consequential and will affect, among other things, coastal communities, fireworks sponsors, tourism, tax revenues, national celebrations, civic traditions, and public safety. We believe that there are so many different stakeholders and the issues so consequential that any proposed first-in-the-nation regulation of coastal fireworks calls for, at a minimum, a public work shop and sufficient time to analyze the Regional Water Quality Control Board staff's proposal. We therefore request that the staff hold a work shop to allow all stakeholders to present the legal, scientific, economic and other public policy considerations not presently reflected in the record. Thank you for your consideration of this request.

Very truly yours,

Robert M. Howard
of LATHAM & WATKINS LLP

Item No. 6
Supporting Document No. 5

Michelle Mata - Order R9-2010-0124, General Permit No. CAG999002 - Request for continuance and workshop.

From: Denny Knox <info@OceanBeachSanDiego.com>
To: <mmata@waterboards.ca.gov>
Date: 10/7/2010 1:23 PM
Subject: Order R9-2010-0124, General Permit No. CAG999002 - Request for continuance and workshop.

TO: Michelle Mata

RE: Tentative Order R9-2010-0124, General Permit No. CAG999002 – Request for continuance and workshop.

We have reviewed the letter from Latham & Watkins, and on behalf of the Ocean Beach MainStreet Association (450 business members) we also respectfully request an extension of the October 18, 2010 public comment period and the November 10, 2010 Regional Board hearing on the Tentative Order and General Permit. We believe that the proposed Order and General Permit raise significant legal, scientific, economic, civic and free speech issues that could be thoroughly addressed in a workshop prior to any action. We do not believe that the three week comment period is sufficient to address these issues. We are part of a very large stakeholder group and we were notified late last week of the proposed Order and General Permit. A continuance would give all of these stakeholders the necessary time to comment and provide meaningful input on this important issue.

Thank you for your consideration in this matter.

Denny (Denise) Knox
Executive Director
Ocean Beach MainStreet Association
1868 Bacon Street, Suite A
San Diego, CA 92107
Telephone: 619-224-4906
Fax: 619-224-4976
Email: info@oceanbeachsandiego.com
Website: www.OceanBeachSanDiego.com

Twitter:
http://www.oceanbeachsandiego.com/images/02_obma-tweets.png
Facebook:
http://www.oceanbeachsandiego.com/images/02_obma-facebook.png

THE CREADORE LAW FIRM P.C.
305 BROADWAY – FOURTEENTH FLOOR
NEW YORK, NEW YORK 10007

Donald E. Creadore - NY, MO, CT, DC
Thomas D. Seymour – MA

Tel. 212.355.7200
Fax. 212.583.0412
Efax 212.822.1459

October 8, 2010

VIA ELECTRONIC MAIL dgibson@waterboards.ca.gov
AND REGULAR MAIL
David W. Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court – Suite 100
San Diego, CA 92123

In reply refer to:
Reg. Measure ID 375971: MMATA
Place 656901

Re: Tentative Order No. R9-2010-0124, General Permit No. CAG99902
General Waste Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson:

This law firm represents the National Fireworks Foundation ("NFA"), and we write to respectfully request an extension of the public comment period and the hearing relating to Tentative Order R9-2010-0124 and General Permit CAG99902 for the following reasons.

The NFA writes to advise that Tentative Order No. R9-2010-0124 and General Permit CAG99902 is a first-in-the-nation regulation and, according to our membership and their customers, its adoption will have adverse impact upon fireworks displays nationwide. In addition to the direct consequences to be suffered by the display fireworks industry, there are also significant indirect consequences to be suffered by all the businesses (e.g., restaurants, hotels, charter boats) that earn revenues simply as a result of operating in proximity to a fireworks display. Locally, fireworks displays within the San Diego Region represent a primary source of revenues for local display fireworks businesses and their employees, in addition to being a lucrative source of revenues for scores of San Diego merchants, and all of this translates into a reliable source of jobs and generous tax revenues for the City.

The NFA currently has nearly four hundred members nationwide, and our local members that conduct business in and around the San Diego Region have been fielding a large number of inquiries from their customers regarding the regulation contemplated by this Board. As this issue will likely influence fireworks display operators and their customers throughout the United States, any decision rendered by this Board will, according to a consensus of our members, definitively have a demonstrative adverse impact throughout the San Diego region; this harm is real and credible and has the potential to spread to countless other localities, in terms of cancellation of fireworks display contracts and the resultant loss of the revenues, jobs and taxes they generate for their local communities, as well. In this sense, the Board's decision has nationwide reach and, consequently, any final determination should be well-informed and well-considered.

In light of the foregoing, we urge this Board to exercise restraint in making any determination and permit a full dialogue and examination of all issues and remedies. To that end, we respectfully request that the October 18, 2010 deadline to submit responses, as well as the November 10, 2010 hearing, be adjourned. By virtue of the fact that there is no known exigent circumstance creating urgency to the matter or immediate threat to health or property of a demonstrable nature to weigh against an extension, the objectives of a fair and open forum are best served by adjusting the present deadlines. The present deadline, October 18, 2010, is only seventeen (17) business days after issuance of the proposed regulations, dated September 23, 2010, and the NFA contends that this time period is inadequate for it to acquire the facts and documents necessary to prepare and furnish this Board with a comprehensive and meaningful response.

The NFA respectfully makes the additional request that, in the interim, the Regional Water Quality Control Board consider holding a workshop to analyze the competing interests and to allow all stakeholders the opportunity to present all legal, scientific, civic and economic policy considerations not already in the record.

Thank you for your consideration of our requests.

Very truly yours,

Donald E. Creadore

Item No. 6
Supporting Document No. 5

HUNSUCKER GOODSTEIN & NELSON PC
HGNLAW.COM

Brian L. Zagon
Attorney
Phone: 925-299-5105
bzagon@hgnlaw.com

October 8, 2010

VIA EMAIL AND U.S. MAIL

David W. Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Re: Tentative Order No. R9-2010-0124, General Permit No. CAG999002 General Waste Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson:

We represent Pyro Spectaculars, Inc. ("PSI"). PSI requests an extension of the October 18, 2010 public comment deadline and the November 10, 2010 Board hearing relating to Tentative Order R9-2010-0124 and proposed General Permit CAG999002, which pertains to the public display of fireworks. The Tentative Order and proposed General Permit were first circulated for public comment on September 23, 2010, only three weeks before the current deadline for public comment. The legal, scientific, and other issues presented by the Tentative Order and proposed General Permit are complex and have significant potential consequences involving many stakeholders, including all of the communities in the San Diego Region.

More time is needed for a public workshop process and to analyze Board staff's proposal. Accordingly, we request that Board staff hold a workshop to allow all stakeholders to present the legal, scientific, economic, and other public policy considerations not presently reflected in the record, and that the comment and hearing dates be moved until after the workshop takes place.

We and PSI appreciate the efforts of Board staff. We are optimistic that a workable solution can be reached through a collaborative workshop process with Board staff and other stakeholders.

Thank you for your consideration of this request.

Very truly yours,
Hunsucker Goodstein & Nelson PC

Brian L. Zagon

Item No. 6
Supporting Document No. 5

From: David Gibson
To: Creadore@aol.com
CC: Barker, David, Kelley, Brian
Date: 10/8/2010 10:13 AM
Subject: Re: Tentative Order No. R9-2010-0124; Reg. Measure ID375971: MMATA, Place 656901

Mr. Creadore,
Thank you for your email. We appreciate your comments and as I indicated in the voicemail I just left, we are planning a more extended process and public workshops for this Tentative Order.

I am available today at 858 336 2326 if you would like to discuss this today and I will return to the office on Monday.

Dave Gibson

David W. Gibson
Executive Officer
San Diego Water Board
858-467-4387
-----Original Message-----
From: <Creadore@aol.com>
To: Gibson, David <DGibson@waterboards.ca.gov>
Cc: Mata, Michelle <mmata@waterboards.ca.gov>

Sent: 10/8/2010 9:42:02 AM
Subject: Tentative Order No. R9-2010-0124; Reg. Measure ID375971: MMATA, Place 656901

Re: Tentative Order No. R9-2010-0124; Reg. Measure ID375971: MMATA, Place 656901

Dear Mr. Gibson: On behalf of the National Fireworks Association, please see attached letter of even date requesting an extension and other stated relief.
Kindly confirm receipt of this email and attachment.
I am available to discuss.
Thanks,
Don

Donald E. Creadore
The Creadore Law Firm, P.C.
305 Broadway-Fourteenth Floor
New York, New York 10007
917.226.1881
creadore@aol.com (mailto:pcreadore@aol.com)

Item No. 6
Supporting Document No. 5

From: Connie Anderson
To: Mata, Michelle
CC: Beegan, Chris; Fujimoto, Bruce; Gregorio, Dominic
Date: 10/11/2010 4:13 PM
Subject: Tentative Order No. R9-2010-0124 Fireworks Permit comments due Oct

Dear Michelle Mata,

Thank you for allowing us the opportunity to comment on the San Diego Regional Fireworks Permit Tentative Order No. R9-2010-0124.

Staff supports your efforts in including and considering the requirements of the Sediment Quality Objectives (SQOs) and Areas of Special Biological Significance (ASBS) absolute waste discharge prohibition.

Ocean Unit staff support the Sediment Monitoring Plan as described in VIII.A. and the water quality monitoring component. We feel the water column monitoring component is very vague and would like to see more specific guidelines. Unless monitoring takes place in the water column immediately after the fireworks displays are completed, and with some information about oceanic currents such as obtained from Southern California Coastal Ocean Observing System (SCCOOS) there is the possibility of little linkage between the fireworks fallout and the constituent level in the sediment as well.

The proposed Fireworks Permit prohibits discharges into ASBS, however, we suggest to use the term "State Water Quality Protection Areas" (SWQPA) in order to be more inclusive. Not all SWQPAs are ASBS, and we suggest using "The discharge of fireworks related waste to areas designated by the State Board as being State Water Quality Protection Areas, inclusive of Areas of Special Biological Significance (ASBS), is prohibited."

Staff is pleased to provide any additional support or guidance especially with regard to water quality monitoring and in light of the California Marine Protected Areas and relevant SWQPAs matters.

Sincerely,

Constance S. Anderson, Environmental Scientist
Areas of Special Biological Significance,
Ocean Unit, Division of Water Quality
State Water Resources Control Board
916.341.5280
ASBS listserve sign up link:
http://www.waterboards.ca.gov/resources/email_subscriptions/swrcb_subscribe.shtml (
http://www.waterboards.ca.gov/resources/email_subscriptions/swrcb_subscribe.shtml)

October 12, 2010

David Gibson
Executive Officer
San Diego Regional Water Quality Control Board
9174 Sky Park Ct., Ste. 100
San Diego, CA 92124

Dear Mr. Gibson:

In our recent conversation, you confirmed that the San Diego Regional Board intends to bring forward Tentative Order R9-2010-0124 that would specifically prohibit the discharge of fireworks displays over an Area of Special Biological Significance. The City has monitored and tested the waters off of the Heisler Park area for over ten years. There is no evidence that the fireworks display launched off of Monument Point for our Independence Day celebration has had any measurable effects to the ocean water quality.

The proposed regulation would greatly impact Laguna Beach. The City of Laguna Beach opposes the regulation that would eliminate Fourth of July fireworks displayed off of Monument Point as it has proven to be the safest location for staging the event among tens of thousands of people that attend the celebration. We are requesting that the Regional Board develop an exception for public agencies for a once-a-year discharge for fireworks to celebrate Independence Day.

Sincerely,

Kenneth Frank
City Manager

cc: City Council

505 FOREST AVE. • LAGUNA BEACH, CA 92651 • TEL (949) 497-3311 • FAX (949) 497-0771

Item No. 6
Supporting Document No. 5

CORONADO 4th of July

12 October 2010

David W. Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, Ca 92123

RE: Tentative Order No. R9-2010-0124, General Permit No. CAG999002
General Water Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson

The Coronado 4th of July Committee is requesting an extension of the October 18, 2010 public comment period and the November 10, 2010 Regional Board hearing on the Tentative Order and General Permit. We are also requesting that the California Regional Water Quality Control Board hold a workshop to properly address any significant legal, scientific, economic, civic and free speech issues that have arise from the proposed order and general permit.

We are part of a very large stakeholder group, many of whom are just now being notified of the proposed Order and General Permit. A continuance would give all of these stakeholders the necessary time of comment and provide meaningful input on this important issue.

Thank you for your consideration.

A P Szymanski

Andy Szymanski
President, 4th of July Committee

cc: City of Coronado

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Coronado 4th of July • PO Box 180541 • Coronado, CA 92178-0541
Phone / Fax: (619) 319-5147 • E-Mail: Coronado4thofJuly@usa.net

Item No. 6
Supporting Document No. 5

Michelle Mata - November 10, 2010, RWQCB meeting on Tentative Permit for Fireworks

From: Sandy Purdon <hppurdon@cox.net>
To: <dgibson@waterboards.ca.gov>
Date: 10/12/2010 11:02 AM
Subject: November 10, 2010, RWQCB meeting on Tentative Permit for Fireworks
CC: <mmata@waterboards.ca.gov>, "John Lormon" <john.lormon@procopio.com>, "P...

Dear Mr. Gibson,

On behalf of the San Diego Armed Services YMC charity that produces the annual Port of San Diego Big Bay Boom July 4th Fireworks Show, I would like you to consider postponing the November 10th issue on the Fireworks Permit that the RWQCB will be hearing. It is a challenge to analyze the ramifications of your draft permit and how it will potentially impact our ability to produce the show with the high cost of monitoring that is indicated. I am sure some balanced program can be worked out but the financial impacts of the draft permit and monitoring seem to be the main issue at hand. Most of these fireworks shows are produced by non-profits and the margins are slim for any additional costs. It would be a shame if these shows were to disappear when it seems that while we technically discharge some minor materials in the water there seems to be no damage to the water body. But we are sensitive to the environmental concerns as everyone would be. But let's not "throw the baby out with the bath water."

We are available to meet with you and your staff on this issue anytime that is convenient. We would appreciate your consideration to postpone the hearing on the draft permit until sometime next year.

Sandy Purdon

H. P. "Sandy" Purdon, Executive Producer/Founder
Port of San Diego's Big Bay Boom July 4th Fireworks Show
To Benefit the San Diego Armed Services YMCA
747 Golden Park Avenue
San Diego, CA 92106
619-822-1177
HPPurdon@cox.net

THE CITY OF SAN DIEGO

October 13, 2010

Electronic Submission to: mmata@waterboards.ca.gov

Mr. David Gibson, Executive Director
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Ms. Michelle Mata, WRC Engineer
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Dear Mr. Gibson and Ms. Mata:

Subject: City of San Diego Comments on the Tentative Order No. R9-2010-0124; NPDES Permit No. CAG999002; General Waste Discharge Requirements for Discharges Associated with the Public Display of Fireworks to Surface Waters within the San Diego Region

The City of San Diego, Storm Water Department, is pleased to provide the Regional Water Quality Control Board with comments regarding the tentative General National Pollutant Discharge Elimination System (NPDES) Permit for the firework displays. We understand the need to continue moving forward with water quality improvements, and want to work with you on permit revisions to maximize our water quality efforts in a cost effective and efficient manner.

Discharge Prohibitions

Firework displays are prohibited in Areas of Special Biological Significance (ASBS), Section IV(C). However, it states that discharges must be located a *sufficient distance* from the ASBS. Please define *sufficient distance* to help ensure the ASBS are protected.

Section VI(A)(2)(s) states *"It shall not be a defense for the enrollee in an enforcement action that effluent limitation violations are a result of analytical variability rendering the results inaccurate. The validity of the testing results, whether or not the enrollee has monitored or sampled more frequently that required by this Order, shall not be a defense to an enforcement action."* We recommend that this language be modified requiring the enrollee to comply with the requirements for the Surface Waters Ambient Monitoring Program.

Provisions-Standard Provisions

There is a reference to *permittees, dischargers, and enrollees* in Section VI (A)(2)(d); however, *sponsor* was not included as referenced in Section II(A)(1). Please clarify the association between sponsor and the other identified parties.

The Special Provision Section VI(C)(2) does not call for special studies. The State Water Resources Control Board (State Water Board) Water Quality Control Plan for Enclosed Bays and Estuaries – Part 1 Sediment Quality calls for special studies in Section VII (C)(3) when there are exceedances of the receiving water limits. The proposed Order should allow for special studies to demonstrate that other sources may also be contributing to the degradation of sediment quality.

Attachment A - Definitions

The definition of *Degrade* is not consistent with the definition in the State Water Board Resolution No. 68-16, also known at the *Anti-degradation Policy* which is defined as *"Any actions that can adversely affect water quality in all surface and ground waters must be consistent with the maximum benefit to the people of the state, must not unreasonably affect present and anticipated beneficial use of such water, and must not result in water quality less that that prescribed in water quality plans and policies"*. We recommend using the definition from Resolution 68-16 to be consistent.

Attachment E- Receiving Water Monitoring Requirements- Surface Water

The Sediment Monitoring Plan, Section VIII(A)(3) has several components, one of which is the Conceptual Model. Based on special monitoring studies conducted by the Storm Water Department, Strontium has been identified as a pollutant in storm water runoff. We recommend that a prevailing nighttime indirect deposition zone be added as a Conceptual Model component, and request a definition be included in Attachment A. This request is also based on documentation provided in the US Environmental Protection Agency Frequently Asked Questions About Atmospheric Deposition: A Handbook for Watershed Managers, EPA-453/R-01-009, dated September 2001.

If you have any questions or require more information, please don't hesitate to contact Ruth Kolb at (858) 541-4328.

Sincerely,

Kris McFadden
Interim Director

cc: Chron File

Michelle Mata

Item No. 6
Supporting Document No. 5

CITY OF SANTEE

October 13, 2010
Mr. David T. Barker
California Regional Water Quality Control Board
San Diego Region
9174 Sky Park Court
Suite 100
San Diego, CA 92123-4353

Re: Reg Measure ID 375971: MMATA Place:656901: Tentative Order No. R9-2010-0124 (Fireworks)

Dear Mr. Barker,

Thank you for the opportunity to comment on Tentative Order No. R9-2010-0124 regulating public fireworks displays that discharge pollutant wastes to surface waters in the San Diego Region. Based on a review of the order we would like to make the following recommendations:

Recommendation 1: Fireworks displays on land (where fire deposition zones are at least 50-feet from receiving waters) should be specifically exempted from this Order.

Recommendation 2: Once-a-year events should be exempted from this Order, provided that minimum BMPs are incorporated.

Fireworks displays on the 4th of July are part of our cultural heritage. The City of Santee organizes a 4th of July fireworks display to foster unity and a sense of community identity, as well as to honor those who serve this country. The event is attended by over 7,000 community members. Over half of the attendees surveyed said that participation in this community event was important to them. Nearly half cited a need for an affordable activity. Many municipalities already find it challenging to provide such displays, and this tentative order unnecessarily adds to the burden without providing any net environmental benefit.

Comment 1
It is not clear when a fireworks display would be considered to be causing a discharge to inland surface waters. For example, the Order would apply if fireworks were being discharged over the San Diego River, but would it also apply if the fireworks and associated deposition zone were in an area of land within the San Diego River watershed?

Recommendation 1
Firework shows over land should be specifically exempted from this order. The exemption language should require that the Fire Permit issued by the local agency identify a firework deposition zone limit that maintains a 50 foot buffer from a receiving water and incorporate best management practices to promptly remove any debris originating from the display.

Comment 2
The requirements of the Order are not proportional to the activity. Costs to comply with this Order will effectively double the cost of a once a year fireworks show. The City's annual fireworks display costs $22,500 and is funded by a private donation. A simple sediment sampling plan incorporating a QAPP, three locations sampled for chemical analysis and benthic macroinvertebrate assessment, and reporting, is estimated to cost at least $16,000. The $1,452 fee to obtain the permit and the associated paper work to demonstrate compliance with the permit is likely to take compliance costs close to $20,000. As a result of this disproportionate cost, the Order will likely result in the cancellation of many fireworks shows in the County.

The requirements are not based on evidence that the activity at a once-a-year frequency is polluting in a measurable way. We recommend that the RWQCB take a close look at any data collected to date on fireworks activities (such as that collected to assess the discharges from the Sea World fireworks display that occur nightly during the summer). Based on these findings it may be appropriate to regulate more frequent displays. However it is unlikely to be practicable to measure any pollutants associated with a once-a-year show.

Recommendation 2
Once-a-year events (such as community 4th July displays) should be exempted from this tentative order, provided that minimum BMPs are incorporated.

We support minimum BMPs (section C.3a through g), which are similar to those used by the City of Santee. We support the text on the use of environmentally-friendly fireworks, as we understand that they are not widely available. The City of Santee shares the Regional Board's goal of protecting and improving water quality. It is important that this Order be revised to acknowledge the value of 4th of July fireworks and to recognize that data collected to date does not support any measurable environmental benefit from their elimination. Thank you again for the opportunity to comment on this tentative order. If you have any questions regarding these comments then please contact Helen Perry at (619) 258-4100 x177.

Yours sincerely,

Pedro Orso Delgado, P.E.
Deputy City Manager/Director of Development Services

National Fireworks Association
Office of the President
8101 Fairview Ave
La Mesa, CA 91941

October 18, 2010

California Regional Water Quality Control Board
San Diego Region
9174 Sky Park Court
Suite 100
San Diego, CA 92123-4343

Re: Reg Measure ID 375971: MMATA
Place: 656901

I commend the Board on the decision to postpone the deadlines of October 18, 2010 for written comment and November 10, 2010 for the public hearing. This issue is much too important to be moved through on such a short and rapid process. Passage of this order as is has devastating consequences for sponsors, affiliated businesses and those of us that make our living in the Fireworks Industry. The move to slow this down and open up discussion via workshops is the reasonable thing to do.

I represent the fireworks industry as the president of a local company: Fireworks & Stage FX America, Inc and as the president of the National Fireworks Association. Let me state that the eyes of the country are on this Water Quality Board. SeaWorld San Diego is the only entity in this great country that has a permit such as this for producing fireworks shows over water. That alone should make us take a step back and at least wonder why and maybe rethink this process.

The firework industry has a great deal of information to present at these workshops. We also know that no matter what information is presented, there will be those that will not agree or accept our information. Yet, all that has come from others pressing for this "permit" are threats of lawsuits. They have presented no information scientific or other to justify their insistence on this permit under "Federal Law", which only seems to pertain to the San Diego area.

I find it totally amazing that on September 29, 2010, the Union Tribune publishes an article: "San Diego beaches earn top marks in water quality" and the Associated Press releases an article: "California has 4th straight summer of clean beach water". Does this indicate a severe pollution event from the fireworks being produced over water near our beaches?

Let the workshop begin!

Respectfully,

Joseph R. Bartolotta, President
National Fireworks Association
and
Fireworks & Stage FX America, Inc.

THE CREADORE LAW FIRM P.C.
305 BROADWAY – FOURTEENTH FLOOR
NEW YORK, NEW YORK 10007

Donald E. Creadore - NY, MO, CT, DC
Thomas D. Seymour – MA

Tel. 212.355.7200
Fax. 212.583.0412
Efax 212.822.1459

November 19, 2010

VIA ELECTRONIC MAIL dgibson@waterboards.ca.gov
AND REGULAR MAIL
David W. Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court – Suite 100
San Diego, CA 92123

In reply refer to:
Reg. Measure ID 375971: MMATA
Place 656901

Re: Tentative Order No. R9-2010-0124, General Permit No. CAG99902
General Waste Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson:

As you know, this law firm represents the National Fireworks Foundation ("NFA"), and we write to respectfully request that the scheduling of the workshop, the public comment period and the hearing relating to Tentative Order R9-2010-0124 and General Permit CAG99902 ("Tentative Order") be held in abeyance until a proper and complete order has been duly issued and, further, that the Notice of Public Workshop, dated November 17, 2010 (the "Notice"), be withdrawn.

The NFA registers its objections to the unorthodox and unprecedented demands that the NFA undertake to prepare a proper and authoritative response relating to highly technical, scientific and environmental issues before the California Regional Water Quality Control Board (the "Board") even issues a full and complete explanation of the underlying rules of the game.

By its own admission, the "Board is currently in the process of making modifications to the Tentative Order. Additional modifications include clarifying

language, adding definitions, and modifying receiving water monitoring requirements…" are to come; these are not trivial matters. The stated need to clarify language implies that the current language is defective or inaccurate in some manner and, consequently, requires correction before the intended meaning and expression is accurate.

Similarly, while "adding definitions," has many obvious benefits, at this late juncture it only throws into doubt the commonly-accepted definitions being used by the NFA and its retained experts and consultants, thereby compromising the reliability and accuracy of all work performed to date, causing it great prejudice.

Lastly, "modifying receiving water monitoring requirements" will undoubtedly have an impact upon recommended testing and monitoring protocol, and associated expenses. Surely, you can appreciate the fact that the NFA's experts and consultants cannot perform a professional and reliable investigation, and make meaningful recommendations, without first knowing what the receiving water monitoring requirements are, for example. The Notice implies that the "receiving water monitoring requirements in the Monitoring and Reporting Program." continues to be a work in progress; meaning that, presently, the NFA can only speak in the hypothetical with its experts and consultants regarding these matters, a highly unproductive process of questionable value. Simply stated, there is an inherent unfairness to demanding a person to respond to a set of requirements before they have even been issued and, seemingly, discourages participation.

To the extent that the Notice indicates that at some undetermined time in the future the Board will change the rules in the middle of the game, the workshop should be adjourned and the Notice withdrawn, even more so where no stated urgency is found and, here, none is stated. The NFA respects the fact that the San Diego Water Board is in the process of making modifications to the Tentative Order and awaits a final Tentative Order, and only asks that it and all other affected persons be afforded a reasonable opportunity to respond. In the interim, the Board is encouraged to remain sensitive to the fact that the NFA, and doubtless other persons, prefer to avoid incurring the time and expense of responding to one set of standards only to learn that they are subject to change by the Board.

In conclusion, we respectfully request that the scheduling of the workshop, the public comment period and the hearing relating to Tentative Order be held in abeyance until a proper and complete order has been duly issued and, further, that the Notice be withdrawn.

Thank you for your consideration of our requests.

Very truly yours,

Donald E. Creadore

GW

Item No. 6 Griffey
Supporting Document No. 5

Kelly E. Richardson
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LATHAM & WATKINS LLP

SAN DIEGO REGIONAL WATER QUALITY CONTROL BOARD

2010 NOV 22 P 3:00

File No. 503154-0000

November 19, 2010

VIA E-MAIL & FEDERAL EXPRESS

Executive Officer Gibson and Honorable Board Members
San Diego Regional Water Quality Control Board
9175 Sky Park Court, Suite 100
San Diego, CA 92123-4340

Re: Revised Tentative Order No. R9-2010-0124, NPDES No. CAG999002; General Waste Discharge Requirements for Discharges Associated With The Public Display Of Fireworks To Surface Waters in the San Diego Region

Dear Mr. Gibson and Honorable Board Members:

This letter is submitted in response to the November 17, 2010 public notice regarding a public workshop that the California Regional Water Quality Control Board, San Diego Region ("San Diego Water Board") has scheduled to hold on December 16, 2010 regarding a Revised Tentative Order No. R9-2010-0124 ("Revised Tentative Order"). The notice indicated that a public workshop has been scheduled in advance of any public distribution of a forthcoming Revised Tentative Order, with no specific date provided when such public distribution will be accomplished. As you know, many stakeholders objected to the Tentative Order and the fast-track schedule proposed in October to implement a first-in-the-nation NPDES permitting program for fireworks by November 2010. The Revised Tentative Order will raise significant legal, technical, economic, and practical considerations that will affect many stakeholders. It makes little sense to schedule a workshop without giving the public the benefit of sufficient time to review the yet-to-be released Revised Tentative Order.

We respectfully request that the Regional Board postpone any public workshop on the Revised Tentative Order and provide at least thirty (30) days, excluding the upcoming Thanksgiving holidays, following the forthcoming release to allow all interested persons the reasonable opportunity to review and comment prior to the workshop. Unless the public is allowed sufficient time to thoroughly review and analyze the Revised Tentative Order, any comments submitted or obtained at the public workshop would be premature and incomplete.

ORIGINAL

Please feel free to contact me if you have any questions. Thank you for your consideration of this matter.

Very truly yours,

LBR

Kelly E. Richardson

Kelly E. Richardson
of LATHAM & WATKINS LLP

GW

Item No 6 - Griffey
Supporting Document No. 5

11/19/2010

SAN DIEGO REGIONAL
WATER QUALITY
CONTROL BOARD

2010 NOV 22 P 3:42

S.D. Regional Water Quality Board
David Gibson – Executive Officer
9174 Sky Part Court, Suite 100
San Diego, CA 92123

Dear Mr. Gibson:

I am a 25-year Laguna resident. The annual fireworks display is something my children and grandchildren look forward to and thoroughly enjoy. It seems to be a shame that this simple pleasure that occurs once a year can't be exempted from the water regulations.

Laguna has consistently received high marks for the purity of our coastal water and beaches.

Surely there must be an easy fix to allow fireworks display to continue off Heisler Park's Monument Point. This is the safest and most logical location for viewing. Please, consider an exemption to allow this fine tradition to continue.

Best regards,

Paul Puma

Paul Puma
530 High Drive
Laguna Beach, CA 92651
Phone: 949-494-8842

[redacted] SW M. Mata

Item No. 6
Supporting Document No. 5

City of Mission Viejo

Office of the City Manager

December 1, 2010

Mr. David W. Gibson
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, California 92123

Subject: **Tentative Order No. R9-2010-0124, General Waste Discharge Requirements for Discharges Associated with Public Displays of Fireworks to Surface Waters in the San Diego Region**

Dear Mr. Gibson,

Thank you for the opportunity to comment on Tentative Order No. R9-2010-0124 regulating public fireworks displays that discharge pollutant wastes to surface waters in the San Diego Region.

We understand the issue of discharge of pollutants to surface waters of the State from fireworks displays originated from Sea World's daily summer-time fireworks displays over Mission Bay and the concerns raised over the impacts those pollutants may have on Mission Bay. While the Tentative Order proposes to address these issues by requiring a sediment sampling plan, a chemical analysis, and a benthic macro-invertebrate analysis of surface waters under any fireworks display to gauge the impacts fireworks fall-out have on surface waters, the Tentative Order's proposed requirements are unreasonable for single annual fireworks displays like on the Fourth of July paid for by non-profit organizations and municipalities as a show of patriotic support for our Country.

Fourth of July fireworks in Mission Viejo are primarily paid for by the Mission Viejo Activities Committee, a non-profit, volunteer, civic organization, which also holds a Fourth of July Street Faire on Olympiad Road in conjunction with this holiday. It is part of the community's tradition to hold a Fourth of July fireworks display hosted by the Mission Viejo Activities Committee. Therefore, the City agrees with the City of Santee's October 13, 2010, comments that the potential costs for complying with this Tentative Order for a single annual display of fireworks is overly costly and disproportionate to the cost of the display. Our own analysis suggests that the cost of compliance would easily reach $10,000 plus the proposed Regional Board's Notice of Intent to Discharge (permit) fee of $1,452.

200 Civic Center • Mission Viejo, California 92691
http://www.cityofmissionviejo.org

949/470-3051
FAX 949/859-1386

The City of Laguna Beach in its October 12, 2010 comment letter correctly implies that cities choose fireworks display locations primarily as a function of public safety. Fourth of July fireworks in Mission Viejo are fired off from the Marty Russo Youth Athletic Park adjacent to Olympiad Road under the auspices of the Orange County Fire Authority. This location provides one of the safest locations for the viewing of fireworks by Mission Viejo residents. The fireworks fall-out zone includes an open channel within the Youth Athletic Park. While the channel is a man-made channel, the Permit covers all "waters of the State," which is defined as "any surface water or groundwater, including saline waters, within the boundaries of the state" per Water Code Section 13050(e). Therefore, because Mission Viejo's fireworks fall-out zone is over waters of the State, this channel would need to be monitored and a Notice of Intent to Discharge application would need to be made to the Regional Board. There is no evidence that aerial deposition of fireworks causes pollution in this narrow stretch of channel. If the Tentative Order is issued as-is, the Tentative Order assumes any single fireworks display will impact any water bodies of the State, like this channel, and will place the burden on municipalities and non-profit organizations to prove otherwise. This is not sound public policy.

Therefore, the City of Mission Viejo requests that the Regional Board exempt once-per-year fireworks displays from this Tentative Order, and revise the Tentative Order to exclude inland surface waters. Anything less would unnecessarily add burdensome costs to Fourth of July fireworks displays onto community organizations and municipalities, and may potentially cancel patriotic displays of celebration of our Country.

Questions on this letter may be addressed to Richard Schlesinger, City Engineer, at 949/470-3079 or rschlesinger@cityofmissionviejo.org.

Sincerely,

Dennis R. Wilberg
City Manager

cc: Mayor, Mayor Pro Tem, and City Council Members
Mission Viejo Activities Committee
Mark Chagnon, Director of Public Works
Rich Schlesinger, City Engineer

G:\PW\WP\LETTERS\Joe\Water Quality\Fireworks Displays (2).doc

SW

Item No. 6
Supporting Document No. 5

K. Kelley (M. Mata)

December 3, 2010

SAN DIEGO REGIONAL
WATER QUALITY
CONTROL BOARD

2010 DEC -6 P 2:40

SD Regional Water Quality Board
Attn: David Gibson
9174 Sky Port Court
San Diego, CA 92123

RE: Public Comments, Tentative Order # R9-2010-0124

Dear Director Gibson:

As a disabled veteran who has spent eleven years in the US Army Infantry, Fourth of July fireworks displays to celebrate our country's birth are something very sacred to me and my family. I now understand that the City of Laguna Beach may not be permitted by your agency to have the Heisler Park annual fireworks display next year.

At first I thought "What a bunch of unpatriotic bastards?", but after thinking about it for a while, there seemed to some hidden dirty political motivation for such unreasonable actions.

Fireworks do have unhealthy toxic fallout but the bad effects once a year are almost nothing compared to the huge commercial ***questionable*** use of fireworks. By ***questionable***, I mean amusement parks (Sea World), baseball games, concerts, and other for profit events that use fireworks displays as a sideline to increase attendance, not as a necessary part of their activities. However, fireworks are a necessity to celebrate the birth of our nation.

The Anaheim Disneyland Park has huge fireworks displays almost daily year round. I understand that Disney may be contaminating the nearby large Santa Ana River settlement ponds that are used to refurbish Orange County's underground water supplies.

A reasonable order by your agency would be to eliminate the unnecessary commercial use of fireworks and limit fireworks displays only on the Fourth of July or New Years. So the real purpose of your tentative order could be some sort of a scam to develop a ground swell of pro fireworks support from patriotic groups of Americans. This support then could be misinterpreted to justify the continuing pollution by commercial year round fireworks users.

Denis Fitzgerald

Denis Fitzgerald, P.O. Box 9222, Laguna Beach, CA 92652-9222

SW

Item No. 6
Supporting Document No. 5

CITY of LAGUNA NIGUEL
27801 La Paz Road • Laguna Niguel, California 92677
Phone/949•362•4300 Fax/949•362•4340

December 7, 2010

Michelle Mata
9174 Skypark Court, Ste. 100
San Diego CA 92123
mmata@waterboards.ca.gov

RE: PROPOSED GENERAL WASTE DISCHARGE REQUIREMENTS FOR THE PUBLIC DISPLAY OF FIREWORKS – TENTATIVE ORDER NO. R9-2010-0124, NPDES NO. CAG999002

Dear Ms. Mata:

Thank you for the opportunity to comment on Tentative Order No. R9-2010-014, which would establish Waste Discharge Requirements for the Public Display of Fireworks. The City of Laguna Niguel sponsors one annual public fireworks display on each 4th of July. Pyro Spectaculars, Inc. is our currently contracted fireworks display operator. From our reading of the Draft Tentative Order, it appears that our once-per-year public fireworks display would be regulated under the Order and that the City of Laguna Niguel would be required to provide an annual Notice of Intent and Public Display of Fireworks Event Notification Form, pay applicable filing fees, implement specific Best Management Practices, maintain event-related activity logs, conduct pre-event and post-event water quality and sediment monitoring and submit monitoring reports to the Regional Board.

It is our understanding that the Draft Tentative Order is based on the premise that public fireworks displays contain constituents, particulates and debris that may adversely affect water and sediment quality in receiving waters. The Draft Tentative Order relies exclusively on the results of water quality and sediment monitoring by Sea World San Diego to support this premise.

The City of Laguna Niguel offers the following comments on Draft Tentative Order No. R9-2010-014:

1. ***The Draft Tentative Order is a "One Size Fits All" Approach that Fails to Differentiate between Events of Different Magnitude***

There is clearly a difference between a sponsor like Sea World San Diego which discharges public fireworks on a nightly basis up to 150 times per year and a sponsor like the City of Laguna Niguel that discharges public fireworks once-per-year to commemorate the 4th of July (Independence Day). Any Final Order should recognize such differences and scale or tier new permitting and monitoring requirements accordingly. There is a reasonable argument that all once-per-year public fireworks

displays should be completely exempted from the NOI filing, fee and monitoring requirements of the Draft Tentative Order.

2. ***The Draft Tentative Order Fails to Differentiate between Event Sites of Greater or Lesser Sensitivity and Proximity to Surface Waters***

The City of Laguna Niguel conducts its annual 4th of July public fireworks display from an inland County Regional Park site that is 3.5 miles from the Pacific Ocean and 6.0 miles from the nearest Area of Special Biological Significance (ASBS). Prevailing coastal winds are more likely to carry fireworks-related constituents and particulates away from sensitive surface waters. Any Final Order should recognize such site differences and scale or tier new permitting and monitoring requirements accordingly. There is a reasonable argument that once-per-year public fireworks displays at less sensitive sites should be completely exempted from the NOI filing, fee and monitoring requirements of the Draft Tentative Order.

3. ***The Sea World San Diego Monitoring Studies Suggest that the Affects from Once-Per Year Public Fireworks Displays are Insignificant***

The Draft Tentative Order cites water and sediment quality monitoring that has been conducted by Sea World San Diego since 2001. Despite the frequency of Sea World public fireworks displays (up to 150 times per year) and the unique characteristics of Mission Bay (restricted circulation and shallow depth of bay in the vicinity of the fireworks events), water chemistry sampling showed little evidence of pollutants that exceeded applicable water quality objectives. Sediment chemistry monitoring revealed highly variable and temporal results that were seemingly inconclusive. Based on the Sea World monitoring results, it is reasonable to assume that the pollutant affects from once-per-year public fireworks displays are insignificant.

4. ***The Draft Tentative Order Would Impose Costs for Annual Administrative and Monitoring Compliance that Would Be Burdensome and Out-of-Proportion for Once-A-Year Fireworks Displays***

The City's annual fireworks display currently costs about $32,000 to produce, and the show lasts for 20 to 30 minutes. The Draft Tentative Order would add $1,452 for filing an annual Notice of Intent (NOI). In another comment letter, the City of Santee has estimated a cost of $16,000 for development and implementation of a sampling and quality assurance plan and the sampling and analytical analysis of the water column, sediment chemistry, and benthic fauna at strategic locations. It has been suggested that all of a region's fireworks event sponsors form a coalition to share the costs of a regional monitoring program. It is questionable that such an approach would reduce individual monitoring costs given the diversity of fireworks launch locations, fireworks deposition zones and surface and receiving waters. As a result, adding 50% or more to the current cost of once-per-year public fireworks displays, as well as new staffing burdens, may cause future fireworks displays to be significantly cut back or cancelled.

5. *The Draft Tentative Order Requires Further Consideration and Consultation with All Affected Stakeholders*

The Draft Tentative Order will potentially affect numerous public agencies, non-profit organizations and associations and private commercial enterprises that are either sponsors or operators of public fireworks displays in the San Diego region. It is not clear that the Regional Board has identified the full range of public and private stakeholders who will be affected by the regulation of public fireworks displays as a pollutant waste discharge. Additional time should be taken to gather information on the number of public and private fireworks display sponsors in the San Diego region, the location and frequency of fireworks events, and the proximity of such events to sensitive water bodies. Any Final Order should not take effect until January 1, 2012 to avoid disruption to events that are already planned and/or contracted for in 2011 including the 4th of July.

In summary, the City of Laguna Niguel respectfully requests that the Regional Board:

1. Reconsider the necessity and merits of the Draft Tentative Order to regulate public fireworks displays.
2. Consider an exemption or waiver for sponsors of once-per-year public fireworks displays.
3. Recognize the significant differences in event size/magnitude/frequency and event locations/proximity to surface waters and modify the Draft Tentative Order accordingly.
4. Defer the effective date of any Final Order to January 1, 2012 to avoid impacts on events that are already planned or scheduled for 2011.

Thank you for your consideration of the City's comments.

Yours truly,

Tim Casey
City Manager

Cc: Mayor and City Council
Terry Dixon, City Attorney
Pam Lawrence, Deputy City Manager
John Banks, Deputy Director of Recreation
Nancy Palmer, Environmental Programs Manager

CITY of LAGUNA NIGUEL
27801 La Paz Road • Laguna Niguel, California 92677
Phone/949•362•4300 Fax/949•362•4340

December 7, 2010

Michelle Mata
9174 Skypark Court, Ste. 100
San Diego CA 92123
mmata@waterboards.ca.gov

RE: PROPOSED GENERAL WASTE DISCHARGE REQUIREMENTS FOR THE PUBLIC DISPLAY OF FIREWORKS – TENTATIVE ORDER NO. R9-2010-0124, NPDES NO. CAG999002

Dear Ms. Mata:

Thank you for the opportunity to comment on Tentative Order No. R9-2010-014, which would establish Waste Discharge Requirements for the Public Display of Fireworks. The City of Laguna Niguel sponsors one annual public fireworks display on each 4th of July. Pyro Spectaculars, Inc. is our currently contracted fireworks display operator. From our reading of the Draft Tentative Order, it appears that our once-per-year public fireworks display would be regulated under the Order and that the City of Laguna Niguel would be required to provide an annual Notice of Intent and Public Display of Fireworks Event Notification Form, pay applicable filing fees, implement specific Best Management Practices, maintain event-related activity logs, conduct pre-event and post-event water quality and sediment monitoring and submit monitoring reports to the Regional Board.

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displays should be completely exempted from the NOI filing, fee and monitoring requirements of the Draft Tentative Order.

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4. ***The Draft Tentative Order Would Impose Costs for Annual Administrative and Monitoring Compliance that Would Be Burdensome and Out-of-Proportion for Once-A-Year Fireworks Displays***

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5. *The Draft Tentative Order Requires Further Consideration and Consultation with All Affected Stakeholders*

The Draft Tentative Order will potentially affect numerous public agencies, non-profit organizations and associations and private commercial enterprises that are either sponsors or operators of public fireworks displays in the San Diego region. It is not clear that the Regional Board has identified the full range of public and private stakeholders who will be affected by the regulation of public fireworks displays as a pollutant waste discharge. Additional time should be taken to gather information on the number of public and private fireworks display sponsors in the San Diego region, the location and frequency of fireworks events, and the proximity of such events to sensitive water bodies. Any Final Order should not take effect until January 1, 2012 to avoid disruption to events that are already planned and/or contracted for in 2011 including the 4th of July.

In summary, the City of Laguna Niguel respectfully requests that the Regional Board:

1. Reconsider the necessity and merits of the Draft Tentative Order to regulate public fireworks displays.
2. Consider an exemption or waiver for sponsors of once-per-year public fireworks displays.
3. Recognize the significant differences in event size/magnitude/frequency and event locations/proximity to surface waters and modify the Draft Tentative Order accordingly.
4. Defer the effective date of any Final Order to January 1, 2012 to avoid impacts on events that are already planned or scheduled for 2011.

Thank you for your consideration of the City's comments.

Yours truly,

Tim Casey
City Manager

Cc: Mayor and City Council
Terry Dixon, City Attorney
Pam Lawrence, Deputy City Manager
John Banks, Deputy Director of Recreation
Nancy Palmer, Environmental Programs Manager

December 8, 2010

Attn: Michelle Mata
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Via Electronic Mail
mmata@waterboards.ca.gov

RE: **Tentative Order No. R9-2010-0124, NPDES Permit No. CAG999002**
General Waste Discharge Requirements For Discharges Associated With Public Displays of Fireworks To Surface Waters In The San Diego Region

Dear Ms. Mata and Regional Board members:

Please accept these comments in strong support of the proposed water quality protections to be provided upon adoption of Tentative Order No. R9-2010-0124 by the Regional Water Quality Control Board.

As Board staff is well aware, when fireworks explode over or adjacent to surface waters, chemicals contained in the fireworks are discharged directly into the water bodies themselves. The chemical-laden paper casings of spent fireworks, as well as unexploded "dud" fireworks also fall from the sky into the surface waters, and are often not recoverable. Both the federal and state Clean Water Acts require that permits be obtained before such discharges occur, and we appreciate the Board finally addressing this mandate.

Fireworks generally contain perchlorate salts, aluminum, magnesium, titanium, barium copper, chloride, and potassium nitrates. Other hazardous chemical compounds often associated with fireworks include, but are not limited to: arsenic salts, strontium salts, lithium salts, calcium salts, sodium, barium, cadmium, copper, aluminum, titanium, lead, mercury and magnesium. These chemicals are widely regulated by the Board, and no other circumstance exists where such intentional point source discharges are tolerated unpermitted. Failure to regulate these obvious discharges not only threatens water quality, it also calls into question the integrity of the Board's entire permitting scheme.

I would urge the Board to resist the pressures of fireworks purveyors and local municipalities to water down the proposed Waste Discharge Requirements. It is critical that the permitting, reporting, and monitoring provisions of the draft permit be maintained until the full impacts of these discharges are understood.

Sincerely,

Joel Goldberg

Jgoldberg76@gmail.com

O C E A N B E A C H

MAINSTREET ASSOCIATION

December 8, 2010

Regional Water Quality Control Board
9174 Sky Park Ct # 100
San Diego, CA 92123-4353

Dear Hon. Members of the Board:

Thank you for the opportunity to comment on the **proposed Tentative Order R9-2010-0124 regarding the public display of fireworks.** Ocean Beach MainStreet Association is the sponsor of the Annual 4th of July Fireworks off the OB Pier event. Our organization has been putting on fireworks at this event for 32 and are concerned that the proposed Tentative Order raises many complicated and vague requirements and threatens to end important traditions that thousands of citizens count on each year. We are also not aware of any evidence that our show has caused or is otherwise a threat to the environment.

First, we believe that the extensive permitting, monitoring and BMPs proposed in the Tentative Order could add tens of thousands of dollars and make the economics of shows prohibitive. Several people have estimated that the water and sediment monitoring alone, could cost between $30,000 and $100,000 per event, depending on the amount of testing required to prove a negative. The cost of our show is $25,000 and these requirements would more than double the cost of putting on this show for the public. The added cost would likely end our fireworks show tradition. Our show is funded with community and small business donations, and each year we work very hard to raise the funds necessary to put on this display for the public. We do not believe that we would be capable of raising the additional funds necessary to implement these regulations.

Second, we do not understand why once or twice yearly fireworks shows are being singled out for regulation. These shows have been going on for decades, and no one has ever suggested that these patriotic and civic displays present a water quality problem. In fact, your order provides no evidence that once or twice a year fireworks shows, cause any problems. Massive fireworks shows are done each year over bodies of water throughout the United States, but do not require the monitoring and permitting suggested in the proposed Tentative Order. We do not believe that there is a problem, and ask that you exempt our short and occasional shows from these regulations. We understand no other fireworks event in the country other than SeaWorld San Diego has a fireworks permit under the Clean Water Act, and our show is much smaller than SeaWorld's summer-long show. We also understand inland fireworks over inland water

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info@OceanBeachSanDiego.com

O C E A N  B E A C H

MAINSTREET ASSOCIATION

bodies are exempted. The scientific evidence, even at SeaWorld, shows no significant environmental impacts.

Lastly, fireworks are part of the social fabric and economic engine of our community. It is hard to imagine that a single American has not watched fireworks at some point on the Fourth of July or New Years Eve. They are part of a public celebration of our collective liberty that brings the entire community together. Also, our tourist business booms during the first week of July with visitors staying in the area 3 to 5 days which helps support our local small businesses.

We are at a loss to understand why the Regional Board would want to regulate out of existence something that is so important to so many. We hope the Regional Board will reconsider the merit of this proposed regulation, or find that infrequent , professionally administered fireworks events have minimal impact and do not require any onerous regulation.

Sincerely,

Denise Knox

Denise Knox
Executive Director

1868 Bacon St., Ste. A
San Diego, CA 92107

PH: 619-224-4906
FAX: 619-224-4976

OceanBeachSanDiego.com
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City of San Clemente
Beaches, Parks and Recreation Department
Sylvia Rosenthal, Interim Recreation Manager
Phone: (949) 361-8343 Fax: (949) 361-8280
Email: RosenthalS@san-clemente.org

December 8, 2010

Ms. Michelle Mata
California Regional Water Quality Control Board
9174 Sky Park Court
San Diego, CA 92123

Re: Tentative Order R9-2010-0124

Ms. Mata;

The City of San Clemente has recently become aware that the California Regional Water Quality Control Board is considering new regulations for fireworks displays.

Our City offers only one show per year, in celebration of Independence Day. We do not expect our display to grow beyond what we have historically done. We are concerned that the costs of these new regulations could be prohibitive and possibly result in the cancellation of our traditional celebratory show.

The City of San Clemente respectfully requests that the California Regional Water Quality Control Board withdraw the proposed Tentative Order, or at least review and adjust the requirements to not affect shows that are only one time per year.

Thank you for your time and consideration.

Sylvia N. Rosenthal
Interim Recreation Manager

HUNSUCKER GOODSTEIN & NELSON PC
HGNLAW.COM

Brian L. Zagon
Attorney
Phone: 925-299-5105
bzagon@hgnlaw.com

December 9, 2010

VIA EMAIL AND U.S. MAIL

Michelle Mata
California Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123
Email: mmata@waterboards.ca.gov

Re: Tentative Order No. R9-2010-0124, General Permit No. CAG999002 General Waste Discharge Requirements for the Public Display of Fireworks

Topics of Discussion for December 16, 2010 Public Workshop

Dear Ms. Mata:

We represent Pyro Spectaculars, Inc. ("PSI"). PSI is a family-owned fireworks display company that creates and sells fireworks shows to public, private and non-profit customers around the country, including in the San Diego Region. Representatives of PSI will attend the December 16, 2010 Public Workshop ("Workshop") regarding Tentative Order No. R9-2010-0124 (the "Tentative Order"). The purpose of this letter is to provide the Regional Board and its staff with some of the topics that should be discussed at the Workshop. PSI requests that one of its representatives have the opportunity to speak at the Workshop. PSI looks forward to a productive discussion.

Before setting forth the individual topics, it is important to focus on big picture ramifications of the Tentative Order. The Tentative Order will create a permit process for annual fireworks display shows that does not exist anywhere else in California or the United States. The application process, consultant workplans, repeated water and benthic sampling requirements and consultant reporting that appear to be required by the Tentative Order for each fireworks display show over water will so dramatically increase the costs that the shows will not take place. While no doubt an unintended consequence of the Tentative Order, the traditional public 4th of July celebrations in the San Diego Region, enjoyed by hundreds of thousands of residents, will no longer take place. This issue has been addressed in numerous comment letters already submitted to the Regional Board.

We now turn to some of the specific issues that PSI wants to discuss at the Workshop.

I. LEGAL BASIS FOR ISSUING WDRS FOR FIREWORKS DISPLAYS

We would like to spend some time during the Workshop discussing the Regional Board's legal basis for issuing Waste Discharge Requirements ("WDRs") for fireworks displays as proposed in the Tentative Order. PSI, as well as the fireworks industry trade organizations, does not think that the Regional Board has the legal authority to regulate fireworks displays through the issuance of WDRs. No similar permit has been required anywhere else in California or the United States.

The federal Clean Water Act's National Pollutant Discharge Elimination System and the California WDR requirements are applicable only to "point sources" of pollution. City of Arcadia, et al. v. State Water Resources Control Board, 135 Cal.App.4th 1392, 1404-05 (2006). "Point Source" is defined as "any discernible, confined and discrete conveyance ... from which pollutants are or may be discharged." 33 U.S.C. 1362; Cal. Water Code § 13373. Fireworks displays are not "discernible, confined and discrete conveyances" for the discharge of pollutants to navigable waters. Fireworks displays are seasonal entertainment events where pyrotechnics are launched into the air from a mortar. Fireworks, not "pollutants," are shot out of the mortars.

The legislative history of the Clean Water Act suggests that Congress meant only to cover discharges that were at least "frequent," or that resulted in some "measurable" waste entering the water. NW. Envtl. Def. Ctr. v. Brown, 617 F.3d 1176, 1183 (9th Cir. 2010). The definition of "point source" and the examples given by Congress "evoke images of physical structures and instrumentalities that systematically act as a means of conveying pollutants from an industrial source to navigable waterways." Plaza Health Labs., Inc., 3 F.3d 643, 646 (2d Cir. 1993) (emphasis added). Seasonal fireworks displays, however, take place only once or twice per year and are not an industrial source of "pollutants." The evidence shows that occasional public fireworks displays are not "frequent" nor do they result in any "measurable" amount of material entering the water. Any small amount of debris that may fall to the water surface is incidental to the primary entertainment purpose of the show. Incidental discharges from airborne deposition have been considered "non-point discharges" under the Clean Water Act. For these reasons, there is no legal basis for the issuance of the WDRs in the Tentative Order.

II. THERE IS NO SCIENTIFIC BASIS FOR ISSUING WDRS

PSI requests that the Regional Board explain the scientific basis for its decision to regulate fireworks displays within the San Diego Region through the issuance of WDRs. The Regional Board's decisions must be based on science, not speculation or

fear of litigation. The data from the sampling by SeaWorld clearly demonstrates that no significant impact to water quality is associated with the vast number of fireworks shows (80 or more shows) put on by SeaWorld each year. The data is compelling when one considers the unique features of Mission Bay that are unlike most other waterbodies in the San Diego Region. This point is recognized by the Regional Board in the Tentative Order: "SeaWorld's public fireworks events represent the highest level of water and sediment effects because ... Mission Bay is unique due to the restricted circulation of waters within the bay [and] the shallow depth of the bay in the vicinity of the fireworks events." (Tentative Order, p. 7.) According to the terms of the Tentative Order, both the Regional Board and the United States EPA have classified fireworks displays as very minor discharges. (Tentative Order, p. 1, Table 1.) Further, the Tentative Order states that "the threat to water quality and complexity of the discharge is determined to be category 3C" under Title 23, Section 2200 of the California Administrative Code. Category 3C is the lowest possible threat discharge. There is no scientific basis here to support issuance of the Tentative Order. At a minimum, the proposed water quality and benthic sampling requirements, which are substantial and extremely expensive, should be eliminated.

III. ANY DISCHARGE FROM FIREWORKS DISPLAYS IS *DE MINIMUS*

State law defines a *de minimis* discharge as "types of wastes that have low pollutant concentrations and are not likely to cause or have a reasonable potential to cause or contribute to an adverse impact on the beneficial uses of receiving waters yet technically must be regulated under an NPDES permit." 23 Cal. Code of Regs. § 2200, n. 15. While we believe that the Regional Board lacks legal authority to regulate fireworks displays in the first instance, if the Regional Board continues to take a contrary position it should exercise its discretion and, based on science and the available sampling data, make a finding that fireworks displays are a *de minimis* discharge that requires no permit.

IV. WAIVERS UNDER SECTION 13269 OF THE WATER CODE

Section 13269 of the Cal. Water Code allows the Regional Board to waive WDRs as to a specific discharge or type or discharge – in this case, fireworks displays over water – if two criteria are met: (1) the Regional Board determines that the waiver is consistent with applicable state or regional water quality control plans; and, (2) the waiver is in the public interest. Both of these criteria are met. In addition, because the SeaWorld and other available sampling data shows that the proposed discharges do not pose a significant threat to water quality, the Regional Board should waive the monitoring requirement. Cal. Water Code § 13269(a)(3). If the Regional Board ultimately decides to regulate this issue, it should include a clear, easy to use, and inexpensive conditional waiver process.

V. ECONOMIC CONSIDERATIONS MUST BE TAKEN INTO ACCOUNT

The Regional Board is required by law to consider both the economic and social impacts of its actions including the issuance of WDRs. Cal. Water Code §§ 13000, 13241 and 13263(a). The United States Supreme Court confirmed that it is proper to compare costs and benefits when applying the Clean Water Act in situations where the benefits are significantly disproportionate to the costs - especially when the benefits are *de minimis*, as here. Entergy Corp. v. Riverkeeper, Inc., 129 S. Ct. 1498, 1510 (2009).

The sponsors of the vast majority of fireworks displays in the San Diego Region are cities, towns and small non-profit organizations. These fireworks shows are not typically a source of revenue to the sponsor and are generally held to express patriotism, celebrate individual and group accomplishments and to benefit charitable organizations and local small businesses. Most Independence Day committees, non-profit organizations and community groups have neither the expertise to comply with the technical requirements of the Tentative Order nor the financial resources to fund the substantial increases in their costs if it is adopted.

There is social value to these fireworks displays. They provide a form of free entertainment to the community, which is desperately needed in these tough economic times. Fireworks shows attract tourists and draw patrons to local hotels, restaurants and other establishments. The unique relationship between the greater San Diego area and the U.S. military makes the region a great place to hold fireworks displays.

If the Tentative Order is adopted, the cities, towns and small non-profit organizations that sponsor the vast majority of fireworks displays in the San Diego Region will be subject to substantial and costly monitoring and reporting requirements. It has been estimated that the cost to comply with just the monitoring and reporting requirements for a single fireworks display could reach into the six figure range – multiples of what it costs to actually put on the average fireworks display. The likely result is the end of public 4th of July fireworks celebrations.

These substantial costs clearly outweigh any benefit to water quality or the environment that may come by the Regional Board's adoption of the Tentative Order. These displays have already been categorized by the Regional Board as a very low threat discharge.

VI. THE TENTATIVE ORDER THREATENS THE RIGHTS OF CITIZENS TO DEMONSTRATE THEIR PATRIOTISM

The rights of the citizens in the San Diego area to demonstrate their patriotism on Independence Day and to respect our country's Founding Fathers, who pledged their lives, their fortunes and their honor to fight for the very freedoms at issue here, are

matters of Constitutional dimension, of free speech and of the right to peaceably assemble. These are matters of national tradition that necessarily include and revolve around 4th of July fireworks displays. No less are the rights of these same citizens to express themselves through the arts, such as in presentations by the symphony and in other public celebrations and tributes throughout the year.

The application of the Tentative Order to these citizens will result in the elimination of most, if not all, of their fireworks displays, including annual 4th of July fireworks shows in the San Diego area. This result will greatly impair the rights of these San Diego citizens, while leaving other citizens of California and the rest of the country free to express their patriotism and artistic views without such extraordinary financial, technical or logistical burdens.

The Tentative Order, if made final, would also end the availability of a safe public venue for citizens to peaceably assemble in the spirit of community. It would impair their rights to express themselves through the arts, and virtually extinguish their ability to celebrate our nation's most patriotic holiday, Independence Day, in their own communities and in the manner envisioned by our forefathers at the inception of this great country. The harshest consequences of adoption of the Tentative Order will fall upon those of lesser financial means.

PSI appreciates the opportunity to discuss these and other issues with Regional Board staff and the other interested parties at the December 16, 2010 Workshop.

Very truly yours,
Hunsucker Goodstein & Nelson PC

Brian L. Zagon/mm

Brian L. Zagon

BLZ:mm

COASTAL ENVIRONMENTAL RIGHTS FOUNDATION

December 9, 2010

Attn: Michelle Mata
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Via Electronic Mail
mmata@waterboards.ca.gov

Re: Tentative Order No. R9-2010-0124, NPDES Permit No. CAG999002
General Waste Discharge Requirements For Discharges Associated With Public Displays of Fireworks To Surface Waters In The San Diego Region

Dear Ms. Mata and Regional Board members:

Please accept these comments on behalf of the Coastal Environmental Rights Foundation (CERF) in strong support of the proposed water quality protections to be provided upon adoption of Tentative Order No. R9-2010-0124 by the Regional Water Quality Control Board. CERF is a nonprofit environmental organization founded by surfers in North San Diego County and active throughout California's coastal communities. CERF was established to aggressively advocate, including through litigation, for the protection and enhancement of coastal natural resources and the quality of life for coastal residents.

As Board staff is well aware, when fireworks explode over or adjacent to surface waters, chemicals contained in the fireworks are discharged directly into the water bodies themselves. The chemical-laden paper casings of spent fireworks, as well as unexploded "dud" fireworks also fall from the sky into the surface waters, and are often not recoverable. Both the federal and state Clean Water Acts require that permits be obtained before such discharges occur, and we appreciate the Board finally addressing this mandate.

Fireworks generally contain perchlorate salts, aluminum, magnesium, titanium, barium copper, chloride, and potassium nitrates. Other hazardous chemical compounds often associated with fireworks include, but are not limited to: arsenic salts, strontium salts, lithium salts, calcium salts, sodium, barium, cadmium, copper, aluminum, titanium, lead, mercury and magnesium. These chemicals are widely regulated by the Board, and no other circumstance exists where such intentional point source discharges are tolerated unpermitted. Failure to regulate these obvious discharges not only threatens water quality, it also calls into question the integrity of the Board's entire permitting scheme.

I would urge the Board to resist the pressures of fireworks purveyors and local municipalities to water down the proposed Waste Discharge Requirements. It is critical that the permitting, reporting, and monitoring provisions of the draft permit be maintained until the full impacts of these discharges are understood.

Thank you for your consideration of these comments, and for addressing this important water quality issue.

Sincerely,

COASTAL ENVIRONMENTAL RIGHTS FOUNDATION

SARA S. HONADLE
Programs Director

Encl.:

- 6/30/09 Mother Nature Network "Are fireworks bad for the environment?"
- 7/03/09 ABC Science "Fireworks to become a little greener"
- American Chemical Society "How Fireworks Work"
- 06/25/10 Big Bay Boom Water Sampling Results
- 06/30/08 Chemical & Engineering News "Pyrotechnics for the Planet"
- RWQCB Conditional Waiver No. 11 - Aerially Discharged Wastes Over Land
- Environmental Research Letters "Do pyrotechnics contain radium?"
- July 2006 Department of Toxic Substances Control "DTSC's Perchlorate Best Management Practices"
- Earth Island Journal "Fallout Over Disneyland"
- 2008 New Hampshire Department of Environmental Services "Environmental Fact Sheet - Fireworks and New Hampshire's Lakes"
- 02/27/08 Water Air Soil Pollution "The Fallout from Fireworks: Perchlorate in Total Deposition"
- American Chemical Society "'Green' fireworks may brighten eco-friendly Fourth of July displays in future"
- 3/25/10 Gualala Festivals Committee v California Coastal Commission Ruling
- June 2009 Shore Stewards News "A Kaleidoscope of Colors"
- "Status of Outstanding Issues for California's Least Tern Nesting Sites"
- June 2006 Environmental Assessment (NOAA), Fireworks Displays withing the Monterey Bay National Marine Sanctuary
- 2010 Journal of Hazardous Materials "Effect of fireworks events on urban background trace metal aerosol concentrations: is the cocktail worth the show?
- 03/02/10 USDC NRDC v County of Los Angeles - Minutes
- 04/01/06 NOAA letter to City of San Diego re Seal Harassment
- 11/30/07 NOAA letter to City of San Diego re Seal Harassment
- 05/14/10 NOAA letter to City of San Diego re Seal Harassment
- 2007 Environ. Sci. Technol. "Perchlorate Behavior in a Municipal Lake Following Fireworks Displays"
- 07/27/07 "World record rocket launch attempt - an assessment of pollution to controlled waters and toxicity"
- 04/05/10 RWQCB Letter to Port District re: Regulation of Wastewater Discharges from Fireworks Events
- 12/12/07 Executive Officer Summary Report (re SeaWorld San Diego fireworks)
- 12/12/07 Tentative Addendum No. 1
- 06/07/07 Atmospheric Environment "Impact of Fireworks on Airborne Particles"

Are fireworks bad for the environment?

Fourth of July fireworks unleash a shower of toxins into the soil and water, and scientists are only beginning to figure out what that means for human health.
By Russell McLendon
Tue, Jun 30 2009 at 8:30 AM EST
Read more: TOXINS & CHEMICALS, WATER POLLUTION

The rockets' red glare on the Fourth of July can fill onlookers with patriotism and awe. Unfortunately, it can also fill them with particulates and strontium.

Fireworks get their flamboyance from a variety of chemicals, many of which are toxic to humans. From the gunpowder that fuels them to the metallic compounds that color their explosions, fireworks often contain radioactive, carcinogenic or endocrine-disrupting substances that seep into soil and water, potentially threatening animals throughout the food chain.

But fireworks shows are woven into the fabric of the United States — they were popular here even before the country won its independence — and it's not like they happen every day. Is an occasional peppering of perchlorates in the Potomac really a big deal compared with all the industrial pollution it and other U.S. waterways have been dealt over the years?

Maybe not, but it's still not entirely clear how fireworks affect ecological health. While they haven't been directly linked to any widespread outbreaks of disease, it's not always easy to pin down why someone developed hypothyroidism, anemia or cancer.

What we do know is that, although they're fleeting and infrequent, fireworks shows spray out a toxic concoction that rains down quietly into lakes, rivers and bays throughout the country. Many of the chemicals in fireworks are also persistent in the environment, meaning they just stubbornly sit there instead of breaking down. That's how mercury from coal emissions winds up in fish, and it's how DDT thinned bald eagles' eggshells in the '70s. There's scant evidence that fireworks are having similar effects, but the possibility has been enough to raise concern in many communities.

Here's a look at what's in fireworks, how it can affect people, and what kinds of alternatives exist:

Perchlorates and particulates
For fireworks and other pyrotechnics to blow up, they need to blow up *something* — usually a blend of charcoal and sulfur fuel. They also need an ingredient that can inject oxygen to speed up the explosion, historically relying on potassium nitrate. These three chemicals are mixed together into a sooty substance known as gunpowder.

When a spark hits gunpowder, the potassium nitrate feeds oxygen to the fire, helping it quickly burn the charcoal-sulfur fuel. This produces volumes of hot, rapidly expanding solids and gases that can be used to fire a bullet, explode an artillery shell or launch a Roman candle.

The original blends of black powder can be a bit too unstable and messy for some uses, though, so the potassium nitrate is often replaced by perchlorates, a family of chemicals all featuring a central chlorine atom bonded by four oxygen atoms. Two types in particular — potassium perchlorate and ammonium perchlorate — have become the go-to oxidizers of the pyrotechnics industry.

Perchlorates may have introduced a new problem, though: In high enough doses, they limit the human thyroid gland's ability to take iodine from the bloodstream, potentially resulting in hypothyroidism. The thyroid needs iodine to make hormones that control a variety of body functions, and people running too low on these hormones can develop a wide range of disorders. Children, infants and especially fetuses suffer the worst from hypothyroidism, since thyroid hormones are crucial for normal growth. Perchlorates have also been shown to cause thyroid cancer in rats and mice, but scientists believe humans are less vulnerable to this effect.

Low doses of perchlorates don't seem to hurt healthy adults — volunteers who took 35 milligrams for 14 days or 3 milligrams for six months showed no thyroid-related problems, and studies of workers exposed to similar amounts for years also failed to uncover any major side effects. Plus, perchlorate advocates often point out that it should theoretically all be incinerated in the sky before any can fall down to contaminate the ground.

But a 2007 study of an Oklahoma lake following fireworks displays overhead found that perchlorate levels spiked more than 1,000 times above the baseline level for 14 hours after a show. While the maximum concentration detected was 44.2 micrograms — less than 1 milligram — per liter, the study was still the most concrete evidence yet that fireworks release perchlorates into waterways.

Another study by the Massachusetts Department of Environmental Protection found perchlorate levels up to 62 micrograms per liter at eight groundwater-monitoring wells on the Dartmouth campus, near where fireworks are regularly fired.

EPA spokesman Skip Anderson cautions that these weren't health-effects studies, and points out that more data is needed to determine how great a risk perchlorates pose in surface water around the country. Still, he says, their results "suggest that some perchlorate in fireworks is not combusted and therefore can wind up in the environment."

The smoke from fireworks' burned charcoal and sulfur fuel also contains particulate matter that can get lodged in people's lungs, an immediate danger for those with asthma or chemical sensitivities. Prolonged exposure to similar airborne particles from diesel exhaust has also been shown to cause lung cancer. Air-quality monitors reportedly spike for about three hours after a fireworks show.

One positive of both perchlorates and particulates is that they most likely don't pose a long-term threat. Particulates fade away after a few hours, and perchlorates dissipate days or weeks after being released. Unfortunately, the same can't be said about some other chemicals that help light up the sky.

Metallic compounds
In addition to gunpowder, fireworks are packed with heavy metals and other toxins that produce their sparkling shower of colors. Like perchlorates, the exact effect of fireworks' heavy-metal fallout is still mainly a mystery, but scientists do know that the metals themselves can wreak havoc in the human body.

• **Strontium (red):** This soft, silvery-yellow metal turns red when it burns, is extremely reactive with both air and water, and can be radioactive. Some strontium compounds dissolve in water, and others move deep into soil and groundwater; radioactive strontium has a half-life of 29 years. While low levels of stable and radioactive strontium haven't been shown to affect human health, they both can be dangerous at high doses. Radioactive strontium can damage bone marrow, cause anemia and prevent blood from clotting correctly, and lab studies have shown it can lead to birth defects in animals. Stable strontium is mainly a threat to children because it can impair their bone growth.

• **Aluminum (white):** Since aluminum is the most abundant metal in Earth's crust — and one of humanity's most widely used — avoiding exposure is almost impossible. Virtually all food, water, air and soil contain some amount of aluminum — the average adult eats about 7 to 9 milligrams of the silvery-white metal every day in food. It's generally safe at these levels, but it can affect the brain and lungs at higher concentrations. People and animals exposed to large amounts of aluminum have performed poorly on mental and physical tests, and some studies suggest aluminum exposure may lead to Alzheimer's disease, although that connection has yet to be proven.

• **Copper (blue):** Fireworks' blue hues are produced by copper compounds. These aren't very toxic on their own, but the copper jump-starts the formation of dioxins when perchlorates in the fireworks burn. Dioxins are vicious chemicals that don't occur naturally and aren't intentionally produced anywhere; they only exist as unwelcome byproducts of certain chemical reactions, one of which happens in blue fireworks. The most noted health effect of dioxin exposure is chloracne, a severe skin disease with acne-like lesions mostly on the face and upper body. Dioxin doesn't stop there, though — the World Health Organization has identified it as a human carcinogen, and it's also been shown to disrupt hormone production and glucose metabolism.

• **Barium (green):** Fish and other aquatic organisms can accumulate barium, which means it can move up the food chain. The silvery-white metal naturally bonds with other elements to form a variety of compounds that all have different effects — none are known to be carcinogenic, but they can cause gastrointestinal problems and muscular weakness when exposure exceeds EPA drinking water standards. Symptoms may include vomiting, diarrhea, breathing trouble, changes in blood pressure, numbness around the face, general muscle weakness and cramps. High levels of barium exposure can lead to changes in heart rhythm, paralysis or death.

• **Rubidium (purple):** This soft, silvery metal is one of the most abundant elements on Earth. It burns purple, melts to a liquid at 104 degrees Fahrenheit and is highly reactive with water, capable of igniting fires even far below the freezing point. It hasn't been reported to cause any major environmental damage, but it can cause skin irritation since it's so reactive with moisture, and it's moderately toxic when ingested, reportedly able to replace calcium in bones (PDF).

• **Cadmium (various):** Used to produce a wide range of fireworks colors, this mineral is also a known human carcinogen. Breathing high levels of cadmium can seriously damage the lungs, and consuming it can fluster the stomach, often resulting in vomiting and diarrhea. Long-term exposure can lead to kidney disease, lung damage and fragile bones. Plants, fish and other animals take up cadmium from the environment, meaning that any released into waterways from a fireworks show can be passed up the food chain.

Alternative fireworks

The most eco-friendly alternative to fireworks is to forgo explosions altogether — go to a parade, go fishing, grill out, camp out or help out.

If you must see the sky festively illuminated, you might want to try a laser light show, which create dazzling displays of color without launching dangerous chemicals into the air. They may consume lots of energy, but so does the rampant production of single-use fireworks. Here's an example of lasers in lieu of fireworks on the Fourth of July, from Stone Mountain, Ga., in 2008:

In 2004, Disney began using compressed air to launch fireworks at Disneyland in Anaheim, Calif., reducing at least the issues of smoky particulates in the air and perchlorates in the water. Researchers have also been fine-tuning alternative propellants that use nitrogen-rich materials in place of perchlorates, but those are still likely several years away from hitting the market.

Related on MNN: Our advice columnist weighs in on fireworks as well.

UPDATE: Several readers have commented that toxins in fireworks are insignificant, or are combusted before they can contaminate the ground. Both are valid arguments — this article doesn't claim that fireworks are definitively dangerous to environmental health; it simply

highlights the concern that known toxins are unnaturally entering the environment and scientists don't know exactly what ecological effects they have. The potential dangers alone have been enough to spur Disneyland and some communities to explore other options, and researchers are pursuing alternatives to perchlorates due to the possibility of health effects. As for combustion, the article cites two studies that found perchlorates can still make it into lakes, either from shells being overstuffed or from duds that fail to combust. Only a small amount was found, but only two lakes were studied. Again, this article aims simply to point out the potential dangers since scientific understanding is still limited.

The explanations of the chemicals that give fireworks their colors have also drawn some fire from commenters. The environmental impact of these hasn't been studied well enough for anyone to know their ecological effects; rather than speculating how they might affect ecological health, the article summarizes the toxicological profiles of these chemicals, primarily using information from the CDC's Agency for Toxic Substances and Disease Registry. The article makes clear at several points that it's merely presenting the potential dangers inherent to the materials in question. While common chemicals like table salt that contain otherwise dangerous components are known to be safe, the effects of fireworks' toxins in the environment are much less understood.

Thanks to everyone who's commenting, and please feel free to include links to any data or studies that conflict with information presented here. We scrutinized this article to ensure its accuracy, but we will certainly correct any errors or clarify any unclear statements.

Happy Fourth of July (and Canada Day)!

Item No. 6
Supporting Document No. 5

News in Science (/science/news/?site=science)

Fireworks to become a little greener

Emily Sohn
Discovery News (http://dsc.discovery.com/news/news.html)

Friday, 3 July 2009

Scientists are working on a new generation of kinder, gentler pyrotechnics that produce less smoke and use fewer toxic metals.

Fireworks are fun and exciting, but the flashing displays can harm the environment and pose risks to human health.

Researchers at the Los Alamos National Laboratory (http://www.lanl.gov/) in New Mexico have been developing a new type of pyrotechnic, which should be welcome news for people who operate or watch fireworks on a regular basis.

"Everyone at or downwind of a pyrotechnic display is getting subjected to levels of these metals that aren't natural levels," says David E Chavez, a chemist at Los Alamos National Laboratory. "Whether that really is going to cause health effects is up for debate."

Disneyland provided the initial impetus for Chavez's group to start investigating cleaner burning fireworks. Night after night, as the theme park put on spectacular fireworks displays, neighbours began to complain about smoke that was enveloping their homes and irritating their lungs.

Previous studies have shown a rise in asthma attacks during fireworks-filled festivals. While particle-filled smoke may be the most obvious concern, it's not the only issue.

Firework displays, such as this one exploding over Sydney Harbour, may contain high levels of toxic material *(Source: Tim Wimborne/REUTERS)*

Related Stories

Hollywood movies contribute to smog (/science/articles/2006/11/15/1788910.htm) , Science Online, 15 Nov 2006

Lawn clippings may help make smog (/science/articles/2004/11/29/1252461.htm) , Science Online, 06 Nov 2008

Fireworks leave polluting afterglow (/science/articles/2001/06/28/320412.htm) , Science Online, 09 Dec 2008

Toxic ingredients

Some of the metals that make fireworks colourful may also be poisonous when heated. For example, antimony, which is sometimes used to produce the colour white, can harm the lungs, heart, stomach and other organs.

Barium, which provides a green hue, "does something really nasty to your insides and gastrointestinal tract," says Michael Hiskey, an explosive chemist at DMD Systems, a pyrotechnic research and development company. Barium can also be toxic to the heart.

Then there are perchlorates - oxygen-rich molecules that allow the fuel in fireworks to burn. Perchlorates appear in nuclear missiles, flares and rocket fuel for spacecraft.

So far, the US Environmental Protection Agency has not set an upper limit for perchlorates in soil or water, even though the chemicals have been detected in drinking water, as well as in breast milk and in store-bought cow's milk.

Animal studies have linked perchlorates, such as potassium perchlorate and ammonium perchlorate, to thyroid problems. Repeated pyrotechnic displays, especially ones that occur every day at theme parks, can take their toll.

Build-up

In a 2007 study, researchers found that perchlorate levels spiked more than 1000 times higher than normal in parts of a small Oklahoma lake within 14 hours after a Fourth of July fireworks show.

It took up to 80 days for levels of the chemical to return to where they started, the team reported in the journal *Environmental Science & Technology* (http://pubs.acs.org/journal/esthag) .

"If you have a place where pyrotechnics are shot over and over again for years and years," says Chavez, "you will have a build-up of these toxins in the environment."

In the last two or three years, scientists have come up with some "greener" alternatives.

For example, Hiskey's company has developed fireworks that burn nitrogen-based fuels instead of carbon-based versions, making perchlorate unnecessary.

The result is a cleaner burn, and with less smoke to obscure the colour. These fireworks also contain 10 times less barium than the standard kind.

For now, eco-friendly fireworks are only being used by companies that put on regular displays, especially indoors, where smoke is particularly unacceptable.

For most neighbourhood shows, cost is still too limiting at this point. Prices won't come down, experts say, unless the EPA decides to place strict regulations on levels of toxic chemicals involved.

In the meantime, Hiskey has a simple message for anyone watching an upcoming fireworks display, such as this weekend's Fourth of July celebration.

"Be safe. Don't be downwind from fireworks. And call it good."

Tags:
air-pollution (/science/tag/browse.htm?site=science&topic=latest&tag=air-pollution)
,
land-pollution (/science/tag/browse.htm?site=science&topic=latest&tag=land-pollution)
,
chemistry (/science/tag/browse.htm?site=science&topic=latest&tag=chemistry)

FIRE

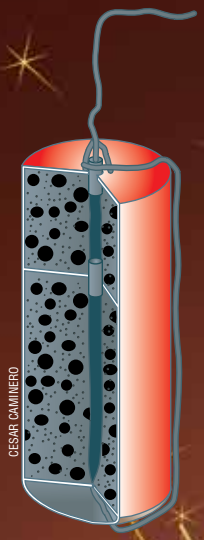
By Kathy De Antonis

Fireworks are one of the most spectacular outdoor shows. They produce amazing bursts of colors that take a variety of shapes. But how do they work? How do they burn into so many colors and patterns? And why, if not handled properly, can they cause serious injuries or even death?

What's inside a firework?

The source of most fireworks is a small tube called an aerial shell that contains explosive chemicals. All the lights, colors, and sounds of a firework come from these chemicals.

An aerial shell is made of gunpowder, which is a well-known explosive, and small globs of explosive materials called stars (Fig. 1). The stars give fireworks their color when they explode. When we watch fireworks, we actually see the explosion of the stars. They are formed into spheres, cubes, or cylinders that are usually 3–4 centimeters (1–1½ inch) in diameter.



CESAR CAMINERO

Figure 1. Structure of an aerial shell. The black balls are the stars, and the gray area is gunpowder. The stars and the powder are surrounding a bursting charge, which also contains black powder.

Each star contains four chemical ingredients: an oxidizing agent, a fuel, a metal-containing colorant, and a binder. In the presence of a flame or a spark, the oxidizing agent and the fuel are involved in chemical reactions that create intense heat and gas. The metal-containing colorant produces the color, and the binder holds together the oxidizing agent, fuel, and colorants.

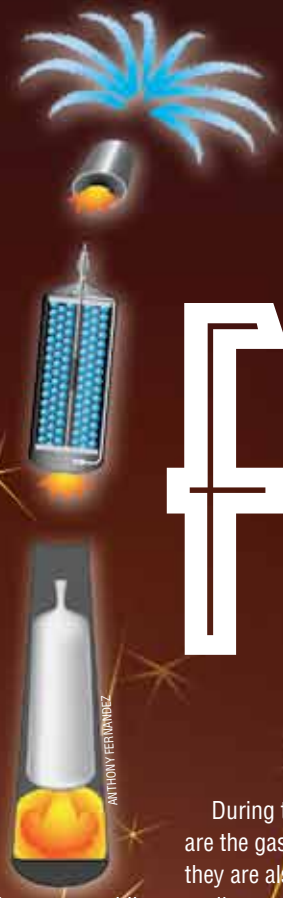
At the center of the shell is a bursting charge with a fuse on top. Igniting the fuse with a flame or a spark triggers the explosion of the bursting charge and of the entire aerial shell.

How fireworks explode

The explosion of a firework happens in two steps: The aerial shell is shot into the air, and then it explodes in the air, many feet above the ground.

To propel the aerial shell into the air, the shell is placed inside a tube, called a mortar, which is often partially buried in sand or dirt. A lifting charge of gunpowder is present below the shell with a fuse attached to it. When this fuse, called a fast-acting fuse, is ignited with a flame or a spark, the gunpowder explodes, creating lots of heat and gas that cause a buildup of pressure beneath the shell. Then, when the pressure is great enough, the shell shoots up into the sky.

After a few seconds, when the aerial shell is high above the ground, another fuse inside the aerial shell, called a time-delay fuse, ignites, causing the bursting charge to explode. This, in turn, ignites the black powder and the stars, which rapidly produce lots of gas and heat, causing the shell to burst open, propelling the stars in every direction.



ANTHONY FERNANDEZ

During the explosion, not only are the gases produced quickly, but they are also hot, and they expand rapidly, according to Charles' Law, which states that as the temperature of enclosed gas increases, the volume increases, if the pressure is constant (Fig. 1). The loud boom that accompanies fireworks is actually a sonic boom produced by the expansion of the gases at a rate faster than the speed of sound!

If the stars are arranged randomly in the aerial shell, they will spread evenly in the sky after the shell explodes. But if the stars are packed carefully in predetermined patterns, then the firework has a specific shape—such

as a willow, a peony, or a spinner—because the stars are sent in specific directions during the explosion.

The timing of the two fuses is important. The fast-acting fuse ignites first, propelling the shell into the air, and then the time-delay fuse ignites to cause the aerial shell to explode when it is high in the sky. If the timing of the fuses is not just right, the shell can explode too close to the ground, injuring people nearby.

More often, light from fireworks is produced by luminescence. When fireworks explode in the sky, the gunpowder reactions create a lot of heat, causing the metallic substances present in the stars to absorb energy from the heat and emit light. These metallic substances are actually metal salts, which produce luminescent light of different colors when they are dispersed in the air.

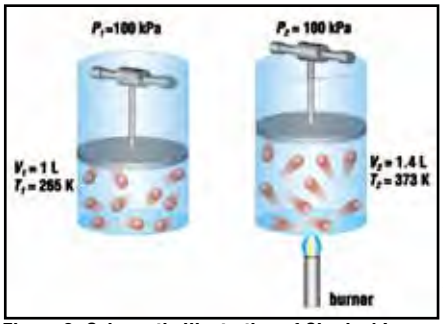
WORKS!

carefully and have an adult in charge," says John Conkling, an adjunct professor of chemistry at Washington College, Chestertown, Md., and former executive director of the American Pyrotechnics Association.

Color	Compound
red	strontium salts, lithium salts lithium carbonate, Li_2CO_3 = red strontium carbonate, $SrCO_3$ = bright red
orange	calcium salts calcium chloride, $CaCl_2$
yellow	sodium salts sodium chloride, NaCl
green	barium compounds + chlorine producer barium chloride, $BaCl_2$
blue	copper compounds + chlorine producer copper(I) chloride, CuCl
purple	mixture of strontium (red) and copper (blue) compounds

HTTP://WWW.SCIFUN.ORG/ ; PROF. BASSAM Z. SHAKHASHIRI, UNIVERSITY OF WISCONSIN-MADISON

Table 1. Colorant compounds used in fireworks and the colors they produce.



ANTHONY FERNANDEZ

Figure 2. Schematic illustration of Charles' Law. When the pressure of a volume of gas is constant, an increase in temperature leads to a proportional increase in the volume of the gas. The gas molecules move faster at higher temperatures.

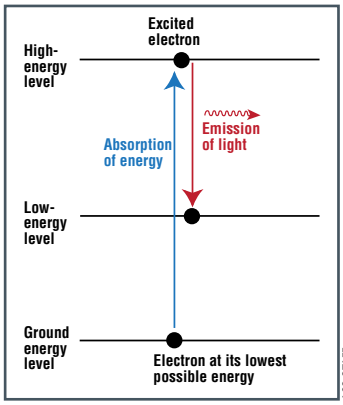
Where do fireworks' colors come from?

What makes fireworks so special is the beautiful colors they produce. These colors are formed in one of two ways: luminescence and incandescence.

Incandescent light is produced when a substance is heated so much that it begins to glow. Heat causes the substance to become hot and glow, initially emitting infrared, then red, orange, yellow, and white light as it becomes increasingly hotter. When the temperature of a firework is controlled, the glow of its metallic substances can be manipulated to be a desired color at the proper time.

This light is produced by electrons inside the metal atoms (Fig. 3). These electrons absorb energy from the heat, which causes them to move from their original ground-energy state to an excited state. Then, nearly immediately, these electrons go to a lower energy state and emit light with a particular energy and characteristic color.

The color of the light emitted by the electrons varies depending on the type of metal or combination of metals. So, the colors are specific to the metals present in the fireworks. The metal-containing colorants for some common fireworks are listed in Table 1.



ACS STAFF

Figure 3. Principle of luminescence. Heating atoms causes electrons to move from their ground-energy level to a higher energy level (blue arrow). When the excited electrons move to a lower energy level (red arrow), they emit light with a specific energy and characteristic color.

Fireworks' safety

Fireworks are a lot of fun to watch, but they must be handled with great care because they can be dangerous. "When using fireworks, one should follow the label directions very

Knowing the rules and regulations is important, too. According to Conkling, fireworks that are publicly available in stores are legally allowed in 41 of the 50 U.S. states. So, you may not be able to purchase fireworks if your state does not allow it.

Also, regulations require that consumer fireworks should have no more than 50 milligrams (about 1/500th of an ounce) of gunpowder. This may seem like a relatively small amount. But don't be fooled. Even 50 milligrams of gunpowder or less can cause serious injuries. "You would be surprised by how powerful fireworks can be," says Doug Taylor, president of Zambelli Fireworks, one of the largest fireworks companies in the United States.

Some fireworks contain more than the limited amount of 50 milligrams. Although they are illegal, such fireworks—which include the "cherry bombs" and "M-80s"—can be found in some stores or on the black market and cause even more damage.

ISTOCK

Despite people's attention to safety, accidents still happen. Most injuries are caused by the mishandling of firecrackers. When they burn, they can reach temperatures of up to 1,000 °C (1,800 °F). Many people, especially children, are burned by them.

Accidents involving fireworks occur every year. They cause field and house fires and result in injuries and deaths. Many of the accidents involve young people. For instance, in 2009, a 17-year-old boy in Latrobe, Pa., lost his right hand and leg after an M-80 firework exploded in his lap.

Another case involved teenagers who were playing with fountain fireworks—aerial fireworks that shoot up tall fountains of sparks—on the front porch of a duplex home in St. Paul, Minn., when a fire broke out. The flames burned through the second floor and reached the roof, resulting in nine people being displaced from their homes.

Because of the danger associated with consumer fireworks, the American Academy of Pediatrics recommends that children and young adults avoid them altogether and attend local aerial fireworks demonstrations instead. Taylor says watching aerial fireworks can be very moving. "One of the grandchildren of the founder of Zambelli Fireworks was known for saying, 'Grandpa, I like your fireworks because I can feel them in my heart,'" he says. "That's so true! It's really an emotional experience."

SELECTED REFERENCES

Chemistry of Firework Colors: http://chemistry.about.com/od/fireworkspyrotechnics/a/fireworkcolors.htm [accessed June 2010].

Fireworks! NOVA Online/Public Broadcasting Service (PBS): http://www.pbs.org/wgbh/nova/fireworks/ [accessed June 2010].

Fireworks Safety: http://pediatrics.about.com/od/safety/a/0607_fireworks.htm?p=1 [accessed June 2010].

Finn, R. The Island; Finding Refuge in the Family Fireworks. *The New York Times,* July 2, 2006: http://query.nytimes.com/gst/fullpage.html?res=9A0DE4DE1430F931A35754C0A9609C8B63 [accessed June 2010].

Kathy De Antonis is a science writer who lives in Old Saybrook, Conn. Her latest *ChemMatters* article, "Space Food," appeared in the December 2009 issue.


JOHN CONKLING

Interview with Pyrotechnic Chemist John Conkling

During the past 30 years, John Conkling, a fireworks expert at Washington College, Chestertown, Md., has made more than 40 trips to China—the world's major producer of fireworks—to meet with officials from the Chinese fireworks industry. He is the author of *The Chemistry of Pyrotechnics—Basic Principles and Theory*, which many consider the most definitive reference on pyrotechnics, and he holds nine patents dealing with energetic chemical systems. Conkling explains what pyrotechnic chemists do.

What do pyrotechnic chemists do?

They combine compounds to make a mixture that can explode to produce color, light, and audible effects, such as the sizzles, pops, and booms of fireworks. When these compounds are lit by a spark or a flame, explosive chemical reactions occur, creating the light and sound effects seen in fireworks.

The mixtures made by pyrotechnic chemists are used not only for entertainment, but also for emergency signaling–such as pink flares that people put on the road next to car accidents—and military applications, such as mixtures that produce effects visible only with night vision goggles.

How did you become a pyrotechnic chemist?

I was interested in all kinds of science as a child, and eventually, chemistry became my focus. I went to graduate school at Johns Hopkins University, Baltimore, Md., to pursue a Ph.D. in physical organic chemistry. The topic of my thesis (unusual reaction mechanisms involving "nonclassical" pathways) doesn't have much to do with what I do now, but it taught me the discipline of doing research and recording observations.

In 1969, I went on to teach undergraduate chemistry at Washington College, Chestertown, Md., which is where I pursued my undergraduate studies. Soon after that, I was approached by a fireworks company that wanted to hire me for a side project on developing chemical compositions for fireworks that are safe to carry and store. I became really interested in the chemistry of fireworks.

Later, the U.S. Army asked me if I was interested in working on some military pyrotechnic applications involving the production of brightly colored smoke for signaling purposes, and my pyrotechnic chemistry career shot off. Nowadays, I do training seminars for people interested in anything that explodes—from people who design and manufacture fireworks to people who dispose of bombs.

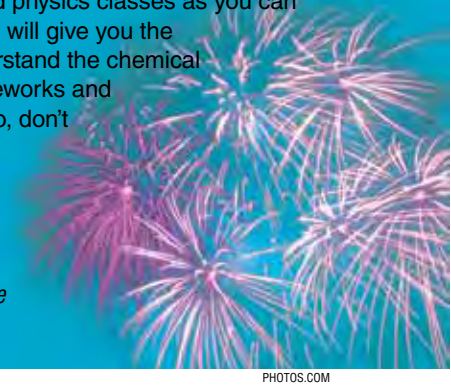
How do you make sure that fireworks are safe?

Mainly, you don't want compounds that explode as they fall on the ground. It's important to develop stable compounds that ignite only in the sky. Fireworks were invented hundreds of years ago, and we have learned through the centuries to avoid certain chemicals and mixtures that are too easy to ignite accidentally. There is also a big push now to make fireworks as environmentally friendly as possible.

Do you have any advice for students who want to become pyrotechnic chemists?

Take as many chemistry and physics classes as you can while in school. These classes will give you the background you need to understand the chemical reactions that take place in fireworks and other pyrotechnic devices. Also, don't experiment on your own with explosive materials! There are many easy ways to make explosives, but that does not mean they are safe.

—Christen Brownlee


PHOTOS.COM

Nautilus Environmental

June 25, 2010

Mr. Sandy Purdon
Administrative Headquarters - Murphy Canyon:
San Diego Armed Services YMCA Paul Hartley Complex
3293 Santo Road
San Diego, CA 92124
Via email: hppurdon@cox.net, paul@asymcasd.org

SUBJECT: 4 July 2010 Fireworks Monitoring Results

Dear Mr. Purdon,

This letter transmits analytical data for San Diego Bay seawater samples collected to monitor selected pollutant concentrations in relation to fireworks shows conducted on 4 July 2010. Monitoring was conducted at Shelter Island, Harbor Island, the Embarcadero, and at Seaport Village before fireworks shows commenced and within 1 to 2 hours following the end of the shows. Eight samples were analyzed for the following metals in seawater: Barium, Copper, Manganese, Molybdenum, Nickel, Selenium, and Zinc (EPA Method 1640); and for Perchlorate (in Seawater, EPA method 331.0(M)) by Calscience Environmental Laboratories, Inc.

We have reviewed the data and found that the vast majority of metals analyses results indicated that total concentrations either declined between the pre-firework and post-firework sampling events, or increased less than 10 percent (an arbitrary value). Exceptions included:

- Embarcadero: Selenium incresed from non-detectable levels (<0.0500 micrograms per Liter [ug/L]) before the show to 0.344 ug/L post-show; manganese increased from 6.78 ug/L (pre) to 7.95 ug/L (post); and
- Seaport Village: Nickel, selenium, and manganese all increased relative to pre-show conditions at (0.584 ug/L to 0.648 ug/L, 0.852 ug/L to 1.56 ug/L, and 8.00 to 10.7 ug/L, respectively);

Perchlorate was detected in 3 of the 4 post-show samples and ranged from 0.13 ug/L to 6.3 ug/L. These results represent an increase over the pre-show conditions, which were all below the detection limit (0.10 ug/L). Perchlorates were not detected in either of the Shelter Island samples.

Please refer to the attached original chemistry report, which includes a summary of detected analytes, results of all analyses, and quality control data. Field logs (listing locations, times of sample collection, and other pertinent field conditions) are also attached.

We very much appreciate the opportunity to work with you. Please feel free to contact me anytime with any questions you might have at 858-587-7333 x210 (office), 619-985-9111 (mobile) or at nick@nautilusenvironmental.com.

Best regards,

Nick Buhbe, M.S.
Environmental Scientist & Program Manager
Attachments
Cc: Katie Flocken, Nautilus Environmental, LLC

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5009 Pacific Highway East
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British Columbia
8664 Commerce Court
Burnaby, British Columbia
V5A 4N7
604.420.8773
fax: 604.603.9381

Calscience Environmental Laboratories, Inc.

July 14, 2010

Nick Buhbe
Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121-4798

Subject: **Calscience Work Order No.: 10-07-0270**
Client Reference: 4th of July Fireworks 2010

Dear Client:

Enclosed is an analytical report for the above-referenced project. The samples included in this report were received 7/6/2010 and analyzed in accordance with the attached chain-of-custody.

Unless otherwise noted, all analytical testing was accomplished in accordance with the guidelines established in our Quality Systems Manual, applicable standard operating procedures, and other related documentation. The original report of subcontracted analysis, if any, is provided herein, and follows the standard Calscience data package. The results in this analytical report are limited to the samples tested and any reproduction thereof must be made in its entirety.

If you have any questions regarding this report, please do not hesitate to contact the undersigned.

Sincerely,

Calscience Environmental
Laboratories, Inc.
Danielle Gonsman
Project Manager

Calscience Environmental Laboratories, Inc.

The difference is service

Client:	Nautilus Environmental	Work Order:	10-07-0270
	5550 Morehouse Drive, Suite 150	Project name:	4th of July Fireworks 2010
	San Diego, CA 92121-4798	Received:	07/06/10 15:50
Attn:	Nick Buhbe		

DETECTIONS SUMMARY

Client Sample ID / Analyte	Result	Qualifiers	Reporting Limit	Units	Method	Extraction
Shelter Island Pre						
Barium	**8.78**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**3.64**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.0**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.660**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**8.40**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**5.44**		1.00	ug/L	EPA 1640	EPA 3005A Total
Shelter Island Post						
Barium	**8.82**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**2.87**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.1**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.527**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**7.44**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**5.28**		1.00	ug/L	EPA 1640	EPA 3005A Total
Harbor Island Pre						
Barium	**9.16**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**3.55**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.2**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.584**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**9.02**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**6.80**		1.00	ug/L	EPA 1640	EPA 3005A Total
Harbor Island Post						
Barium	**9.26**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**3.38**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.3**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.574**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**8.95**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**7.04**		1.00	ug/L	EPA 1640	EPA 3005A Total
Perchlorate	**6.3**		0.10	ug/L	EPA 331.0 (M)	Cartridge

*MDL is shown.

Calscience Environmental Laboratories, Inc.

The difference is service

Client:	Nautilus Environmental 5550 Morehouse Drive, Suite 150 San Diego, CA 92121-4798	Work Order:	10-07-0270
		Project name:	4th of July Fireworks 2010
		Received:	07/06/10 15:50
Attn:	Nick Buhbe		

DETECTIONS SUMMARY

Client Sample ID Analyte	Result	Qualifiers	Reporting Limit	Units	Method	Extraction
Embarcadero Pre						
Barium	**9.13**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**3.25**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.3**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.542**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**8.61**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**6.78**		1.00	ug/L	EPA 1640	EPA 3005A Total
Embarcadero Post						
Barium	**9.32**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**3.55**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.3**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.590**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Selenium	**0.344**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**9.39**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**7.95**		1.00	ug/L	EPA 1640	EPA 3005A Total
Perchlorate	**0.13**		0.10	ug/L	EPA 331.0 (M)	Cartridge
Seaport Village Pre						
Barium	**9.35**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**3.44**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.5**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.584**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Selenium	**0.852**		0.500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**7.49**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**8.00**		1.00	ug/L	EPA 1640	EPA 3005A Total
Seaport Village Post						
Barium	**9.86**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Copper	**3.71**		0.0300	ug/L	EPA 1640	EPA 3005A Total
Molybdenum	**14.4**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Nickel	**0.648**		0.0500	ug/L	EPA 1640	EPA 3005A Total
Selenium	**1.56**		0.500	ug/L	EPA 1640	EPA 3005A Total
Zinc	**8.10**		1.00	ug/L	EPA 1640	EPA 3005A Total
Manganese	**10.7**		1.00	ug/L	EPA 1640	EPA 3005A Total
Perchlorate	**0.30**		0.10	ug/L	EPA 331.0 (M)	Cartridge

Subcontracted analyses, if any, are not included in this summary.

*MDL is shown.

Calscience Environmental Laboratories, Inc.

Analytical Report

Nautilus Environmental		Date Received:	07/06/10
5550 Morehouse Drive, Suite 150		Work Order No:	10-07-0270
San Diego, CA 92121-4798		Preparation:	Cartridge
		Method:	EPA 331.0 (M)

Project: 4th of July Fireworks 2010

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Shelter Island Pre	**10-07-0270-1-A**	**07/04/10 19:33**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 20:32**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	ND	0.10	1		ug/L

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Shelter Island Post	**10-07-0270-2-A**	**07/04/10 22:31**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 19:26**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	ND	0.10	1		ug/L

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Harbor Island Pre	**10-07-0270-3-A**	**07/04/10 19:41**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 19:35**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	ND	0.10	1		ug/L

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Harbor Island Post	**10-07-0270-4-A**	**07/04/10 22:46**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 19:45**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	6.3	0.10	1		ug/L

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Embarcadero Pre	**10-07-0270-5-A**	**07/04/10 19:53**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 19:54**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	ND	0.10	1		ug/L

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Embarcadero Post	**10-07-0270-6-A**	**07/04/10 22:56**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 20:03**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	0.13	0.10	1		ug/L

RL - Reporting Limit , DF - Dilution Factor , Qual - Qualifiers

Calscience Environmental Laboratories, Inc.

Analytical Report

Nautilus Environmental		Date Received:	07/06/10
5550 Morehouse Drive, Suite 150		Work Order No:	10-07-0270
San Diego, CA 92121-4798		Preparation:	Cartridge
		Method:	EPA 331.0 (M)

Project: 4th of July Fireworks 2010

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Seaport Village Pre	**10-07-0270-7-A**	**07/04/10 20:04**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 20:13**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	ND	0.10	1		ug/L

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Seaport Village Post	**10-07-0270-8-A**	**07/04/10 23:04**	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10 20:22**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	0.30	0.10	1		ug/L

Client Sample Number	Lab Sample Number	Date/Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Method Blank	**099-12-400-53**	**N/A**	**Aqueous**	**LC/MS 1**	**07/07/10**	**07/12/10 18:48**	**100712L01**

Parameter	Result	RL	DF	Qual	Units
Perchlorate	ND	0.10	1		ug/L

RL - Reporting Limit , DF - Dilution Factor , Qual - Qualifiers

Calscience Environmental Laboratories, Inc.

Analytical Report

Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121-4798

Date Received: 07/06/10
Work Order No: 10-07-0270
Preparation: EPA 3005A Total
Method: EPA 1640
Units: ug/L

Project: 4th of July Fireworks 2010

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Shelter Island Pre	**10-07-0270-1-A**	**07/04/10 19:33**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 19:14**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	8.78	0.0500	1		Selenium	ND	0.0500	1	
Copper	3.64	0.0300	1		Zinc	8.40	1.00	1	
Molybdenum	14.0	0.0500	1		Manganese	5.44	1.00	1	
Nickel	0.660	0.0500	1						

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Shelter Island Post	**10-07-0270-2-A**	**07/04/10 22:31**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 19:25**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	8.82	0.0500	1		Selenium	ND	0.0500	1	
Copper	2.87	0.0300	1		Zinc	7.44	1.00	1	
Molybdenum	14.1	0.0500	1		Manganese	5.28	1.00	1	
Nickel	0.527	0.0500	1						

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Harbor Island Pre	**10-07-0270-3-A**	**07/04/10 19:41**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 19:37**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	9.16	0.0500	1		Selenium	ND	0.0500	1	
Copper	3.55	0.0300	1		Zinc	9.02	1.00	1	
Molybdenum	14.2	0.0500	1		Manganese	6.80	1.00	1	
Nickel	0.584	0.0500	1						

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Harbor Island Post	**10-07-0270-4-A**	**07/04/10 22:46**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 20:34**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	9.26	0.0500	1		Selenium	ND	0.0500	1	
Copper	3.38	0.0300	1		Zinc	8.95	1.00	1	
Molybdenum	14.3	0.0500	1		Manganese	7.04	1.00	1	
Nickel	0.574	0.0500	1						

RL - Reporting Limit , DF - Dilution Factor , Qual - Qualifiers

Calscience Environmental Laboratories, Inc.

Analytical Report

Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121-4798

Date Received: 07/06/10
Work Order No: 10-07-0270
Preparation: EPA 3005A Total
Method: EPA 1640
Units: ug/L

Project: 4th of July Fireworks 2010

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Embarcadero Pre	**10-07-0270-5-A**	**07/04/10 19:53**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 20:45**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	9.13	0.0500	1		Selenium	ND	0.0500	1	
Copper	3.25	0.0300	1		Zinc	8.61	1.00	1	
Molybdenum	14.3	0.0500	1		Manganese	6.78	1.00	1	
Nickel	0.542	0.0500	1						

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Embarcadero Post	**10-07-0270-6-A**	**07/04/10 22:56**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 20:57**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	9.32	0.0500	1		Selenium	0.344	0.0500	1	
Copper	3.55	0.0300	1		Zinc	9.39	1.00	1	
Molybdenum	14.3	0.0500	1		Manganese	7.95	1.00	1	
Nickel	0.590	0.0500	1						

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Seaport Village Pre	**10-07-0270-7-A**	**07/04/10 20:04**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 21:08**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	9.35	0.0500	1		Selenium	0.852	0.500	10	
Copper	3.44	0.0300	1		Zinc	7.49	1.00	1	
Molybdenum	14.5	0.0500	1		Manganese	8.00	1.00	1	
Nickel	0.584	0.0500	1						

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Seaport Village Post	**10-07-0270-8-A**	**07/04/10 23:04**	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10 21:20**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	9.86	0.0500	1		Selenium	1.56	0.500	10	
Copper	3.71	0.0300	1		Zinc	8.10	1.00	1	
Molybdenum	14.4	0.0500	1		Manganese	10.7	1.00	1	
Nickel	0.648	0.0500	1						

RL - Reporting Limit , DF - Dilution Factor , Qual - Qualifiers

Calscience Environmental Laboratories, Inc.

Analytical Report

Nautilus Environmental		Date Received:	07/06/10
5550 Morehouse Drive, Suite 150		Work Order No:	10-07-0270
San Diego, CA 92121-4798		Preparation:	EPA 3005A Total
		Method:	EPA 1640
		Units:	ug/L
Project: 4th of July Fireworks 2010			Page 3 of 3

Client Sample Number	Lab Sample Number	Date /Time Collected	Matrix	Instrument	Date Prepared	Date/Time Analyzed	QC Batch ID
Method Blank	**099-13-067-10**	**N/A**	**Aqueous**	**ICP/MS 03**	**07/08/10**	**07/12/10 15:24**	**100708L04**

Parameter	Result	RL	DF	Qual	Parameter	Result	RL	DF	Qual
Barium	ND	0.0500	1		Selenium	ND	0.0500	1	
Copper	ND	0.0300	1		Zinc	ND	1.00	1	
Molybdenum	ND	0.0500	1		Manganese	ND	1.00	1	
Nickel	ND	0.0500	1						

RL - Reporting Limit , DF - Dilution Factor , Qual - Qualifiers

Calscience Environmental Laboratories, Inc.

Quality Control - Spike/Spike Duplicate

Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121-4798

Date Received: 07/06/10
Work Order No: 10-07-0270
Preparation: EPA 3005A Total
Method: EPA 1640

Project 4th of July Fireworks 2010

Quality Control Sample ID	Matrix	Instrument	Date Prepared	Date Analyzed	MS/MSD Batch Number
Shelter Island Pre	**Sea Water**	**ICP/MS 03**	**07/08/10**	**07/12/10**	**100708S04**

Parameter	MS %REC	MSD %REC	%REC CL	RPD	RPD CL	Qualifiers
Barium	4X	4X	50-150	4X	0-20	Q
Copper	4X	4X	50-150	4X	0-20	Q
Molybdenum	4X	4X	50-150	4X	0-20	Q
Nickel	126	118	50-150	3	0-20	
Selenium	113	105	50-150	7	0-20	
Zinc	144	139	50-150	2	0-20	
Manganese	4X	4X	50-150	4X	0-20	Q

RPD - Relative Percent Difference , CL - Control Limit

Calscience Environmental Laboratories, Inc.

Quality Control - Spike/Spike Duplicate

Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121-4798

Date Received: 07/06/10
Work Order No: 10-07-0270
Preparation: Cartridge
Method: EPA 331.0 (M)

Project 4th of July Fireworks 2010

Quality Control Sample ID	Matrix	Instrument	Date Prepared	Date Analyzed	MS/MSD Batch Number
Shelter Island Pre	**Sea Water**	**LC/MS 1**	**07/07/10**	**07/12/10**	**100712S01**

Parameter	MS %REC	MSD %REC	%REC CL	RPD	RPD CL	Qualifiers
Perchlorate	110	102	80-120	7	0-15	

RPD - Relative Percent Difference , CL - Control Limit

Calscience Environmental Laboratories, Inc.

Quality Control - LCS/LCS Duplicate

Nautilus Environmental
5550 Morehouse Drive, Suite 150
San Diego, CA 92121-4798

Date Received: N/A
Work Order No: 10-07-0270
Preparation: EPA 3005A Total
Method: EPA 1640

Project: 4th of July Fireworks 2010

Quality Control Sample ID	Matrix	Instrument	Date Prepared	Date Analyzed	LCS/LCSD Batch Number
099-13-067-10	**Aqueous**	**ICP/MS 03**	**07/08/10**	**07/12/10**	**100708L04**

Parameter	LCS %REC	LCSD %REC	%REC CL	RPD	RPD CL	Qualifiers
Barium	101	101	70-130	0	0-20	
Copper	106	105	70-130	1	0-20	
Molybdenum	122	124	70-130	2	0-20	
Nickel	93	93	70-130	0	0-20	
Selenium	87	88	70-130	2	0-20	
Zinc	100	96	70-130	4	0-20	
Manganese	93	92	70-130	2	0-20	

RPD - Relative Percent Difference , CL - Control Limit

Calscience Environmental Laboratories, Inc.

Quality Control - LCS/LCS Duplicate

ACCREDITED IN ACCORDANCE WITH nelac

Nautilus Environmental		Date Received:	N/A
5550 Morehouse Drive, Suite 150		Work Order No:	10-07-0270
San Diego, CA 92121-4798		Preparation:	Cartridge
		Method:	EPA 331.0 (M)

Project: 4th of July Fireworks 2010

Quality Control Sample ID	Matrix	Instrument	Date Prepared	Date Analyzed	LCS/LCSD Batch Number
099-12-400-53	**Aqueous**	**LC/MS 1**	**07/07/10**	**07/12/10**	**100712L01**

Parameter	LCS %REC	LCSD %REC	%REC CL	RPD	RPD CL	Qualifiers
Perchlorate	97	105	85-115	7	0-15	

RPD - Relative Percent Difference , CL - Control Limit

Calscience Environmental Laboratories, Inc.

Glossary of Terms and Qualifiers

Work Order Number: 10-07-0270

Qualifier	Definition
*	See applicable analysis comment.
<	Less than the indicated value.
>	Greater than the indicated value.
1	Surrogate compound recovery was out of control due to a required sample dilution, therefore, the sample data was reported without further clarification.
2	Surrogate compound recovery was out of control due to matrix interference. The associated method blank surrogate spike compound was in control and, therefore, the sample data was reported without further clarification.
3	Recovery of the Matrix Spike (MS) or Matrix Spike Duplicate (MSD) compound was out of control due to matrix interference. The associated LCS and/or LCSD was in control and, therefore, the sample data was reported without further clarification.
4	The MS/MSD RPD was out of control due to matrix interference. The LCS/LCSD RPD was in control and, therefore, the sample data was reported without further clarification.
5	The PDS/PDSD or PES/PESD associated with this batch of samples was out of control due to a matrix interference effect. The associated batch LCS/LCSD was in control and, hence, the associated sample data was reported without further clarification.
B	Analyte was present in the associated method blank.
E	Concentration exceeds the calibration range.
J	Analyte was detected at a concentration below the reporting limit and above the laboratory method detection limit. Reported value is estimated.
ME	LCS Recovery Percentage is within LCS ME Control Limit range.
ND	Parameter not detected at the indicated reporting limit.
Q	Spike recovery and RPD control limits do not apply resulting from the parameter concentration in the sample exceeding the spike concentration by a factor of four or greater.
X	% Recovery and/or RPD out-of-range.
Z	Analyte presence was not confirmed by second column or GC/MS analysis.
	Solid - Unless otherwise indicated, solid sample data is reported on a wet weight basis, not corrected for % moisture.

Calscience Environmental Laboratories, Inc.

CHAIN OF CUSTODY RECORD

Date 7/6/10

Page 1 of 1

SoCal Laboratory
7440 Lincoln Way
Garden Grove, CA 92841-1427
(714) 895-5494

NorCal Service Center
5063 Commercial Circle, Suite H
Concord, CA 94520-8577
(925) 689-9022

LABORATORY CLIENT: Nautilus Environmental

ADDRESS: 5550 Morehouse Dr. Ste 150

CITY: San Diego STATE: CA ZIP: 92121

TEL: 858-587-7333 E-MAIL: nick@nautilusenvironmental.com

CLIENT PROJECT NAME / NUMBER: 4th of July Fireworks 2010

P.O. NO.:

PROJECT CONTACT: Nick Burbe

LAB USE ONLY: 07-0269 10 K

SAMPLER(S): (PRINT) Katie Flocken

COELT LOG CODE:

COOLER RECEIPT TEMP= °C

TURNAROUND TIME: ☐ SAME DAY ☐ 24 HR ☐ 48 HR ☐ 72 HR ☒ STANDARD

SPECIAL REQUIREMENTS (ADDITIONAL COSTS MAY APPLY)
☐ RWQCB REPORTING FORMS ☐ COELT EDF ☐ _____

SPECIAL INSTRUCTIONS:
* TOTAL METALS: Ba, Cu, Mn, Mo, Ni, Se, Zn

REQUESTED ANALYSES

LAB USE ONLY	SAMPLE ID	FIELD POINT NAME (FOR COELT EDF)	SAMPLING DATE	SAMPLING TIME	MATRIX	NO. OF CONT.	TPH (g)	TPH (d) or (C6-C36) or (C6-C44)	TPH ( )	BTEX / MTBE (8260B) or ( )	VOCs (8260B)	Oxygenates (8260B)	Encore Prep (5035)	SVOCs (8270C)	Pesticides (8081A)	PCBs (8082)	PNAs (8310) or (8270C)	T22 Metals (6010B/747X)	Cr(VI) [7196A or 7199 or 218.6]	VOCs (TO-14A) or (TO-15)	TPH (g) [TO-3]+	Perchlorate (331.0M)	Metals (1640) *
1	Shelter Island Pre		7/4/10	1933	Aq	2																X	X
2	Shelter Island Post			2231		2																	
3	Harbor Island Pre			1941		2																	
4	Harbor Island Post			2246		2																	
5	Embarcadero Pre			1953		2																	
6	Embarcadero Post			2256		2																	
7	Seaport Village Pre			2004		2																	
8	Seaport Village Post		↓	2304	↓	2																	

Relinquished by: (Signature)	Received by: (Signature/Affiliation)	Date:	Time:
N. Burbe	CEC	7/6/10	1208
Rudy Hyp		7/6/10	1550
		Date:	Time:

DISTRIBUTION: White with final report, Green and Yellow to Client.
Please note that pages 1 and 2 of 2 of our T/Cs are printed on the reverse side of the Green and Yellow copies respectively.

05/01/07 Revision

Calscience Environmental Laboratories, Inc.

WORK ORDER #: 10-07-0269

SAMPLE RECEIPT FORM

Cooler 1 of 1

CLIENT: NAUTILUS ENVIRONMENTAL DATE: 07/6/10

TEMPERATURE: Thermometer ID: SC1 (Criteria: 0.0 °C – 6.0 °C, not frozen)

Temperature 2.2 °C + 0.5 °C (CF) = 2.7 °C ☑ Blank ☐ Sample

☐ Sample(s) outside temperature criteria (PM/APM contacted by: _____).

☐ Sample(s) outside temperature criteria but received on ice/chilled on same day of sampling.

☐ **Received at ambient temperature, placed on ice for transport by Courier.**

Ambient Temperature: ☐ Air ☐ Filter ☐ Metals Only ☐ PCBs Only **Initial:** BM

CUSTODY SEALS INTACT:

☐ Cooler ☐_____ ☐ No (Not Intact) ☑ Not Present ☐ N/A **Initial:** BM

☐ Sample ☐_____ ☐ No (Not Intact) ☑ Not Present **Initial:** _____

SAMPLE CONDITION:	Yes	No	N/A
Chain-Of-Custody (COC) document(s) received with samples	☑	☐	☐
COC document(s) received complete	☑	☐	☐
☐ Collection date/time, matrix, and/or # of containers logged in based on sample labels.			
☐ No analysis requested. ☐ Not relinquished. ☐ No date/time relinquished.			
Sampler's name indicated on COC	☑	☐	☐
Sample container label(s) consistent with COC	☑	☐	☐
Sample container(s) intact and good condition	☑	☐	☐
Proper containers and sufficient volume for analyses requested	☑	☐	☐
Analyses received within holding time	☑	☐	☐
pH / Residual Chlorine / Dissolved Sulfide received within 24 hours	☐	☐	☑
Proper preservation noted on COC or sample container	☑	☐	☐
☐ Unpreserved vials received for Volatiles analysis			
Volatile analysis container(s) free of headspace	☐	☐	☑
Tedlar bag(s) free of condensation	☐	☐	☑

CONTAINER TYPE:

Solid: ☐4ozCGJ ☐8ozCGJ ☐16ozCGJ ☐Sleeve (_____) ☐EnCores® ☐TerraCores® ☐_____

Water: ☐VOA ☐VOA**h** ☐VOA**na$_2$** ☐125AGB ☐125AGB**h** ☐125AGB**p** ☐1AGB ☐1AGB**na$_2$** ☐1AGB**s**

☐500AGB ☐500AGJ ☐500AGJ**s** ☐250AGB ☐250CGB ☐250CGB**s** ☐1PB ☐500PB ☐500PB**na**

☐250PB ☐250PB**n** ☐125PB ☐125PB**znna** ☐100PJ ☐100PJ**na$_2$** ☑125 mL ☑500PB n ☐_____

Air: ☐Tedlar® ☐Summa® **Other:** ☐_____ **Trip Blank Lot#:**_____ **Labeled/Checked by:** SO

Container: C: Clear A: Amber P: Plastic G: Glass J: Jar B: Bottle Z: Ziploc/Resealable Bag E: Envelope **Reviewed by:** _____

Preservative: h: HCL n: HNO_3 na$_2$: $Na_2S_2O_3$ na: NaOH p: H_3PO_4 s: H_2SO_4 znna: $ZnAc_2$+NaOH f: Field-filtered **Scanned by:** _____

Nautilus Environmental

4th of July Fireworks Water Sampling
Field Data Sheet

Site ID	Date	Time	Latitude & Longitude	Pre or Post Event	Collected by	Notes
Shelter Island Barge	7/4/10	1933	32° 42.810 N 117° 13. 243 W	Pre	AM/KF	64.5°F water temp.
Harbor Island Barge	7/4/10	1941	32° 43.331 N 117° ~~12~~ 11. 958 W	Pre	AM/KF	67.5°F
Embarcadero Barge	7/4/10	1953	~~32° 4 KF~~ 32° 43.032 N 117° 10. 807 W	Pre	AM/KF	67.5°F
Seaport Village Barge	7/4/10	2004	32° 42.228 N 117° 10.130 W	Pre	AM/KF	69.0°F

Nautilus Environmental

4th of July Fireworks Water Sampling
Field Data Sheet

Site ID	Date	Time	Latitude & Longitude	Pre or Post Event	Collected by	Notes
Shelter Island Barge	7/4/10	~~2131~~ KF 2231	~~34~~ KF 32° 42.810 117° 13.243	Post	~~AM/KF~~	68.5°F Water temp barge moved
Harbor Island Barge	7/4/10	~~2146~~ KF 2246	32° 43.331 117° ~~12~~ KF .958 11	Post	~~AM/KF~~	68.5°F barge moved
Embarcadero Barge	7/4/10	2256	32° 43.032 117° 10.807	Post	AM/KF	68.0°F
Seaport Village Barge	7/4/10	2304	32° 42.228 117° 10.130	Post	~~AM~~/KF	68.5°F barge moved


Unified Port of San Diego
Port of San Diego
Big Bay Boom
An Independence Day Spectacular
July 4, 2010 • 9:00 P.M
300 YARD SAFETY ZONE
PLEASE STAY CLEAR
Interstate 5
San Diego Int'l Airport
Harbor Drive
Pacific Hwy
Broadway
Kettner Blvd.
Harbor Dr.
National
Laurel
Ash St.
A St.
8th Ave.
5th Ave.
Harbor Island Dr.
Rosecrans St.
Nimitz Blvd.
McCain Blvd.
1st St.
2nd St.
3rd St.
4th St.
Alameda Blvd.
Orange Ave.
Glorietta Blvd.
Canon St.
North Island Naval Air Station
EMBARCADERO BARGE
32° 43.00' North
117° 10.80' West
SEAPORT VILLAGE
CORONADO LANDING
BARGE
32° 42.20' North
117° 10.04' West
HARBOR ISLAND BARGE
32° 43.33' North
117° 12.00' West
SHELTER ISLAND BARGE
32° 42.83' North
117° 13.20' West
Imperial Beach
Palm Ave.
Imperial Beach Blvd.
Seacoast Dr.
IMPERIAL BEACH
PIER
Enjoy the biggest fireworks show in San Diego!
Simulcast LIVE on
FOX 5 SAN DIEGO
105.7 The Walrus
TO BENEFIT
ARMED SERVICES YMCA
For more information on this event, please go to: www.thebigbay.com

Item No. 6
Supporting Document No. 5

Cover Story

June 30, 2008
Volume 86, Number 26
pp. 14-18

Pyrotechnics For The Planet

Chemists seek environmentally friendlier compounds and formulations for fireworks and flares

Bethany Halford

WHETHER THEY'RE lighting up Roman candles or basking in the glow of a fireworks extravaganza this Fourth of July, chemists are entitled to feel a certain amount of professional pride along with their patriotism. After all, it's chemistry that gives a humble bottle rocket its pop and makes a chrysanthemum shell bloom into a crowd-pleasing explosion of colored sparks.

U.S. Navy
View Enlarged Image

Flare Up Chemists are targeting military pyrotechnics, such as the deployed decoy flares shown here, for more eco-friendly formulations.

Even so, when it comes to pyrotechnics, students of chemistry would be wise to bear in mind this old adage from physics: What goes up must come down. The complex brew of oxidizers, propellants, fuels, binders, and coloring agents is what makes each firework's burst brilliant. But it leaves behind a smoky ghost of combustion products and particulate matter, which waft their way into the nearby soil and water.

The same thing happens when real rockets give off their red glare. Military pyrotechnics, which encompass everything from missile propellants to handheld flares, release a plume of smoke and potentially toxic products that pose a health hazard to the men and women of the armed forces who may breathe them in.

Consequently, chemists have been working to make new pyrotechnic compounds and formulations so that bombs bursting in air do so more benignly.

Typical pyrotechnics function by burning, so their basic chemical components consist of an oxidant and a fuel. Black powder, the original pyrotechnic, blends potassium nitrate oxidizer with charcoal and sulfur fuel. Set this witch's brew alight, and in a flash the nitrate oxidizes the charcoal and sulfur, producing glowing solids and a vast volume of hot gases. Other components, such as colorants, binders, and propellants, can be added to the mix, depending on the task the pyrotechnic has to perform.

Over the years, perchlorate has become the oxidizer of choice for most pyrotechnic applications, supplanting less stable chlorate oxidants that were the cause of numerous deadly explosions. "Potassium perchlorate is the ideal oxygen donor to use in pyrotechnics in terms of safety, cost, and reproducibility," says John A. Conkling, a pyrotechnics expert and adjunct professor of chemistry at Washington College, in Chestertown, Md.

Unfortunately, perchlorate has also been identified as a potential human health hazard. Studies suggest that it inhibits the thyroid's

ability to take up iodine from the bloodstream and can reduce the production of thyroid hormone. And because the anion is highly water soluble, it readily slips into groundwater. "The major effort in most areas of environmentally friendly pyrotechnics research is to find perchlorate replacement materials," Conkling says.

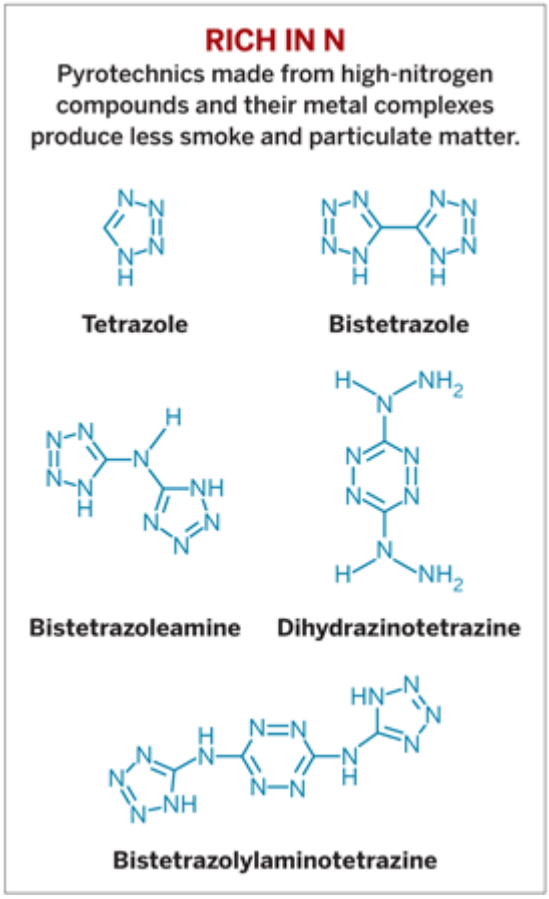
Conkling points out that in a working pyrotechnic—for example, a firework or a roadside flare—combustion should consume the majority of the perchlorate. In practice, however, that doesn't always happen. Pyrotechnics can be loaded with excess perchlorate to ensure burning; burning can snuff out prematurely; and, inevitably, there are duds that don't burn at all.

A team led by the Environmental Protection Agency's Richard T. Wilkin studied the concentration of perchlorate in the surface waters of a small lake in Ada, Okla., where there is an annual Fourth of July fireworks show (*Environ. Sci. Technol.* **2007**, *41*, 3966). Within 14 hours of the pyrotechnic display, the perchlorate level in the lake spiked as high as 1,000 times its baseline value. The researchers found that it took anywhere from 20 to 80 days for the perchlorate level to come down to its background level.

"THE PRESSURE is on to eliminate future perchlorate contamination by eliminating the perchlorate ingredient from as many rocket propellant and pyrotechnic compositions as possible," says Robert G. Shortridge, a scientist in the Pyrotechnic Operations Branch at the Crane Division of the Naval Surface Warfare Center.

Pyrotechnic flares have numerous roles in military operations, Shortridge notes. Aviators routinely carry red flares in their flight suits and life rafts to use as distress signals if their aircraft go down. Ground-based troops frequently use green and yellow flares to mark their locations, and all different types of flares are used on training grounds so soldiers become accustomed to the frequent explosions they're likely to encounter on the battlefield.

Aircraft also use decoy flares to thwart heat-seeking missiles. Such pyrotechnics give off an infrared signal that mimics the aircraft engine's infrared signature, so the missile goes after the flare rather than the plane or helicopter. With pyrotechnics being so vital to military operations, the Department of Defense's Strategic Environmental Research & Development Program and Environmental Security Technology Certification Program sponsor an extensive series of efforts to make pyrotechnic materials friendlier to the environment.



For example, Shortridge and his colleagues have been working to replace the perchlorate in colored signal flares. So far, they've had the most success with red signal flares that use strontium-based oxidants. Their perchlorate-free formulation is about to undergo safety testing, as well as tests in which the flares will be loaded into the signal hardware and subjected to the environmental rigors they would experience in service. "We intend to pass all of them while making the environment a little safer too," Shortridge says of the tests.

The other area in which pyrotechnics could improve from an environmental standpoint is their use of coloring agents. To achieve colored fireworks and flares, pyrotechnic makers employ metals or metal compounds that emit light in the visible spectrum. Red hues come from strontium, sodium glows yellow, barium burns green, and blues and greens come from copper.

At one time, mercury and lead compounds were used as colorants, but they were phased out long ago. Ironically, the modern pyrotechnic components that could use some "greening" are the barium compounds that give fireworks and flares their green color.

While tromping through a fresh snowfall in Vienna on New Year's Eve, Georg Steinhauser decided to find out just how much of these metal combustion products make their way into the environment from a typical fireworks display. Steinhauser, a licensed pyrotechnician and chemistry postdoc at Vienna University of Technology, scooped samples of snow in the city and countryside before and after the holiday pyrotechnics show and tested them for combustion products.

Postpyrotechnics snow from the city had significant concentrations of barium and strontium, indicating that the fireworks had left a chemical signature behind. By comparison, snow from the countryside, which was well out of fireworks range, remained clean after the show.

Before you break out the plastic sheeting and duct tape in anticipation of this year's Independence Day pyrotechnics extravaganza, you should know that most experts think the level of pollution from shooting off fireworks outdoors a couple of times per year is actually

pretty small. Steinhauser is quick to point out that with the exception of barium, the metallic combustion products he found are harmless. The particulate matter and combustion products from fireworks quickly disperse, and the amount of perchlorate that fireworks give off is relatively little.

Pollution from fireworks becomes more problematic when the pyrotechnics are being used indoors, such as at concerts or sporting events, or when they're set off in the same spot night after night. "In places where you're doing displays multiple days per week, pollution can definitely become a major issue," says David E. Chavez, a chemist at Los Alamos National Laboratory (LANL). "If you have pyrotechnic devices being used day after day, show after show, then you'll gradually build up a certain amount of toxic metals."

The problem came to LANL's attention about a decade ago, when the national lab was approached by Walt Disney Co. Disneyland's neighbors in Anaheim, Calif., were complaining about pollution from the amusement park's nightly fireworks show, and the company wondered whether LANL's explosives experts could develop environmentally friendlier fireworks.

"Smoke was essentially the main issue," Chavez explains. The black powder used to propel the fireworks skyward left a trail of smoke, as did pyrotechnic combustion products, such as potassium chloride from the potassium perchlorate oxidant, and metal oxides from metallic fuels, such as magnesium.

Disney was able to solve the black powder problem with an engineering solution. The company built a system that uses compressed air to send pyrotechnics aloft, eliminating the need for black powder. "The other problem is a chemistry problem, which is eliminating the smoke once it gets up there," says Mike Hiskey, an energetic materials expert who was part of the LANL team and now runs his own pyrotechnics company, DMD Systems.

In the late 1990s, the LANL team tried to address the smoke problem while making compounds with high nitrogen content as potential explosives and propellants. "We took one of our high-nitrogen materials and mixed it with a little strontium nitrate just to see what it would look like, and we got a very quickly burning, beautifully colored flame with absolutely no smoke," Hiskey recalls. "We thought, 'We're on to something here.' "

Le Maitre Special Effects

Clowning Around The circus uses DMD Systems' low-smoke, perchlorate-free fireworks to make sparks indoors.

Unlike traditional pyrotechnics, which get their energy from oxidizing carbon or metal fuels, high-nitrogen materials store their energy in their N–N and N–H bonds. "When they give off their energy, it's not an oxidizing process," Chavez explains. Instead, the molecules release energy as they break up into N_2 and H_2. Very little carbon is present in these nitrogen-rich molecules, he continues, so much smaller amounts of oxidizers, such as perchlorate, are needed.

Less carbon and less perchlorate also mean less smoke, Chavez adds. With less smoke to obscure color, pyrotechnic makers can cut down on the amount of coloring agent they need in a firework or flare. "You can reduce the amount of metal pretty dramatically," Chavez says. In a traditional pyrotechnic blend, the coloring agent can account for as much as 20–30% of the mixture by weight. In a high-nitrogen pyrotechnic formulation, the colorant makes up just 2–5 wt % of the blend.

Nitrogen-rich pyrotechnics also offer chemists the opportunity to combine the metal coloring agent and fuel in a single compound. The high-nitrogen compounds readily associate with popular colorant metals, such as strontium or copper.

"In a regular pyrotechnic mixture, you just have a metal compound that has to be vaporized somehow—generally through heat generated in the oxidizing process," Chavez explains. "We thought if you could actually vaporize individual atoms of metals using metal complexes of bistetrazole or bistetrazoleamine, for example, it would make for a much more efficient method of getting the color-producing metals in the gaseous form."

IN THE PAST 10 years or so that chemists have been pursuing high-nitrogen pyrotechnics, they've managed to create a vast menagerie of these nitrogen-rich compounds. Thomas M. Klapötke, a chemistry professor at Germany's University of Munich, and Vienna University of Technology's Steinhauser recently published a comprehensive review on the topic (*Angew. Chem. Int. Ed.* **2008**, *47*, 3330).

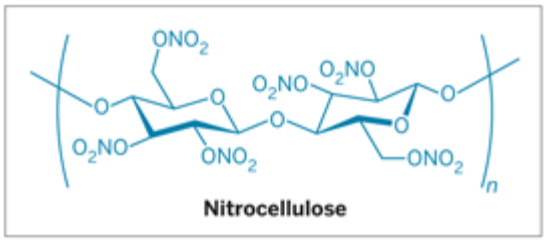
Looking at these compounds—salts based on tetrazole, bistetrazole, bistetrazoleamine, dihydrazino tetrazine, and bistetrazolylamino tetrazine, to name a few—one would think that the goal is to string as many nitrogen atoms together in a compound as is possible. But much more goes into making a good high-nitrogen pyrotechnic, Klapötke says.

"You try to make a molecule with lots of nitrogen that is kinetically stable enough so that it can be handled in a safe way," Klapötke tells C&EN. "It's easy to make a compound with a lot of nitrogen that is friction, impact, or electrostatically sensitive. We don't want that for flares or civil fireworks. We want them to be safe to handle." To that end, the nitrogen atoms are often contained in an aromatic or pseudoaromatic ring system, so that delocalization lends the molecule some kinetic stability.

Klapötke's lab has been pursuing nitrogen-rich compounds for use as military flares. Such specialized military applications are probably the first place that high-nitrogen pyrotechnics will find a practical use, chemists working in the field agree. They may also make lovely fireworks, but they're simply not cost competitive with commercially available pyrotechnics, most of which come from China and are assembled from inexpensive starting materials with very low labor costs.

Darren Naud says he and Hiskey learned that lesson the hard way eight years ago when they founded DMD Systems as a weekend enterprise away from their full-time jobs at LANL. "We originally started the business thinking we could use bistetrazoleamine," Naud says, alluding to the work that he and Hiskey had done with the group at LANL.

To start playing around with commercial pyrotechnic formulations of bistetrazoleamine, however, they needed to get a drum of the stuff. The compound isn't commercially available, so Naud and Hiskey sought a contractor to make the material. Originally, they were given an estimate of $30 per lb—expensive but within reach. When the contractor came back with a revised estimate of $300 per lb, they knew they would have to look elsewhere.



Nitrocellulose

"WE KNEW we had to use stuff that was commercially available and dirt cheap, so we settled on propellant-grade nitrocellulose, which is used by the military in millions of millions of tons every year as a gun propellant," Hiskey says.

"Nitrocellulose is probably one of the best low-smoke ingredients," Naud adds. "It burns with little smoke, and there's no fallout or residual combustion by-products that are nasty. There's just CO_2, water, and nitrogen."

Nitrocellulose has most of the oxygen it needs for complete, clean combustion already in the molecule as nitrate esters, Hiskey explains. There's no need to load it up with perchlorate oxidizers. Because the material produces little smoke, only small amounts of coloring agents are required to get vividly hued pyrotechnic effects.

Two-and-a-half years ago, Hiskey and Naud left LANL to devote themselves to DMD full time. In the remote reaches of northern New Mexico, the company's five employees do both pyrotechnics R&D and production, frequently shooting off fireworks beneath their warehouse's 25-foot-high ceilings.

DMD's low-smoke pyrotechnics have found a nice niche with customers who want indoor fireworks. "We crank out mainly theatrical pyro stuff," Hiskey says. They supply fireworks for Las Vegas shows, rock bands, circuses, Disney, and the folks at World Wrestling Entertainment.

Hiskey estimates that a fireworks display from DMD costs about twice as much as a traditional show, but customers are willing to pay extra for pyrotechnics that are safer for indoor use. "How do we make a product that would be able to compete with the Chinese pyrotechnics?" Hiskey asks. "The answer is that we can't, unless the customer demands that it's perchlorate-free or low-smoke." And that demand is on the rise, Hiskey and Naud tell C&EN. Their clients are also starting to ask them for perchlorate-free pyrotechnics for outdoor shows.

It's going to take that kind of demand, combined with tougher regulation, if eco-friendly pyrotechnics are going to light up the skies, scientists say. Despite the competition from inexpensive, traditional pyrotechnic formulations, they think there is a future for greener products. "Anything that we do for the environment initially costs money, but you have to take into account that all the cleanup processes often cost even more," Klapötke notes. In the long run, he says, it could be cheaper to go with pyrotechnics that maintain their dazzle and glow while minimizing their environmental fizzle.

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Conditional Waiver No. 11 – Aerially Discharged Wastes Over Land

Conditional Waiver No. 11 is for wastes that have been discharged aerially over land, which may be a source of pollutants that can adversely affect the quality of waters of the state.

The following types of discharge not regulated or authorized under WDRs may be eligible for Conditional Waiver No. 11:

- Discharges of wastes related to fireworks displays over land
- Other wastes discharged aerially over land that may adversely affect the quality of the groundwaters of the state, but determined to be "low threat" by the San Diego Water Board

These types of discharge can have similar environmental settings and potential threat to water quality. Therefore, wastes discharged aerially over land were grouped into one discharge classification. Wastes discharged aerially over land that comply with the waiver conditions are not expected to pose a threat to the quality of waters of the state.

For waste discharges related to fireworks displays, available studies suggest annual or infrequent fireworks displays present a low threat to groundwater quality. However, there may be potential water quality impacts that are cumulative for shallow groundwaters used as drinking water sources with recurring fireworks displays. With proper planning and management, the potential treat to groundwater quality from wastes related to fireworks discharged over to land can be eliminated. Therefore, waiver conditions must require proper planning and management of fireworks displays over land to minimize or eliminate the discharge of pollutants to waters of the state.

There may be other aerially discharged wastes in the San Diego Region that are determined to pose a low threat to the quality of groundwaters of the state. These aerially discharged wastes would likely require the same minimum conditions to be protective of the quality of groundwaters of the state.

The permitting process and permits issued by other public agencies (e.g., air pollution control districts, municipalities, fire departments) can provide preliminary information and data to the San Diego Water Board to determine compliance with conditions of a waiver for aerially discharged wastes. Obtaining the proper permits, licenses, or certifications from appropriate public agencies can be a waiver condition that serves as the method of enrollment for a conditional waiver.

However, waiver conditions should be developed in order for members of the public, cities, counties, local agencies and organizations, and/or the San Diego Water Board to determine if aerially discharged wastes are in conformance with the conditional waiver, or causing significant adverse effects on the waters of the state. Significant adverse effects include, but are not limited to, one-time observations of exceedences of drinking water maximum contaminant levels in reservoirs and groundwater source water wells,

persistent pollutant concentrations in the water column that exceed water quality objectives for surface waters, and persistent pollutant concentrations in the sediments of surface water bodies that exceed sediment screening levels or sediment criteria.

If dischargers are not in compliance with waiver conditions, they can be issued a Notice of Violation and required to correct deficiencies in order to be eligible for Conditional Waiver No. 11. If dischargers violate any waiver conditions, the San Diego Water Board has the option to terminate the conditional waiver for the discharge and begin regulating the discharge with individual WDRs and/or take other enforcement actions.

In order to be eligible for Conditional Waiver No. 11, discharges must comply with certain conditions to be protective of water quality. The waiver conditions applicable to wastes discharged aerially over land include the following:

11.I.A. General Waiver Conditions for Aerially Discharged Wastes Over Land

11.II.A. Specific Waiver Conditions for Discharges of Waste Related to Fireworks Displays Over Land

Wastes discharged aerially over land that comply with the general and specific waiver conditions in Conditional Waiver No. 11 are not expected to pose a threat to the quality of waters of the state.

11.I.A. General Waiver Conditions for Aerially Discharged Wastes Over Land

1. Aerially discharged wastes cannot be discharged directly over and/or into surface waters of the state (including ephemeral streams and vernal pools).
2. Aerially discharged wastes must not cause or threaten to cause a condition of contamination, pollution, or nuisance.
3. Aerially discharged wastes must not impact the quality of groundwater in any water wells or surface water in any drinking water reservoirs.
4. Dischargers must comply with any local, state, and federal ordinances and regulations and obtain any required approvals, permits, certifications, and/or licenses from authorized local agencies.
5. Discharger must submit a Notice of Intent or technical and/or monitoring program reports when directed by the San Diego Water Board.

11.II.A. Specific Waiver Conditions for Discharges of Waste Related to Fireworks Displays Over Land

1. No more than one fireworks display may be conducted from a launch site or within 1.0 mile of another launch site within a 48-hour period.[1] If the organizer will have more than one fireworks display within a 48-hour period, the organizer must file a Notice of Intent containing information about the fireworks to be used, location of launch area and nearby water bodies and groundwater basins, surrounding land uses, planned period of and frequency

[1] This condition is intended to alleviate spatial and temporal accumulation of fireworks-related chemical contaminants.

of discharge, copies of any permits obtained from other public agencies, and measures that will be taken to minimize or eliminate the discharge of pollutants that might affect surface water and groundwater quality. Sufficient information must be submitted before the discharge may begin.

2. All fireworks-related debris must be cleaned up from land surface areas.
3. Launch areas and deposition areas of fireworks displays may not be located within areas designated as Zone A for groundwater source area protection, as defined by the California Department of Public Health's Drinking Water Source Assessment Protection Program. This condition may be waived if the owner or operator of a groundwater drinking water source, through a permit, specifically allows the fireworks display launch area and/or deposition area within an area designated as Zone A for groundwater source area protection.
4. Launch areas and deposition areas of fireworks displays may not be located within areas designated as Zone A for surface water source protection, as defined by the California Department of Public Health's Drinking Water Source Assessment Protection Program. This condition may be waived if the owner or operator of a surface water source reservoir or intake structure, through a permit, specifically allows the fireworks display launch area and/or deposition area within an area designated as Zone A for surface water protection.
5. The fireworks display must be permitted by all relevant public agencies that require permits for fireworks displays (e.g., fire departments, municipal governments, law enforcement, water supply agencies). Copies of any permits must be available on site for inspection.
6. The San Diego Water Board and/or other local regulatory agencies must be allowed reasonable access to the site in order to perform inspections and conduct monitoring

Fact Sheet, July 2006

Department of Toxic Substances Control

Preventing environmental damage from hazardous waste, and restoring contaminated sites for all Californians.

State of California

California Environmental Protection Agency

DTSC's Perchlorate Best Management Practices (BMPs)

INTRODUCTION

The California Legislature passed the Perchlorate Contamination Prevention Act of 2003 requiring the Department of Toxic Substances Control (DTSC) to adopt best management practices regulations for perchlorate materials. DTSC adopted the Perchlorate Best Management Practices (BMP) regulations on December 31, 2005, and the regulations are effective July 1, 2006.

If you are in the Department of Defense, or you are in an industry centered on aerospace, fireworks, pyrotechnics, safety flares, amusement parks, automobile air bag and safety restraint, lithium perchlorate batteries, or if you are in a public safety agency, this information is of use to you. Others affected by these regulations include farmers who use perchlorate-containing fertilizer, laboratories, bleach manufacturers and users, and Publicly Owned Treatment Works (POTWs). Even households may be affected.

This fact sheet provides some background information to help understand the new regulations. This fact sheet also lists the types of perchlorate-containing products that may be subject to these requirements and describes the perchlorate best management practices.

Why regulate perchlorate?

There are existing hazardous material regulations for perchlorate in its pure form because it is used to enhance combustion. In recent years, environmental agencies have found more and more instances of perchlorate appearing in drinking water, groundwater, surface water and soil. In light of the risks to public health and the environment posed by perchlorate releases, the California Legislature directed DTSC to establish best management practices for the prevention of perchlorate contamination. DTSC wrote regulations establishing standards for handling materials, products, and waste that contain perchlorate.

What is perchlorate?

Perchlorate is a chemical that is both manufactured and naturally-occurring. Most commonly found as an ionic salt, when dissolved in water it easily moves through and travels with the flow of water on or beneath the ground. Ammonium perchlorate and sodium perchlorate are examples of manufactured salts. Environmental agencies

attribute its presence in the environment to past waste handling practices at facilities that manufacture or use this perchlorate and materials containing the chemical. It may also be present in the environment as a consequence of using perchlorate-containing products such as those identified below.

How is perchlorate used?

Perchlorate is used primarily as an ingredient in solid rocket propellant. The Department of Defense, the National Aeronautics and Space Administration (NASA), and the defense industry use, and have for decades used, perchlorate in manufacturing, testing, and firing rockets and missiles. On the basis of 1998 manufacturer data, U.S. EPA estimated that manufacturing demand for the military and NASA is 90 percent of the perchlorate salt produced in the United States.

Private industry has used perchlorate to manufacture products such as fireworks, flares, automobile airbags, coin-cell batteries, and commercial explosives. Perchlorate is also found as an impurity in manufactured chemicals and products. Perchlorate can also occur as an impurity in some natural minerals used in some fertilizers.

How do I know if I am using products that contain perchlorate?

You can find perchlorate in a variety of materials. The new perchlorate regulations require that those who manufacture products, or who bring products into California for distribution, label those products to inform consumers of the perchlorate content. The information may appear on the product itself, on the product label, in a Material Safety Data Sheet (MSDS), or on a product insert. A MSDS is a detailed informational document of a hazardous material. If the material already has a perchlorate label, you can assume that the material contains perchlorate.

In what products can I expect to find perchlorate?

Solid Rocket Motors: The amount of ammonium perchlorate required in a given motor varies by the type of solid rocket or missile propellant. For example, model rockets are fueled by single-use rocket motors may contain perchlorate. These motors are professionally manufactured and available to the general public for purchase.

Flares: Both road and marine flares contain perchlorate salts.

Fireworks: Sodium perchlorate and potassium perchlorate are often ingredients in fireworks.

Pyrotechnic Devices: Pyrotechnics, used to produce light, smoke, heat, or sound effects, all contain an oxidizer component that is often a perchlorate material.

Explosives: Perchlorate salts have been used as detonators, initiators, and propellants in military explosives. A newer class of explosives now includes ammonium perchlorate in the formulations to reduce accidental ignitions due to shock.

Blasting Agents: Some blasting agents, mostly water gels, and emulsions, can contain substantial amounts of perchlorate salts. Perchlorate-containing blasting agents are especially useful in construction and mining when conditions are wet or water-saturated.

Common Batteries: These include small button batteries which are the size and shape of coins. The battery numbers will start with “CR” and they may be found in watches, appliances, keyless entry systems, and any device that is able to retain memory after the power supply is cut off.

Air Bag Initiators: Airbag initiators are part of a car’s safety system and they may contain perchlorate. If the air bag is deployed during an accident, the perchlorate is used up in the process.

Bleach: Hypochlorite solutions may contain perchlorate as an impurity. The concentration may increase as the product ages.

Fertilizers: Perchlorate has been found in measurable amounts as an impurity in some fertilizers made with natural minerals such as bloodmeal, certain nitrate, fishmeal, hanksite, kelp, and potash. The fertilizer label and the MSDS should be reviewed to determine the presence of perchlorate in the fertilizer product.

What are the human health effects of perchlorate?

Perchlorate exposure at certain levels can disrupt the function of the thyroid gland by interfering with the iodide uptake and thyroid hormone production. This interference may lead to developmental defects. Scientists consider pregnant women, children, infants, and individuals with thyroid disorders to be the populations most at risk of harm from being exposed to perchlorate. These health threats are the reason agencies set standards for perchlorate.

When do the perchlorate BMPs apply?

Perchlorate materials include all forms of matter, goods, products, or waste that contain perchlorate. The perchlorate best management practices regulation specifically excludes hazardous waste, materials with perchlorate concentrations below 6 parts per billion (ppb), food, crops, irrigation water, combustion residuals, and contaminated media.

The regulations apply to any person or business that manages perchlorate materials or waste in any manner including use, processing, generation, transportation, storage, and disposal.

How can I determine the concentration of perchlorate?

You may use industry or chemical knowledge, or a MSDS, to determine the perchlorate concentration. You can also determine the concentration by using various analytical methods. To comply with the Safe Drinking Water Act standards, a lab must use EPA Method 314.0 - Determination of Perchlorate in Drinking Water by Ion Chromatography. U.S EPA and others are developing additional analytical methods. As examples, EPA's Office of Solid Waste is working on a Method 6850 for analyzing perchlorate in various wastes; and the U.S. Food and Drug Administration published a draft analytical method for perchlorate in water, milk, and lettuce.

What are the Perchlorate Best Management Practices?

DTSC established perchlorate best management practices in regulations to address various aspects of handling perchlorate-containing material to minimize the threat of release and resulting public health or environmental harm. Key requirements of these regulations include:

Labeling

- Businesses need to inform purchasers of perchlorate materials or products about the item's perchlorate content.
- Businesses that manufacture perchlorate materials, repackage perchlorate materials, distribute perchlorate materials for sale, receive perchlorate materials for resale or use in California, or who generate a perchlorate-containing waste need to ensure that these perchlorate materials are properly labeled or marked with the following, "Perchlorate Material – special handling may apply."
- There are alternatives to using a label in the BMPs which include shipping documents, MSDS, and training.

Packaging

- Businesses that manufacture, package and distribute perchlorate materials must ensure they are properly contained in water-resistant packaging and labeled.

Containment

- Businesses must adopt additional containment procedures when materials or products are not contained in durable, water-resistant containers. For example, during manufacturing or repackaging, there may be times when perchlorate-containing material is not in a container – transferring from one container to another, for example - so that activity needs to be occur in weather-resistant structures on floors that do not contain drains.

One-Time Notification

- Businesses managing more than 500 pounds of solid perchlorate material or 55 gallons of liquid perchlorate material at any one time must submit to DTSC a one-time notification about their perchlorate materials and related activities. Send that notification to DTSC on or before September 1, 2007, to cover activities occurring between July 1, 2006 and June 30, 2007. This is in addition to the required hazardous material business plan. Certain exemptions may apply, see California Code of Regulations, title 22, section 67384.7(c).

Special Practices

- Use road safety flares in a way to minimize release of perchlorate into the environment. Businesses that use road flares should limit the duration and number of flares as necessary to ensure safety.
- Road flares should be allowed to burn completely.
- All personnel who routinely use road flares in the normal course of employment should receive instruction on the potential environmental hazards associated with using perchlorate materials and on the perchlorate best management practice requirements.
- Use marine safety flares in a manner that minimizes releases of perchlorate to the environment. Do not throw them into the water or into normal garbage. You cannot burn flares to dispose of them.
- Collect un-ignited pyrotechnics within 24 hours of a fireworks display and manage them as hazardous waste.

Spill Response

- Businesses are responsible for cleaning up any spills of perchlorate-containing materials. You must first contain the spill, then clean it up to prevent the chemical from going into storm drains.

Discharge and Disposal

- Businesses can only dispose of perchlorate-containing solid material to either a hazardous waste landfill or a composite-lined portion of a non-hazardous waste landfill.
- Landfills and Publicly Owned Treatment Works (POTWs, wastewater treatment facilities owned by a state or municipality) that accept non-hazardous perchlorate wastes must notify the appropriate Regional Water Quality Control Board of any perchlorate discharge and comply with any modifications to existing environmental monitoring programs.
- Businesses that discharge non-hazardous liquid perchlorate-containing waste or wastewater must notify the overseeing regulatory agency of the discharge. Typically, this is the POTW having jurisdiction in their area, and the business must notify the local Regional Water Quality Control Board. This allows regulatory agencies the opportunity to evaluate these discharges and determine whether the business should include perchlorate in its monitoring program.

Pollution Prevention

- On or before January 1, 2008, and every five years thereafter, a business that uses perchlorate-containing fertilizers, safety flares, explosives, or blasting agents, in an amount greater than 500 pounds in any given month (the same “trigger” used in the Business plan) must review the use of these products determine for themselves if a non-perchlorate-containing alternative is available. These businesses also need to review and implement as appropriate pollution prevention measures to prevent releases of perchlorate. Certain exemptions may apply, see California Code of Regulations, title 22, section 67384.11(a).
- On or before January 1, 2008, a business using fireworks with more than 4,000 pounds of pyrotechnic composition or

8,000 pounds of solid rocket motors during any calendar year must submit to DTSC any existing environmental monitoring for perchlorate in the soil or water around the area of use.

How do the perchlorate BMPs apply?

Perchlorate BMPs for Households

Households are subject to these regulations but have the following minimal requirements.

- Households need to maintain proper packaging. The best way to do that is to keep perchlorate-containing materials in the original containers.
- If you keep the materials in durable, waterproof packaging, you do not have to have a second or backup way to contain it.
- If you use safety flares, keep the duration and number of flares to what is necessary to ensure safety.
- If you use marine safety flares, do not throw them in the water or in the normal garbage. You cannot burn them as a way to dispose of them. Contact your local household hazardous waste center for directions on management.
- Any spills of perchlorate products, spent fireworks, or spent model rockets need to be collected and may be disposed in the garbage.

Perchlorate BMPs for Businesses

For all other businesses, the requirements depend on how the business uses or manages perchlorate materials and/or waste. The following highlight requirements for businesses that sell perchlorate-containing products or handle pyrotechnics, safety flares, solid rocket motors, or fertilizers:

Perchlorate BMPs for Retailers

- Retailers who distribute perchlorate-containing materials for sale, resale or use in California are responsible to ensure that products are properly labeled or marked with the following, "Perchlorate Material – special handling may apply." There are alternatives to using a label in the BMPs which include shipping documents, MSDS, and training.
- Retailers need to ensure that perchlorate-containing products are in packaging or containers that are durable and water-resistant.

Perchlorate BMPs for Special Event Organizers or Amusement Parks using Fireworks

- Pyrotechnics operators are responsible for collecting any "stars" and un-ignited pyrotechnic material found during the inspection of the firing range after a public display of fireworks. The collected material must be managed as hazardous waste.
- On or before January 1, 2008, a business that uses fireworks in amounts greater than 4,000 pounds of pyrotechnic composition during any calendar year needs to submit to DTSC any existing environmental monitoring for perchlorate in the soil and/or water around the area of firework use.

Perchlorate BMPs for Law Enforcement, Fire Response and Other Governmental Agencies using Safety Flares

- Agencies that use safety flares should limit the duration and number of flares as necessary to ensure safety.
- All personnel who routinely use flares in the normal course of employment should receive instruction on the potential environmental hazards associated with the use of perchlorate materials and on the perchlorate BMP requirements.
- On or before January 1, 2008, and every five years thereafter, an agency that uses perchlorate-containing safety flares in an amount greater than 500 pounds in any month, needs to review the use of these perchlorate-containing products and determine for itself if a non-perchlorate-

containing alternative is available. Agencies must review and implement as appropriate pollution prevention measures to prevent releases of perchlorate.

Perchlorate BMPs for Businesses Producing, Testing, or Developing Solid Rocket Motors

- Businesses that process, manufacture, or store perchlorate materials, such as solid rocket motors, must contain these materials in a weather-resistant structure without drains and that prevent seepage into or out of the containment area.
- On or before January 1, 2008 and every five years thereafter, a business using solid rocket motors in amounts greater than 8,000 pounds at any given time must submit to DTSC any existing environmental monitoring for perchlorate in the soil and/or water around the area of use.

Perchlorate BMPs for Businesses Using Fertilizer Containing Perchlorate

- Businesses that manufacture, package, or distribute this fertilizer must ensure that products are properly labeled or marked with the following, "Perchlorate Material – special handling may apply."
- Businesses that simply use this fertilizer are exempt from labeling.
- There are alternatives to using a label in the BMPs which include shipping documents, MSDS, and training.
- Businesses that handle or sell these fertilizers need to ensure that these products are in durable and water-resistant packaging, containers, or are stored in weather – resistant structures.
- Businesses that apply the fertilizer are exempt from the containment requirement, if the fertilizer is stored for less than 30 days on the site of intended application.
- If the distributor from which you got it reported the fertilizer as required by the California's Food and Agriculture annual tonnage report on fertilizer sales and distribution, the business using it need not make the one-time notification described elsewhere.
- Fertilizers allowed by the U.S. Department of Food and Agriculture in keeping with the Organic Foods Production Act of 1990 and fertilizers derived from those sources are exempt from pollution prevention requirements.

Where can I get more information about perchlorate?

General

DTSC has a two perchlorate pages on its website. The first page includes general information, fact sheets, and links to other online resources and is at www.dtsc.ca.gov/HazardousWaste/Perchlorate /index.cfm. The second page, found at www.dtsc.ca. gov/LawslegsPolicies/Regs/Perchlorate_regs.cfm includes the regulations and all the background documents that were developed in support of the perchlorate BMPs.

Health

The California Department of Health Services maintains a web page that provides an overview of issues regarding perchlorate in drinking water at http://www.dhs.ca.gov/ps/ddwem/chemicals/perchl/perchlindex.htm.

For additional information on the health effects of perchlorate, DTSC suggests you search Office of Environmental Health Hazard Assessment site at http://www.oehha.org. You will find the Final Technical Support Document for the Public Health Goal for Perchlorate in Drinking Water is located at www.oehha.org/water/phg/pdf/perchlorate3docs.pdf

You can also find health information on the Centers for Disease Control's Agency for Toxic Substances and Disease Registry at http://www.atsdr.cdc.gov/tfacts162.html

Occurrence

U.S. EPA has national occurrence maps available at: www.epa.gov/swerffrr/documents/perchlorate_links.htm#occurrences.

Analytical Methods

DTSC's Testing Guidance at www.dtsc.ca.gov/HazardousWaste/Perchlorate/upload/HML_POL_Guidance_Perchlorate-Testing.pdf.

U.S. EPA guidance at:

www.epa.gov/safewater/methods/sourcalt.html, and www.epa.gov/ncea/perchlorate/references/documents/ref006.pdf.

Acronyms

BMP	Best Management Practice
Cal/EPA	California Environmental Protection Agency
DTSC	Department of Toxic Substances Control
MSDS	Material Safety Data Sheet
ppb	Parts per billion
US EPA	United States Environmental Protection Agency

Glossary

"***Managing perchlorate materials***" means generation, storage, transportation, manufacture, processing, fabrication, packaging, use, reuse, treatment, transfer, pumping, recovery, recycling, spill response, disposal, and discharge.

"***Packaging***" means a receptacle and any other components or materials necessary for the receptacle to perform its containment function in conformance with the minimum packing requirements.

"***Perchlorate material***" means all perchlorate-containing materials including perchloric acid and perchlorate compounds. "Perchlorate material" includes all forms of matter, goods, and products.

Disclaimer

This fact sheet was prepared in July 2006 and is based on statutes and regulations in effect at that time. The reader should not rely solely on this fact sheet for regulatory compliance and should instead review the most current statutes and regulations.

For More Information or Assistance

For assistance or information call DTSC's Regulatory Assistance toll free at 1 800-72 TOXIC (1-800-728-6942) or visit our website at www.dtsc.ca.gov.

The actual text of the Perchlorate BMP regulations is available at www.dtsc.ca.gov/HazardousWaste/Perchlorate/index.cfm.

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Do pyrotechnics contain radium?

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Abstract

Many pyrotechnic devices contain barium nitrate which is used as an oxidizer and colouring agent primarily for green-coloured fireworks. Similarly, strontium nitrate is used for red-coloured pyrotechnic effects. Due to their chemical similarities to radium, barium and strontium ores can accumulate radium, causing a remarkable activity in these minerals. Radium in such contaminated raw materials can be processed together with the barium or strontium, unless extensive purification of the ores was undertaken. For example, the utilization of 'radiobarite' for the production of pyrotechnic ingredients can therefore cause atmospheric pollution with radium aerosols when the firework is displayed, resulting in negative health effects upon inhalation of these aerosols. In this study, we investigated the occurrence of gamma-photon-emitting radionuclides in several pyrotechnic devices. The highest specific activities were due to K-40 (up to 20 Bq g^{-1}, average value 14 Bq g^{-1}). Radium-226 activities were in the range of 16–260 mBq g^{-1} (average value 81 mBq g^{-1}). Since no uranium was found in any of the samples, indeed, a slight enrichment of Ra-226 in coloured pyrotechnics can be observed. Radioactive impurities stemming from the Th-232 decay chain were found in many samples as well. In the course of novel developments aiming at the 'greening' of pyrotechnics, the potential radioactive hazard should be considered as well.

Keywords: fireworks, inhalation, natural radioactivity, ^{238}U decay chain, ^{226}Ra, ^{228}Ra

1. Introduction

Fireworks are probably the application of chemistry with the best resonance with the general public. Nonetheless, fireworks are increasingly raising environmental concerns. Although the problem of pollution caused by fireworks (and other civil and military pyrotechnic applications) had been identified many years ago [1, 2], the number of environmental studies focusing on this problem has dramatically increased quite recently, e.g. [3–11]. Also the search for environmentally benign pyrotechnic formulations exhibits a rapidly expanding scientific field and has not hit its peak yet [5].

Pyrotechnics are thermodynamically metastable mixtures which consist of at least two basic constituents: the reductant/fuel (e.g. magnesium, aluminium, magnalium alloy, sulfur, charcoal, red phosphorus, etc) and the oxidizer (alkali metal or alkaline earth metal nitrates, perchlorates, chromates, metal oxides, etc). Several additives may find application in pyrotechnics in order to obtain a certain intended effect (e.g. colouring agents, propellants, smoke or sound generators, etc). Colours in pyrotechnics are obtained by the addition of compounds of elements with the desired flame colour. For red light, strontium nitrate is used; barium nitrate for green light; sodium oxalate or cryolithe (Na_3AlF_6) for yellow; and any copper/chlorine system (compounds or mixtures) for blue (see table 1 for some typical compositions of pyrotechnics). During combustion, very short-lived and unstable compounds, such as the monochlorides of alkaline earth metals (SrCl, BaCl) are formed, which emit light in the desired spectra [5, 12, 13]. The formation of the monochlorides thus depends on the presence of a chlorine source. If no chlorine donor is added to a pyrotechnic formulation, barium nitrate causes combustion under the emission of almost white light. This is the reason why barium nitrate is not only used as an oxidizer in green or greenish flares (with a chlorine donor, which is typically PVC powder) but also for white and yellow flares (without a chlorine donor), as shown in table 1. In the presence of chlorine, barium nitrate acts as a combined pyrotechnic oxidizer and colouring agent.

Table 1. Some typical barium nitrate-or strontium nitrate-containing pyrotechnic compositions (data taken from [5, 12, 27]). Values in wt%.

Ingredient	Mk 117 green navy flare	Mk 118 yellow navy flare	Turquoise formulation	Chartreuse formulation	White formulation	Mk 124 red navy flare	Red highway flare
Barium nitrate	22.5	20.0	75	75	55	—	—
Strontium nitrate	—	—	—	—	—	34.7	74
Magnesium	21.0	30.3	—	—	—	24.4	—
Potassium perchlorate	32.5	21.0	—	—	—	20.5	6
Sodium nitrate	—	—	—	5	—	—	—
Potassium nitrate	—	—	—	—	25	—	—
PVC	12.0	—	5	10	—	11.4	—
Sodium oxalate	—	19.8	—	—	—	—	—
Copper powder	7.0	—	—	—	—	—	—
Asphaltum	—	3.9	—	—	—	9.0	—
Sulfur	—	—	10	10	20	—	10
Cuprous chloride	—	—	10	—	—	—	—
Binder	5.0	5.0	—	—	—	—	10

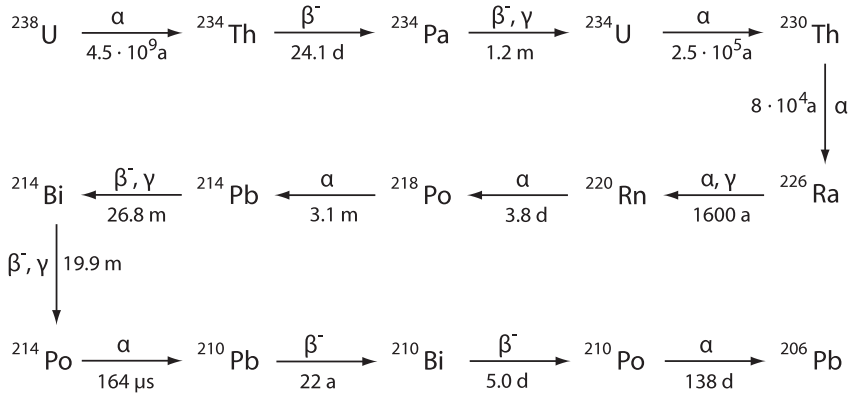
From an environmental and toxicological point of view, the formation of barium-rich aerosols following the display of a firework is a problem. The inhalation of barium-rich aerosols has adverse affects on the lungs and heart and causes muscle cramps [14, 15]. In cases of fireworks and pyrotechnics, barium compounds are set free in the form of mostly water-soluble and thus bioavailable compounds: BaO, $Ba(OH)_2$, $BaCl_2$ and undecomposed $Ba(NO_3)_2$. The raw material of barium compounds is generally barium sulfate (barite). In 2006, approximately 8 million tons barite have been produced by mining worldwide [16]. Only a very minor percentage is used in pyrotechnics. The major amount of barium sulfate is used as a pigment (*Blanc fixe*), or as a filler for paper, paint, varnish, rubber, etc. This mineral is also used as a constituent of heavy concrete for the shielding of ionizing radiation.

To the authors' knowledge, the potential hazard of fireworks due to liberated radionuclides has never been the subject of investigation in the scientific literature before. The radioactive alkaline earth metal radium has very similar chemical properties to barium (and also strontium), as they occur in the same group of the periodic table. This similarity is used, for example, in the preconcentration of radium from water by coprecipitation with barium in the form of $Ba(Ra)SO_4$. Natural sequestering leads to the formation of so-called radiobarite minerals. These minerals accumulate all naturally occurring radium isotopes, in particular the ^{238}U decay chain member ^{226}Ra (half-life $T_{1/2}$ = 1600 a) and the ^{232}Th decay chain member ^{228}Ra ($T_{1/2}$ = 5.76 a). However, the potential accumulation of ^{226}Ra is of higher environmental significance than ^{228}Ra, because the latter is simply too short-lived to be extremely accumulated in barium (or strontium) deposits. If young enough, these minerals and ores have remarkable ^{226}Ra activities. On geological timescales, however, ^{226}Ra has a relatively short half-life. If radiobarite minerals, therefore, are older than 10–20 ka and isolated from any further radium supply, they slowly lose their radioactive properties. The radiobarite-rich sludges and scalings at oil-field-production sites investigated by Zielinski *et al* [17], have ^{226}Ra activities in the range between 3 and 130 Bq g^{-1}, with one sample as active as 4.9 kBq g^{-1}. In their study, the ^{228}Ra activities have been found to be always lower than the ^{226}Ra values. Radiobarite ores in the Ohře Rift (Bohemian Massif) have activities between 0.02 and 7.80 Bq g^{-1} [18]. The scales and tailings in Polish hard coal mining sites were reported to contain radiobarites with activities in the range of 40–100 Bq g^{-1} for ^{226}Ra and 27–62 Bq g^{-1} for ^{228}Ra (barium-rich Rontok scale), and 5.3–6.4 Bq g^{-1} for ^{226}Ra and 6.4–8.5 Bq g^{-1} for ^{228}Ra (barium-poor Bojszowy tailings), respectively [19]. The ambient γ-dose rates are strongly elevated with more than 1 μSv h^{-1} at both sites.

Previous studies [20, 21] have investigated the trace element content of pyrotechnics and their poisoning potential. From an economic point of view, it is clear that raw materials for the production of fireworks are usually not purified beyond the grade which is necessary for the intended effects. This explains why the fireworks investigated in those studies contained significant traces of heavy metals which do not have a pyrotechnic function. The utilization of radium-rich barium and strontium ores would, therefore, involve the risk that radium might be processed together with barium and strontium into the final product. The display of such radium-containing pyrotechnics would set the radioactive material free in the form of easily inhalable aerosols. The incorporation of α-emitting radionuclides (such as ^{226}Ra) is a major health threat in human radiation protection. The ingestion or inhalation of α-emitters should thus be avoided under all circumstances. In order to examine this potential hazard, we applied radioanalytical methods to investigate the radioactivity of pyrotechnics purchasable in Austria.

2. Materials and methods

Fourteen samples of pyrotechnic devices (sky rockets, shell-type rockets, volcanoes) have been investigated with γ-spectrometry in this study (see table 2). The samples were weighed and filled into cylindrical polyethylene (PE) containers (comparable filling level). In principle, for the quantification of the ^{226}Ra activity, two methods are possible: the 186 keV γ-photon emitted by the nuclide itself can be measured. Alternatively, the γ-photons of its decay products ^{214}Pb and ^{214}Bi can be measured, as they are in equilibrium with ^{226}Ra after three or four weeks (due to the short half-lives of ^{214}Pb and ^{214}Bi as well as the intermediate ^{226}Ra-daughter



Scheme 1. Simplified decay scheme of the ^{238}U decay chain, including only the major decay route, and showing the decay types and half-lives of the nuclides. For γ-radiation, only nuclides are marked if significant for our measurement set-up. For exact nuclear data, see table 3.

Table 2. Samples investigated in this study.

Sample code	Sample name	Pyrotechnics type	Potential radium carrier
R1	Weco green glamour	Sky rocket	Barium
R2	Weco green glamour	Sky rocket	Barium
R3	Weco green flower	Sky rocket	Barium
R4	Weco red glamour	Sky rocket	Strontium
R5	Weco red glamour	Sky rocket	Strontium
R6	Weco red flower	Sky rocket	Strontium
R7	Weco pink flower	Sky rocket	Strontium
R8	Weco yellow flower	Sky rocket	Barium
R9	Weco white glamour	Sky rocket	Barium
R10	Wolm Pyrostar Kugelblitz	Shell-type rocket	Barium and/or strontium
R11	Wolm Pyrostar Kugelblitz	Shell-type rocket	Barium and/or strontium
R12	Wolm Pyrostar Kugelblitz	Shell-type rocket	Barium and/or strontium
R13	Weco Riesen Flimmer-Vulkan	Volcano	Barium and/or strontium
R14	Weco Fegefeuer	Volcano	Barium and/or strontium

nuclides ^{222}Rn and ^{218}Po, respectively), see scheme 1. Since radon is known to diffuse through many materials (sample vials), causing a loss of activity, the latter method appears to be the less reliable for our analytical purposes.

When the 186 keV γ peak of ^{226}Ra is used, the possible interference of ^{235}U, which also emits γ photons in this energy region, has to be considered. However, since we can assume that uranium in environmental samples must be present in its natural isotopic ratio, a γ spectrum showing a ^{235}U peak should also show the γ peaks of the short-lived ^{238}U granddaughter ^{234}Pa (with several γ photons at 1001, 743, 786 keV, etc), as shown in scheme 1. Since we did not detect any ^{234}Pa in our samples (detection limit approx. 20 mBq g^{-1}), the uranium content in pyrotechnics can be regarded as negligible. Consequently, any radium in the sample cannot be due to a contamination with uranium minerals being in equilibrium with the daughter ^{226}Ra. Rather, it must be a significant enrichment of radium itself in one of the raw materials.

Gamma-spectrometry was performed on the novel low-level counting facility of the Atominstitut, consisting of a 226 cm^3 HPGe detector (Canberra™, detector model GC5020; 2.0 keV resolution at the 1332 keV ^{60}Co peak; 52.8% relative efficiency), connected to a PC-based multi-channel analyser with preloaded filter. The measurement position of the sample was fixed at a distance of approximately 11 cm on top of the detector. The new detector system is characterized by only approximately one-tenth of the background of the other γ detectors of the radiochemistry group in the same institute. This is due to the improved shielding of the detector by the ORTEC™ HBLBS1 shielding (solid-cast virgin lead with steel casing, total weight 1134 kg). For calibration of the detector's efficiency for ^{226}Ra, 50 μl of QCY48 (Amersham® Ltd) solution in hydrochloric acidic solution (comparable bulk density) was used. The measurement times of the pyrotechnics were at least 1 week, or longer, until no significant improvement of the counting error of the most interesting peaks could be yielded by a—reasonably—longer measurement time. The standard solution was measured for 328 000 s. A background spectrum was recorded (1 816 000 s) and considered for the evaluation of the γ spectra of the pyrotechnics. For quantification, the γ photons with characteristic energies were used as listed in table 3. All nuclear data in this paper are taken from the National Nuclear Data Center [22].

3. Results and discussion

The results of the γ spectrometry are shown in table 4.

The main activity in pyrotechnics is due to ^{40}K (up to 20 Bq g^{-1}, mean value 14 Bq g^{-1}). The presence of ^{40}K

Table 3. Nuclear data of the radionuclides measured by γ spectrometry.

	^{40}K	^{212}Pb	^{214}Pb	^{214}Pb	^{226}Ra	^{228}Ac
Half-life	1.248×10^9 a	10.64 h	26.8 m	19.9 m	1600 a	6.15 h
Principal γ-photon energy (keV)	1460.822	238.632	351.932	609.320	186.211	911.204
γ-photon yield (%)	10.66	43.6	35.60	45.49	3.59	25.8
Decay chain member	—	^{232}Th	^{238}U	^{238}U	^{238}U	^{232}Th

Table 4. Results of the γ-spectrometric measurement of commercially available pyrotechnics. Specific activities are given in mBq g^{-1}, except for ^{40}K (Bq g^{-1}). Errors are due to counting statistics and the efficiency curve error and are given in % relative. 'n.d.' stands for 'not determined'.

	^{40}K		^{212}Pb		^{214}Bi		^{214}Pb		^{226}Ra		^{228}Ac [a]	
Sample (colour)	(Bq g^{-1})	Error	(mBq g^{-1})	Error	(mBq g^{-1})	Error	(mBq g^{-1})	Error	(mBq g^{-1})	Error	(mBq g^{-1})	Error
R1 (green)	11.6	1.6	35	9.2	77	13	79	2.1	110	7.0	29	21
R2 (green)	11.8	1.7	32	4.3	48	3.4	54	8.5	87	43	32	9.2
R3 (green)	17.5	1.7	33	12	48	5.1	41	5.4	16	30	37	14
R4 (red)	12.4	1.7	n.d.		46	3.8	48	3.7	97	12	51	7.0
R5 (red)	11.8	1.7	48	3.5	48	11	41	13	92	63	43	8.1
R6 (red)	17.5	1.8	n.d.		36	17	40	5.2	<50		38	13
R7 (pink)	15.8	1.7	48	18	39	5.5	45	5.1	120	47	41	40
R8 (yellow)	12.9	1.8	45	15	54	12	55	13	120	21	37	38
R9 (white)	11.3	1.8	32	4.6	49	3.6	50	3.5	73	14	20	16
R10 (multi-coloured)	15.9	1.7	11	34	28	20	22	18	<40		12	74
R11 (multi-coloured)	14.0	1.7	150	2.1	110	2.5	120	2.5	260	6.7	120	4.0
R12 (multi-coloured)	11.4	1.7	18	13	67	4.4	66	12	120	15	<25	
R13 (multi-coloured)	19.3	1.7	25	23	26	4.8	21	5.4	42	69	18	16
R14 (multi-coloured)	9.23	1.7	3.9	46	11	5.4	9	23	<20		<10	

[a] In such environmental samples, ^{228}Ac is in secular radioactive equilibrium with its longer-lived mother nuclide ^{228}Ra (a poor γ emitter). The ^{228}Ac activities thus correspond directly to the ^{228}Ra activities.

in the mixtures can easily be explained by the application of oxidizing potassium salts (nitrate, perchlorate) in pyrotechnics. Black powder, for example, consists of some 75% of potassium nitrate; the increased ^{40}K activity, therefore, did not come as a surprise.

Much more noteworthy is the presence of ^{226}Ra (and/or its daughter nuclides ^{214}Pb and ^{214}Bi, respectively) in almost all pyrotechnics investigated. According to the hypothesis of this study, ^{226}Ra in the samples must be the result of the utilization of slightly active radiobarite ores for the production of the pyrotechnic raw material barium nitrate. As stated in the introduction, the use of barium salts in pyrotechnics is not restricted to green luminescent formulations, as it may be applied as an oxidizer with 'neutral colour', if no chlorine donor is added to the mixture. Accordingly, this explains one of the highest specific radium activities (120 mBq g^{-1}) in sample R8—a rocket with a yellow effect.

The highest specific ^{226}Ra activity was 260 mBq g^{-1} (sample R11), whereas only in three samples was the detection limit not exceeded. The average value of all 14 samples was 81 mBq g^{-1}. Pyrotechnics thus exhibit a specific radium activity that is approximately one order of magnitude lower than the lowest active radiobarite sample investigated by Zielinski *et al* [17]. The specific radium activities of our samples, however, correspond to those found in the radiobarites of the Ohře Rift (Bohemian Massif). Some pyrotechnic samples exceeded the lowest active samples from the Ohře Rift (0.02 to 7.80 Bq g^{-1} radium) [18].

We could also find similar levels of ^{226}Ra activity in red luminescent pyrotechnics, where we actually expected much lower activities due to the less obvious chemical similarities of strontium (red colourant) and radium. However, it seems that both alkaline earth ore deposits, strontium and barium, respectively, similarly accumulate radium from the geological environment.

Comparing the ^{214}Pb activities to the respective ^{214}Bi activities shows very good agreement. This observation comes as expected, because the short-lived nuclide ^{214}Bi must be in radioactive equilibrium with its mother ^{214}Pb. Since the principal γ photons of both nuclides have their energy in different regions of the spectrum (352 keV for ^{214}Pb and 609 kev for ^{214}Bi), this observation leads to the conclusion that our simplified approach of 'comparable bulk densities' of samples and QCY48 standard solution is a valid approach for these measurements. If the γ-photon self-absorption properties of sample and standard were significantly different, this would have led to a deviation in the evaluation of γ photons with different energies (and thus different detector efficiencies). Some occasionally higher deviations are due to higher counting errors.

The activities of ^{214}Pb and ^{214}Bi were in the same range (at least in the same order of magnitude) as the ^{226}Ra activities. Remarkably, the ^{226}Ra activities were a little higher than the activities of the daughter nuclides in most cases. This must be, indeed, due to the leaking from the PE sample container.

In contrast to uranium, ^{232}Th and its progeny appeared to be common impurities in pyrotechnics. Since the members of the ^{232}Th decay chain are generally characterized by much shorter half-lives, the thorium decay chain reaches its radioactive equilibrium within only a few decades [23]. The

presence of ^{228}Ra (and its short-lived daughter nuclide ^{228}Ac) in pyrotechnics, therefore, can hardly be the result of a natural, selective incorporation of ^{228}Ra into the barium or strontium ore. Radium-228 ($T_{1/2}$ = 5.75 a), without a supply from the mother nuclide ^{232}Th, would decay completely after a few decades.

The specific activities of ^{228}Ac and ^{212}Pb correlate to a high degree, although, as with the ^{238}U chain, a gaseous decay product lies in between, namely ^{220}Rn. In this specific case, however, we believe that the much shorter half-life of the noble gas ^{220}Rn ($T_{1/2}$ = 55.6 s) does not allow a significant diffusion through the PE container. Consequently, ^{220}Rn and all its decay products are trapped in the container and measured without any losses.

According to the Swiss Administration [24], 1700 tons of pyrotechnics are annually consumed in Switzerland. We can safely assume that these numbers compare to Austria as well. Assuming an average radium content in pyrotechnics of 81 mBq ^{226}Ra g^{-1} and 14 Bq ^{40}K g^{-1}, these numbers will correspond to an annual emission of 138 MBq ^{226}Ra and 23.8 GBq ^{40}K year^{-1} from pyrotechnics only (in countries like Switzerland or Austria).

4. Conclusions

Pyrotechnics do contain radium—not extreme activities but certainly enough to be detected in our new low-level γ spectrometer. The ^{226}Ra activities are in the range of 16–260 mBq g^{-1} and must be due to the selective incorporation of radium into the barium and strontium ore body, as no traces of the mother nuclide (^{238}U and its early daughters) could be found.

In contrast to this, ^{232}Th and its decay chain members (like ^{228}Ra) probably is present in the form of thorium-containing minerals, which are an impurity in the raw materials of pyrotechnics. Due to its much shorter half-life, ^{228}Ra is unlikely to accumulate significantly in the ore body of an alkaline earth metal deposit. This hypothesis is further supported by the very weak or non-existing correlation of the activity concentrations of the two radium isotopes.

Although the radium activities were relatively low, we believe that this potential hazard should be considered as well in the development of environmentally friendly pyrotechnics [5]. The incidental utilization of highly active radiobarite ores as pyrotechnic raw materials would probably exhibit a greater health hazard than the toxicity of the poisonous pyrotechnic constituents (heavy metals, perchlorate) [5]. In particular, when applied as indoor pyrotechnics, the health aspects of pyrotechnic devices should be taken seriously with respect to air pollution by toxic and radioactive substances. This finding justifies the efforts that are currently being undertaken in the search for barium-free pyrotechnics [25]. One possible method to remove radium from the radiobarite ore, however, could be to set it under reducing conditions which causes the complete mobilization of radium from the barite [26].

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Features

Fallout Over Disneyland

BY *AMY DAVIS & GAR SMITH*

Disneyland has been shooting pyrotechnic chemicals into the evening sky since shortly after the amusement park opened in 1955. In the beginning, the fireworks shows were confined to weekends and school vacations. In 2000, the park added its third fireworks show. With the debut of Disneyland's New California Adventures, the blazing tracers and wall-shaking blasts of exploding skyrockets have become nearly nightly occurrences.

Many long-time park neighbors claim that the trajectories of the skyrockets used to be very high but that, in recent years, the rockets have been bursting so low that smoke and cinders become trapped beneath the local inversion layer. Park officials insist that "nothing has changed."

On summer weekends, Disneyland schedules three fireworks shows a night. Park officials recently asked the City of Anaheim for permission to add another 60 nights of pyrotechnics per year. Disneyland officials claim that the park's fire-in-the-sky shows last about five minutes. Neighbors, however, have clocked shows lasting from ten to 15 minutes during the summer tourist season.

In the battle over tourist dollars, pyrotechnics are being dispersed with greater frequency. Southern California tourist attractions like Edison Field, Knott's Berry Farm and the Santa Monica Pier all have adopted pyrotechnics displays to increase attendance. San Diego's Sea World plans to set off fireworks shows 150 nights a year.

University of Utah Meteorology Professor Kevin D. Perry tagged the chemicals used in pyrotechnics and showed that, in mild weather, the heavy metals traveled 100 km (62 miles) downwind over a two-day period. Among the pollutants traced were: strontium, vanadium, potassium, titanium, barium, copper, lead, magnesium, aluminum and zinc. These releases increase toxic levels in the air mix of the Los Angeles, Orange County, Riverside and San Diego basins. The environmental impacts are not confined to the air since these heavy metals also fall into local watersheds.

A study in the June 28, 2001 issue of Nature explained how the superheated sulfuric gasses released into the sky during pyrotechnic reactions can create ozone.

The Southern California Air Quality Management District (SCAQMD) has studied how auto exhaust combines with moist marine air to form airborne irritants. But fireworks, which release significant amounts of sulfur and soot, have not been studied by the SCAQMD staff.

On February 19, in response to persistant citizen complaints, SCAQMD issued a statement declaring that the park's three fireworks shows "did not exceed any acute or chronic Reference Exposure Level established by the state."
In any event, the SCAQMD pointed out that a 1992 ruling "specifically exempts 'pyrotechnic equipment, special effects or fireworks" from air quality permit regulation.

If approved by the California Coastal Commission, SeaWorld would send even more fireworks into the sky, peppering coastal waters with chemical fallout. SeaWorld's existing displays are already plaguing the theme park's neighbors. As resident Dino Russo complained to the *Los Angeles Times*, "Nobody wants to do a chemical analysis of this."

It's a Smelly World, After All
The City of Anaheim has made no attempt to reduce or regulate the fireworks fallout, despite the letters and calls from Disneyland's downwind neighbors who have complained about the noise, smell and debris from the park. The city's lack of action may be explained by the fact that Disneyland provides a significant source of municipal tax revenue. Anaheim pulls in revenue from a sales tax on every item sold in the park.

Anaheim officials respond by noting that Disneyland has broken no laws. The city can say this because there are no laws restricting pyrotechnic pollution. Anaheim defines excessive noise as being a "continuous sound" lasting ten minutes or longer at 60 decibels or higher. Even though the Disneyland displays can last as long as 15 minutes, explosive bursts from fireworks only last a second, so they are not covered by the law.

The National Institutes of Health warns that firecrackers (which can produce 145 decibels) can cause hearing loss at a single exposure. Disneyland, however, is not cited for explosions that are much louder than firecrackers. This noise does not stop at park boundaries.

The removal of sound-muffling urban "softscape" (trees, lawns, bushes) and the increase in "hardscape" (buildings, roads, parking lots) has magnified the "sound bounce" of the exploding rockets throughout the Central Orange County region. Disneyland has planted new palm trees and decorative plants, but these additions have failed to match the sound-softening effect of the original uprooted foliage.

While airports such as Los Angeles International recognize their responsibility to compensate neighborhoods impacted by aircraft noise, for-profit entertainment parks are not held to the same level of accountability.

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In September 2001, Compaq Computer Corp. sponsored the mounting of a spectacular new pyrotechnic show at Disneyland, "Believe... There's Magic in The Stars." A Compaq press release hailed the show as "the largest fireworks display" in the park's history.

While the new show may draw "Oohs" and "Aahs" from park visitors, it elicits "Ughs" and "Yucks" from the park's besieged neighbors. On show nights, it is impossible to see the stars - let alone the "magic" - as clouds of acrid smoke move through the neighborhood, so thick the smoke obscures street lights.

In response to citizen's complaints, Anaheim Fire Department Chief Jeff Bowman replies that his department "does not have the resources, the knowledge or the wherewithal to review the fallout."

Disney, which does have the wherewithal, prepared its own study. It concluded that the fallout would cause a cancer risk to fewer than two people out of a million.

An investigation by the *Daily Titan*, the California State University at Fullerton student newspaper, painted a grim picture of the downwinders' life. "Every night for 200 straight days..., the stench of eye-watering, sulfur-laden black smoke has made living downwind of Disneyland unbearable" for the families whose low-income neighborhood adjoins "The Happiest Place on Earth."

When night falls, families are forced to "shelter in place" as the clear summer night air is replaced with "a dark, smoggy haze often 10-stories-high and half-a-mile wide."

"My wife has asthma, and I have two kids that have asthma," resident Alejandro Robles, told the *Titan*. "When the fireworks go off, it really affects them and they have to go inside the house."

"When the fireworks explode, the neighborhood turns into a wartime movie set," the *Titan* related. "People start to run for cover as ashes from the fireworks rain onto their cars and homes. Car alarms whistle and scream as dogs yelp and babies cry in the darkness."

Some residents have experienced disturbing "flash-backs" to their days as combat soldiers in the Vietnam War.

Disneyland refuses to release any information on the ingredients that go into its nightly fireworks shows. Pyro Spectaculars Inc., which makes Disneyland's fireworks, also refuses to divulge what goes into their products.

Officials at the state's Occupational Health and Safety Commission informed the *Titan's* reporters that "it is not [the agency's] responsibility to handle complaints or to divulge information to the general public." Some politicians, however, are starting to take note of residents' complaints. State Assemblyman Ken Maddox and US Congresswoman Loretta Sanchez have expressed interest in the neighbors' plight.

While Disneyland's managers may not be overly concerned about quality-of-life issues in the neighborhoods surrounding the park, they are very solicitous of the wellbeing of their paying customers. During the shows, large sections of the park are closed to the public because of the danger posed by falling debris. Afterwards, park employees quickly move in with special heavy-duty vacuums to remove all traces of the blackened, smelly debris before visitors are allowed back inside.

Mouse Catarrhs

The damage to the paint jobs on residents' cars is readily apparent but the damage to their health is not. The sulfur in the smoke can combine with moisture to create sulfuric acid, which destroys painted surfaces. The sulfuric acid - mixed with a witch's brew of heavy metals - eventually falls to the ground and seeps into the water table.

These heavy metals bio-accumulate, says California State Fullerton environmental chemist Harold Rogers. "If you are exposed to it over a long period of time... it gets into the body," Rogers told the *Titan*. "It could take years, even decades, before a body starts to show symptoms."

A realistic assessment of the damage to people living downwind would require hearing exams and costly blood tests, which the area's low-income residents cannot afford.

A neighborhood watchdog group called HOME (Home Owners Maintaining their Environment) has complained about the smoke, the ash and the damage to their homes and cars. HOME says these complaints have been ignored.

"SCAQMD officials maintain that they have not received sufficient complaints to conduct an investigation," the *Titan* was told. Anaheim Police Department spokesperson Rick Martinez informed the *Titan* that the police don't handle fireworks complaints. "We ask them to deal with Disneyland directly."

When *Titan* reporters went to Disneyland directly, they discovered that: "As is their standard practice, officials at the theme park would not comment on the possible health hazards of their fireworks."

City officials insist that tourism is a clean industry but they can only make such claims because the pollution these fireworks create has never been adequately measured.

On February 19, SCAQMD finally agreed to begin testing for "airborne particulate matter... at suitable locations in the neighborhood areas bordering the Disneyland Resort."

Amy Davis, a retired teacher, has spent more than $18,000 on insulation and double-paned windows to protect her home from the reverberations of the Magic Kingdom. She has campaigned for regulation of the park's fireworks displays and strict enforcement of the city's anti-noise requirements. Davis, who also is an artist, was surprised to learn that fireworks contain some of the same heavy metals that are used in watercolors. These pigments, Davis notes, "came under regulation in the 1970s." While her watercolors "are contained on paper, behind glass," fireworks allow the same pigments to be "thrown into the winds."

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Item No. 6
Supporting Document No. 5

Water Air Soil Pollut
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The Fallout from Fireworks: Perchlorate in Total Deposition

Jennie Munster · Gilbert N. Hanson · W. Andrew Jackson · Srinath Rajagopalan

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Abstract Recent studies have shown that natural perchlorate may be an important component to the general population exposure. These studies indicate that natural perchlorate is likely deposited by atmospheric deposition. Perchlorate concentration of total (dry + wet) deposition is relatively unstudied yet these measurements will aid in understanding natural levels in the environment. We sampled total deposition monthly at six sites in Suffolk County, Long Island, NY from November 30, 2005 until July 5, 2007. The mean perchlorate concentration is 0.21±0.04 (standard error) μg L^{-1} with a maximum value of 2.78 μg L^{-1}. Here we show up to an 18-fold increase above the mean concentration in July 2006 and July 2007 samples. It appears that this increase in perchlorate in total deposition is associated with Fourth of July fireworks.

Keywords Fireworks · Groundwater · New York · Perchlorate · Precipitation

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1 Introduction

While perchlorate is known to inhibit iodide uptake of the thyroid gland, whether low microgram levels of perchlorate in drinking water are a health concern is still highly debated (Blount and Valentin-Blasini 2006). The US Environmental Protection Agency has yet to establish a national drinking water standard, while many states have set advisory levels. New York State has implemented advisory levels of 18 μg L^{-1} ClO_4 for the public notification level and 5 μg L^{-1} ClO_4 for the drinking water planning level in groundwater. Advisory levels are as low as 1 μg L^{-1} in Massachusetts, Maryland and New Mexico (EPA 2005). Establishing background concentration of perchlorate in precipitation and groundwater, and determining whether the perchlorate is natural or anthropogenic is a prerequisite for determining drinking water standards.

Since the presence of perchlorate in precipitation has only recently been measured (Dasgupta et al. 2005; Barron et al. 2006), the sources of perchlorate in precipitation are not well known. A major source could be the formation of perchlorate in the atmosphere from chlorine species (Dasgupta et al. 2005). Perchlorate in the atmosphere may also be from sea spray since perchlorate is present in seawater (Martinelango et al. 2006). Perchlorate is present in surface soils of the southwest (Rao et al. 2007), thus it is conceivable that perchlorate in dust is picked up by wind, transported and deposited as dry deposition. An anthropogenic source of perchlorate in the atmosphere may be

fireworks. Atmospheric fallout from fireworks consists of fine particles of burnt black powder, paper debris and residue. Perchlorate in paper debris ranges from 302 to 34,200 μg kg^{-1} (DEP 2006). Two studies (Backus et al. 2005; Wilkin et al. 2007) show direct perchlorate contamination of lake water from fireworks displays.

The Massachusetts Dept. of Environmental Protection has determined that historic fireworks displays are the likely source of perchlorate contamination in two of the nine public water supply systems showing levels above 1 μg L^{-1} (Mass. DEP 2006). Although little information is available on the perchlorate content in fireworks their model predicts that groundwater should be contaminated to the tens of μg ClO_4 L^{-1} within 100 meters of the fireworks display. This assumes 1,000–2,000 aerial shells weighing a total of 1,361 kg, of which 40% is ClO_4 and the contaminated area (fireworks fallout area) is equal to 3,600 m^2.

To establish a perchlorate contribution from the atmosphere we collected monthly samples of total deposition at six sites in Suffolk County, Long Island, NY from November 2005 to July 2007 (Fig. 1). We analyzed samples for ClO_4 and also NO_3, NH_4, Cl, Br, I, SO_4, Na, Mg, K, Ca, Sr and B.

2 Methods

One hundred and eight total (wet plus dry) deposition samples were collected monthly for 20 months between November 30, 2005 and July 5, 2007 at six sites in Suffolk County, NY. Suffolk County is the eastern most county on Long Island, which extends east from Queens and Brooklyn. All sample sites are in or near urban areas (Fig. 1).

Samples were collected using All-Weather Precipitation Gauges purchased from Fisher Scientific. These gauges sample both wet and dry (total) deposition since they are not covered during dry periods. The sampling area of the gauge is 10 cm in diameter. The inner sampling device, used to determine rainfall, is 26 cm in height and 3.2 cm in diameter. Evaporation from these samplers is minimal due to the small opening at the top of the gauge. For example, annual rainfall totals for 2006 at our sites ranged from 110 to 130 cm which are only slightly less than the 137.4 cm value for 2006 reported by The National Weather Service for Islip, NY which is in the center of Long Island (http://www.weather.gov/climate). The variation between our sites and Islip, NY could be due to spatial differences as wet

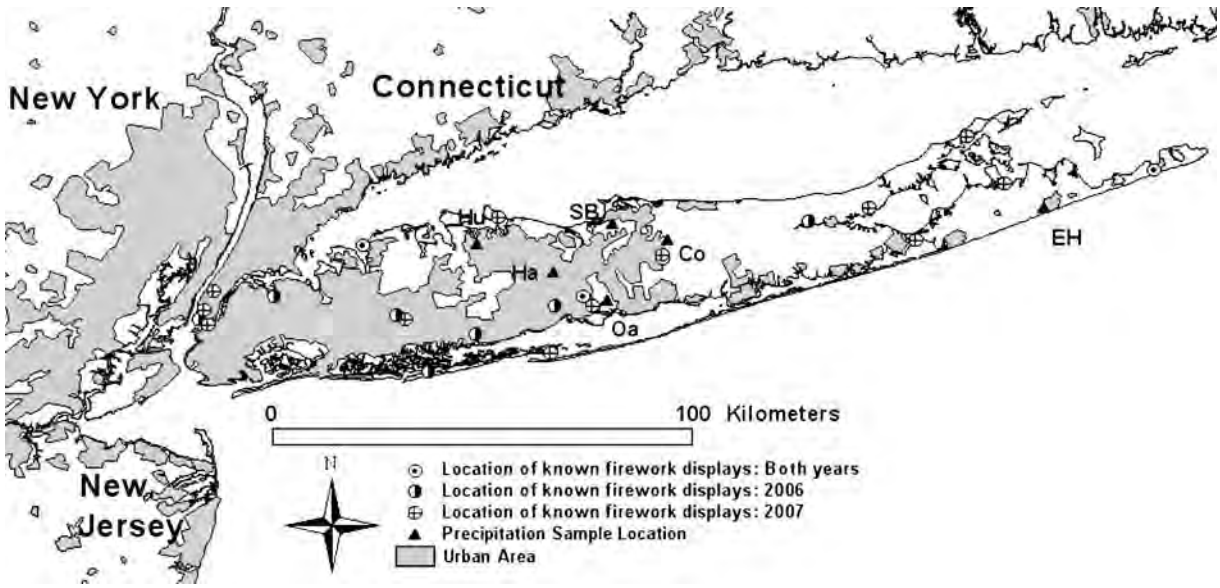

Fig. 1 Location of sample gauges in Suffolk County, Long Island, NY. Site names are abbreviated; *Hu* Huntington, *Ha* Hauppauge, *SB* Stony Brook, *Co* Coram, *Oa* Oakdale, and *EH* East Hampton. *Gray areas* are urban as mapped by the US Geological Survey according to the Digital Chart of the World, revised version of 1998 data. In general, urban areas are a concentration of at least 5,000 persons in continuous collection of houses where the community sense is well developed and the community maintains public utilities, such as, roads, street lighting, water supply, sanitary arrangements etc. Note that two firework display locations overlap near the Coram site. The covered symbol had firework displays both years

precipitation can vary as much as 20 cm (8 in.) across Long Island (Busciolano 2004).

Samples were filtered in the field using a 0.2-μm surfactant-free cellulose acetate (SFCA) filter for perchlorate analysis and 0.45 μm glass fiber filters for all other analysis. Samples were stored in sample rinsed, polypropylene vials untreated for all samples except nitrogen. Vials for nitrogen were acid rinsed with a 10% HCl solution before sample collection. Samples were stored in a cooler while in the field and then at 4°C until analyzed. Samples for nitrogen, once in the laboratory, were frozen until analyzed.

Perchlorate was analyzed using a sequential ion chromatography-mass spectroscopy/mass spectroscopy (IC-MS/MS) technique (Koester et al. 2000) with a method detection limit of 0.005 μg L^{-1}. To account for matrix effects, all samples were spiked with an oxygen-isotope (^{18}O) labeled ClO_4 internal standard. Each sample was measured in duplicate or triplicate and the precision was on average ±5%. B, Br, I, Mg, Na, Ca, K, Sr, Cl, N–NO_3, NH_4 and SO_4, were also analyzed using standard methods.

We used the program Minitab to perform One-way analysis of variance (ANOVA, unstacked) Turkey tests, with a 95% confidence interval. A one-way analysis of variance is a way to test the equality of three or more means at one time by using variances.

The HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory) model was used to model simple air parcel trajectories from known firework displays for 24 h, in 1 h spacing, from July 4, 2006 and July 4, 2007 at 50 m height (Draxler and Rolph 2003).

3 Results

The mean monthly perchlorate concentration of total deposition samples is 0.21±0.04 (standard error) μg L^{-1}. The maximum monthly value is 2.78 μg L^{-1}. The mean value is similar to that reported from Lubbock, TX, 0.20 μg L^{-1} (Dasgupta et al. 2005), while the maximum is similar to the highest value reported in Ireland, 2.82 μg L^{-1} (Barron et al. 2006). What is striking about our data set is the large peak in perchlorate concentrations in the July samples for both 2006 and 2007 collected after the Fourth of July (Fig. 2). Many communities in and around the Metropolitan New York area, which includes Long Island, have large firework celebrations on the evening

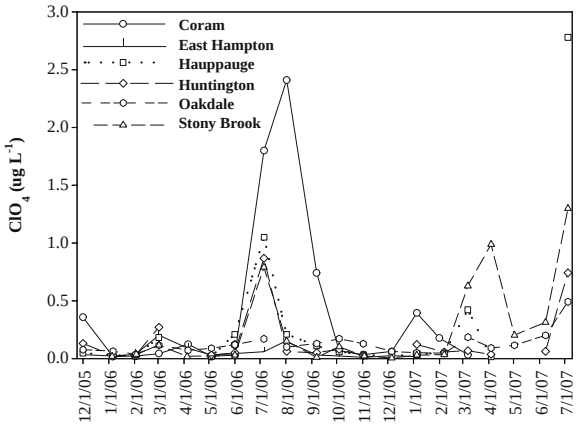

Fig. 2 Monthly perchlorate concentrations for total deposition samples. Collection at Coram was discontinued after March 2007 and discontinued at East Hampton after January 2007

of, and leading up to the Fourth of July. Although fireworks are illegal in New York State, residents also set off fireworks in their neighborhoods. We have located (Fig. 1) known displays during the Fourth of July celebrations reported in Newsday (July 2, 2006 and July 4, 2007), using oral communication with local town clerks, from information on a local fireworks company's website (http://www.grucci.com) and other sources (http://hamptons.plumtv.com; http://www.sagharboryc.com). We have not located all the firework displays, but we believe that we have located the larger ones. Modeled air trajectories, using HYSPLIT, in western Suffolk County, NY, and Atlantic City, NJ, travel in a north to northeast pattern that pass over the rain gauges in Suffolk County. Modeled air trajectories in New York City travel in a similar pattern but do not pass over Suffolk County.

Excluding the samples from July the mean concentration of perchlorate in precipitation is 0.12±0.03 (standard error) μg L^{-1}. Perchlorate concentrations are significantly higher in July compared to all months except August ($p<0.05$). Mean values vary between the six sites, although there was no statistical difference ($p<0.05$). Coram has the highest mean value of 0.40± 0.70 (standard deviation) μg L^{-1}. East Hampton has the lowest mean of 0.06±0.06 μg L^{-1}. Hauppauge has a mean value of 0.27±0.14 μg L^{-1}, Huntington a value of 0.14±0.06 μg L^{-1}, and Stony Brook a mean value of 0.25±0.09 μg L^{-1}. There was no significant correlation (defined as $R^2>0.5$) between ClO_4 and the other ion analyzed.

4 Discussion

In our study area, wet deposition occurred between the Fourth of July and the time of sample collection for both years of this study (http://www.weather.gov/climate). These three storms originated inland and progressed in a west to east direction, moving slightly north during the 2006 events, as noted on NOAA archived radar images (http://www4.ncdc.noaa.gov). The timing of wet deposition combined with modeled air trajectories indicates a high probability that firework fallout is the cause of increased perchlorate concentration in the July samples. The effects of atmospheric pollution from fireworks have been reported by other studies noting increases in SO_2, NO_2, suspended particles and metallic elements (Moreno et al. 2007; Ravindra et al. 2003). Precipitation scavenging can effectively remove pollutants from the atmosphere, with wet deposition being more effective than dry deposition (Loosmore and Cederwall 2004).

Two studies (Backus et al. 2005; Wilkin et al. 2007) which show direct contamination of lake water from firework displays measured perchlorate concentration adjacent to the displays. Our rain gauges are, at the closest, a few km from known displays (Fig. 1). Thus wind properties and storm direction play a role in where the firework fallout eventually settles. Our rain gauges are mostly in areas zoned for business, except for Stony Brook which is on a university campus and Coram, which is in a residential neighborhood. Coram, coincidentally, had the highest concentration in July 2006. Sampling at that site was discontinued after March 2007. Coram is also very near known public firework displays (approximately 1.5 km). Oakdale, which is also near known firework displays, has relatively low concentrations with a value of 0.17 μg L^{-1} on July 6, 2006 and 0.49 μg L^{-1} on July 5, 2007. It is likely that the wind and storm direction did not carry fireworks contamination towards the Oakdale study site in 2006 but that some contamination was received in 2007. Hauppauge measured 2.78 μg L^{-1} on July 5, 2007. There are no known fireworks displays near Hauppauge, yet fireworks fallout from the south is likely influencing Hauppauge rain water. Additionally, there may have been fireworks near Hauppauge that we are unaware of. It is likely that the perchlorate from fireworks in our precipitation samples have traveled some distance in the atmosphere and perchlorate concentrations of precipitation adjacent to large fireworks displays may be much higher than we report.

Our study showed that precipitation concentrations after Fourth of July fireworks displays can be 18 times as much as background levels confirming that, "fireworks constitute a potential source of increasing importance, as fireworks use is rising exponentially with average consumption at 4.5×10^7 kg per year" (Dasgupta et al. 2006). As a result we need to be concerned about the potential impact on our groundwater of increased perchlorate in precipitation associated with fireworks.

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ENVIRONMENTAL Fact Sheet

NEW HAMPSHIRE DEPARTMENT OF Environmental Services

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WD-BB-60 2008

Fireworks and New Hampshire's Lakes

Concerns of Health and Environmental Effects

There are growing concerns about the use of fireworks around New Hampshire's lakes. As fun and enjoyable as fireworks can be, they may be causing more damage then you know. Aside from the obvious danger of operating controlled explosives, what you may not realize is the effects fire works have environmentally, economically and health wise.

Firework Ingredients and their Dangers

Fireworks are composed of many different elements, each contributing to the noise, color or propellant. While these ingredients combine to form a beautiful spectacle, many of them are very dangerous. Here's a list of a few common firework ingredients, their use, and what makes them so dangerous.

Toxic Element	Fireworks Usage	Toxic Effect of Fallout Dust & Fumes
Lead Nitrate/Dioxide/Chloride	oxidizer	Bioaccumulation; developmental danger for children and the unborn; may remain airborne for days; poisonous to plants and animals
Barium	glittering greens	Extremely poisonous, radioactive
Lithium	blazing reds	Slightly toxic
Rubidium	purple colors	Slightly radioactive; can replace calcium in body
Strontium	blazing reds	Can replace calcium in body; can be radioactive
Copper compounds	blues	Dioxin pollution
Aluminum	brilliant whites	Contact dermatitis
Ammonium Perchlorate	propellant	Can contaminate ground and surface waters; can disrupt thyroid functions
Cadmium	firework colors	Extremely toxic, carcinogenic; can bioaccumulate
Potassium Nitrate	in black powder	Toxic dusts, carcinogenic sulfur-coal compounds
Sulfur Dioxide	gaseous byproduct of sulfur combustion	Acid rain from sulphuric acid affects water sources, vegetation and causes property damage

The Effects Fireworks have on You and Nature

The fallout of these different chemicals can affect you both directly and indirectly. Once a firework explodes in the sky, it does many things. The gases from the rocket and the explosion are released into the atmosphere, where they are inhaled by humans and animals, and hurt the ozone layer. In addition to the gases, the debris and burning metals fall back to earth where they litter the area, contaminate aquatic ecosystems, and poison the wildlife, eventually working their way up the food chain.

How Phosphorus in Fireworks Impacts the Water

It has taken years to determine the dangers associated with the many ingredients in fireworks. Up until very recently, phosphorous (also found in fertilizers) was highly popular in fireworks until the realization of its associated problems to the environment. Although most manufacturers no longer incorporate more than trace amounts of phosphorus in fireworks, every little bit added to a lake can influence water quality. Phosphorus accelerates a process called eutrophication, which is the process that results in increased biomass, decreased lake clarity, decreased bottom oxygen, and increases the likelihood of cyanobacteria scums. Algal and cyanobacteria blooms caused by phosphorus introductions impact fisheries, drinking water supplies and impact the health of people who recreate in the waters as well as pets and any animal that drinks these waters.

The Final Impact

Altogether the damaging effect fireworks have is overwhelming. They impact water quality by affecting the odor and taste of drinking water. On the economic side, excessive algal and cyanobacteria growth due to phosphorus or contamination due to firework fallout increases water treatment costs, degrades fishing and boating activities, and impacts tourism and property values. The cost of damage done to property, the litter and the effect upon both wildlife and human life is incalculable. The Department of Environmental Services urges you to consider the effects of fireworks and perhaps find an alternative to a problem that is only growing with time.

For more information, please go to these links:

www.geocities.com/Yosemite/Falls/9200/toxic_fireworks.html
www.serconline.org/phosphorus/background.html

Item No. 6
Supporting Document No. 5

FOR IMMEDIATE RELEASE | June 22, 2009

"Green" fireworks may brighten eco-friendly Fourth of July displays in future

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WASHINGTON, D.C., June 23, 2009 — With millions of people in the United States eagerly awaiting those July 4 fireworks displays — and our Canadian neighbors doing likewise for their July 1 Canada Day celebrations — here's a prospect for those light shows of the future likely to ignite a smile on Mother Nature's face: A new generation of "green" fireworks is quietly making its way toward the sky.

That's "green" as in environmentally friendly.

Fireworks, flares and other so-called "pyrotechnics" traditionally have included potassium perchlorate as the oxidizer, a material that provides the oxygen that fireworks need to burn. Perchlorate, however, is an environmental pollutant with potential adverse effects on people and wildlife. Pyrotechnics contain other ingredients, such color-producing heavy metals, with a similar potential.

Studies have shown that perchlorate from community fireworks displays conducted over lakes, for instance, can lead to perchlorate contamination of the water. For full details about how perchlorate contaminates lakes after fireworks displays, see **a study** published in the American Chemical Society's peer-reviewed journal, *Environmental Science & Technology*.

A spectacular fireworks display on July 4, 2008, over New York City's East Village.
Credit: Wikipedia Commons, David Shankbone
High-resolution version

Researchers, however, have developed new pyrotechnic formulas that replace perchlorate with nitrogen-rich materials or nitrocellulose that burn cleaner and produce less smoke, according to an article in ACS's weekly newsmagazine, *Chemical & Engineering News* (*C&EN*). To read it, click on **fireworks**.

In the article, *C&EN* Associate Editor Bethany Halford says these nitrogen-rich formulas also use fewer color-producing chemicals, dramatically cutting down on the amount of heavy metals used and lowering their potentially toxic effects.

Some of these fireworks have already been used at circuses, rock concerts and other events, but none have been used at large outdoor displays. The problem: cost. The big challenge in launching these "eco-friendly" pyrotechnics into the sky is making them cost-competitive with conventional fireworks while maintaining their dazzle and glow, the article explains.

The article notes that fireworks manufacturers have little incentive to further develop the new green fireworks because no federal regulations currently limit releases of perchlorate from pyrotechnics.

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— Michael Bernstein

The American Chemical Society is a nonprofit organization chartered by the U.S. Congress. With more than 154,000 members, ACS is the world's largest scientific society and a global leader in providing access to chemistry-related research through its multiple databases, peer-reviewed journals and scientific conferences. Its main offices are in Washington, D.C., and Columbus, Ohio.

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CERTIFIED FOR PUBLICATION

IN THE COURT OF APPEAL OF THE STATE OF CALIFORNIA

FIRST APPELLATE DISTRICT

DIVISION THREE

GUALALA FESTIVALS COMMITTEE, Plaintiff and Appellant, v. CALIFORNIA COASTAL COMMISSION et al., Defendants and Respondents.	A125614 (Mendocino County Super. Ct. No. SCUKCVPO08-51671)

Plaintiff Gualala Festivals Committee (the Festivals Committee) appeals from a judgment denying its petition for a writ of mandate seeking to set aside a cease-and-desist order entered by the California Coastal Commission (the Commission). The Commission's order prohibits the Festivals Committee from discharging fireworks over the Gualala River estuary without first obtaining a coastal development permit. The Festivals Committee contends the trial court erred in upholding the Commission's determination that a permit is required because the fireworks display is a development within the meaning of the California Coastal Act of 1976 (Pub. Res. Code,[1] § 30000 et seq.) (the Act). Although such a display may not be a "development" in the ordinary sense of the word, the Commission's interpretation conforms both with the expansive statutory definition of the term and the purpose of the statute. Hence we shall affirm the trial court's judgment upholding the Commission's action.

Factual and Procedural History

The Festivals Committee is an association of business and property owners in Gualala that sponsors community events, including Gualala Patriot Days over the Fourth

[1] All statutory references are to the Public Resources Code unless otherwise noted.

of July weekend. In 2006, the Festivals Committee added a 15-minute fireworks display to the Patriot Days event. The fireworks were launched from private property situated near the Gualala River estuary and Gualala Point Island. Following the 2006 display, the Commission received telephone complaints that the fireworks had disturbed seabirds nesting on Gualala Point Island.

On June 13, 2007, the Commission wrote a letter to the Festivals Committee stating, "It has come to the attention of Coastal Commission staff that the Gualala Festivals Committee . . . is planning a fireworks display scheduled to take place on July 6, 2007 at 9:15 p.m. We understand that the proposed fireworks would be launched from within, or partially within, the public access easement held by the Redwood Coast Land Conservancy . . . and would detonate over the Gualala River estuary. [¶] Commission staff believes that (1) launching fireworks from within the public access easement is inconsistent with the terms and conditions of the easement, (2) the proposed fireworks display above the Gualala River estuary is a form of 'development' as defined by the Coastal Act section 30106 and requires a coastal development permit, and (3) the proposed fireworks display does not qualify as a temporary event exempt from permit requirements because of its potential for adverse impacts to coastal resources." The Commission elaborated on the environmental concerns posed by the fireworks display. "The site of the proposed fireworks display is located approximately one mile from Gualala Point Island which provides nesting and roosting habitat for a variety of seabirds. We understand that a similar fireworks display conducted in 2006 over the Gualala River estuary without the benefit of a coastal development permit resulted in documented disturbance of seabird roosts and rookeries, including observed nest abandonment by several bird species. The Gualala River estuary also provides harbor seal haul-out sites as well as habitat for other marine mammals. Therefore, because the proposed fireworks display would be located in close proximity to known environmentally sensitive habitat areas . . . and has potential for significant adverse impacts . . . , the Executive Director has determined that the proposed temporary event is not excluded from [the coastal development permit] requirements."

In response, the Festivals Committee assured the Commission that the fireworks display would not be launched from the public easement, and public access to the easement would not be blocked during the fireworks display. The Festivals Committee disputed the Commission's claim that its firework display is a "development" within the meaning of the Act and questioned whether there was any evidence of the alleged disturbance of seabird roosts and rookeries. The Commission advised the Festivals Committee that in light of its assurance that the public easement would not be blocked during the display, the Commission would not issue a cease-and-desist order with respect to the 2007 fireworks display. The Commission explained, however, that it considered the fireworks display a development that required a permit and that if the Festivals Committee intended to conduct a similar display the following year a permit application should be filed no later than February 2008. The Commission warned that by not obtaining a permit, the Festivals Committee would be proceeding at its own risk should the fireworks display result in nest abandonment and mortality of seabirds. The Festivals Committee was advised that the federal Bureau of Land Management would be implementing a monitoring protocol to determine whether the 2007 display adversely impacts the nesting seabirds.

On February 12, 2008, the Bureau of Land Management and the federal Fish and Wildlife Service issued a report entitled "Seabird and Marine Mammal Monitoring and Response to a Fireworks Display at Gualala Point Island, Sonoma County, California, May to August 2007." The report documents "a visible response by nesting seabird on Gualala Point Island. Digiscoped and infra-red photography during the 6 July fireworks display showed that Brant's Cormorants quickly changed from resting to erect postures at the first fireworks, followed by birds moving about or departing from the island. . . . During the study period, 90 Brant's Cormorant nests were documented on Gualala Point Island. Of these, seven nests (35% of nest failures) were abandoned in the two days between 5 and 7 July, and another seven nests were abandoned between 7 and 12 July. Those losses contrast with the abandonment of only six nests (30% of nest failures) for

the 30-day period from 5 June to 5 July." The report concludes that the high rate of Brant's Cormorants nest abandonments "likely resulted from fireworks disturbance."

On May 28, 2008, the Commission was notified that the Festivals Committee was planning another fireworks display during the Fourth of July weekend. On April 1, the Commission notified the Festivals Committee of its intent to issue a cease–and-desist order prohibiting it from conducting any unpermitted development within its jurisdiction, including the proposed fireworks display. A hearing on the proposed cease-and-desist order was set before the Commission for June 11, 2008.

On May 29, 2008, the Festivals Committee initiated the present action against the Commission and Peter Douglas, in his capacity as the Executive Director of the Commission, by filing a complaint for declaratory and injunctive relief seeking to prohibit the Commission from issuing the proposed order. At the June 11 hearing, the Commission asserted jurisdiction over the fireworks display and issued a cease-and-desist order prohibiting the Festivals Committee "from undertaking or threatening to undertake development without the necessary coastal development permit, including but not limited to, conducting a fireworks display over the Gualala River estuary." Thereafter, the Festivals Committee filed an amended complaint for declaratory relief and petition for writ of administrative mandate challenging the Commission's jurisdiction over the fireworks display. On May 11, 2009, the trial court denied the Festivals Committee's writ petition and on June 1 entered judgment in favor of the Commission. The court held that the proposed fireworks display is a "development" within the meaning of the Act and that the Commission, therefore, had jurisdiction to require a permit and to issue the cease-and-desist order. The court also found that substantial evidence supports the Commission's findings that (1) the 2007 fireworks display resulted in the closure of a public access trail during the time that it was to remain open; (2) the 2007 fireworks display resulted in placement of debris on the areas of the public easement that remained after the conclusion of the display; and (3) the 2007 fireworks display had adverse impacts on nesting seabirds. The Festivals Committee filed a timely notice of appeal.

Discussion

Section 30600, subdivision (a) of the Act provides: "Except as provided in subdivision (e), and in addition to obtaining any other permit required by law from any local government or from any state, regional, or local agency, any person . . . wishing to perform or undertake any *development* in the coastal zone, . . . shall obtain a coastal development permit." (Italics added.) Section 30106 of the Act defines "development" as follows: "'Development' means, on land, in or under water, the placement or erection of any solid material or structure; discharge or disposal of any dredged material or of any gaseous, liquid, solid, or thermal waste; grading, removing, dredging, mining, or extraction of any materials; change in the density or intensity of use of land, including, but not limited to, subdivision pursuant to the Subdivision Map Act (commencing with Section 66410 of the Government Code), and any other division of land, including lot splits, except where the land division is brought about in connection with the purchase of such land by a public agency for public recreational use; change in the intensity of use of water, or of access thereto; construction, reconstruction, demolition, or alteration of the size of any structure, including any facility of any private, public, or municipal utility; and the removal or harvesting of major vegetation other than for agricultural purposes, kelp harvesting, and timber operations which are in accordance with a timber harvesting plan submitted pursuant to the provisions of the Z'berg-Nejedly Forest Practice Act of 1973 (commencing with Section 4511). [¶] As used in this section, 'structure' includes, but is not limited to, any building, road, pipe, flume, conduit, siphon, aqueduct, telephone line, and electrical power transmission and distribution line." The parties agree that the scope of the Commission's jurisdiction turns on the proper interpretation of "development" as defined in the Act.

"When jurisdiction involves the interpretation of a statute, the issue of whether an agency acted in excess of its jurisdiction is a question of law reviewed de novo on appeal. [Citations.] Moreover, courts do not defer to an agency's determination when deciding whether the agency's action lies within the scope of authority delegated to it by the Legislature." (*Burke v. California Coastal Com.* (2008) 168 Cal.App.4th 1098, 1106.)

Nonetheless, although final responsibility for interpreting the statute resides in the courts, the agency's interpretation of its governing statute is entitled to "great weight." (*La Fe, Inc. v. County of Los Angeles* (1999) 73 Cal.App.4th 231, 240; *Coronado Yacht Club v. California Coastal Com.* (1993) 13 Cal.App.4th 860, 868.)

"When we interpret the meaning of statutes, our fundamental task is to ascertain the aim and goal of the lawmakers so as to effectuate the purpose of the statute. We begin by examining the statutory language, giving the words their usual and ordinary meaning. If we find no ambiguity, we presume that the lawmakers meant what they said, and the plain meaning of the language governs. [Citation.] If, on the other hand, the statutory language is unclear or ambiguous and permits more than one reasonable interpretation, we may consider various extrinsic aids to help us ascertain the lawmakers' intent, including legislative history, public policy, settled rules of statutory construction, and an examination of the evils to be remedied and the legislative scheme encompassing the statute in question. [Citation.] In such circumstances, we must select the construction that comports most closely with the aim and goal of the Legislature to promote rather than defeat the statute['] s general purpose and avoid an interpretation that would lead to absurd and unintended consequences. [Citation.] [¶] When a provision of the Coastal Act is at issue, we are enjoined to construe it liberally to accomplish its purposes and objectives, giving the highest priority to environmental considerations." (*McAllister v. California Coastal Com*. (2008) 169 Cal.App.4th 912, 928.)

We do not question that a fireworks display is not what is commonly regarded as a development of real property. Nonetheless, the Act does not simply use the term "development," leaving the Commission and the courts to ascertain its meaning from common usage. Rather, the statute provides an expansive definition of the activities that constitute development for purposes of the Act. It is the language of that definition that must be applied and interpreted, giving the words "their usual and ordinary meaning." (Cf., e.g., *LT-WR, L.L.C. v. California Coastal Com.* (2007) 152 Cal.App.4th 770, 776, 804-805 [installation of gates with "no trespassing" signs is development]; *La Fe, Inc. v. County of Los Angeles, supra,* 73 Cal.App.4th 231, 239-240 [lot line adjustment is

development]; *Stanson v. San Diego Coast Regional Com.* (1980) 101 Cal.App.3d 38, 47-48 [remodel of existing structure is development]; *California Coastal Com. v. Quanta Investment Corp.* (1980) 113 Cal.App.3d 579, 605-609 [conversion of existing apartments into a stock cooperative is development]; *Monterey Sand Co. v. California Coastal Com.* (1987) 191 Cal.App.3d 169, 176 [offshore sand extraction is development].)

At the hearing before the Commission, staff reported the following information with respect to the debris that results from a fireworks display: "Aerial shells are launched from tubes (called mortars), using black powder charges, to altitudes of 200 to 1000 feet where they explode and ignite internal burst charges and incendiary chemicals. Most of the incendiary elements and shell casings burn up in the atmosphere; however, portions of the casings and some internal structure components and chemical residue fall back to the ground or water, depending on prevailing winds." The Redwood Coast Land Conservancy confirmed that following the 2007 display, its members removed fireworks debris from the Gualala Bluff Trail. This evidence supports the finding that the 2007 fireworks display resulted in placement of solid debris within the coastal zone. The report prepared by the Bureau of Land Management quoted above amply supports the finding that the 2007 fireworks display had adverse impacts on nesting seabirds.[2]

The trial court found that the proposed fireworks display is a development within the meaning of the statute because it would result in the discharge of solid and chemical waste within the coastal zone. We agree. Section 30106 includes within the definition of development, bringing within the Commission's jurisdiction, the "discharge . . . of *any* . . . gaseous . . . [or] solid . . . waste." The statute does not require that a minimum

[2] The trial court also found, based on statements made by representatives of the Redwood Coast Land Conservancy, that public access to the easement was limited during the 2007 show. The Festivals Committee argues that irrespective of past displays, the undisputed evidence establishes that all future fireworks displays would be organized so as not to interfere with public access to the easement. Because we conclude that the displays come within the Commission's jurisdiction on other grounds, we need not decide whether the potential restriction of public access to the easement provides an additional basis for deeming the displays to be a development because they result in a "change in the density or intensity of use of land."

amount of waste be discharged to qualify as a development. Thus, a fireworks display that produces both solid and gaseous waste, as the Festivals Committee acknowledges occurs from its display, is a development under the plain language of the Act.

The Festivals Committee argues that this "literal construction is fatally flawed" and that to avoid absurd results the term "development" must be construed to imply two limitations: "First, an activity must itself physically alter—or be a necessary precondition to an activity that physically alters—land or water within the coastal zone. Second, the physical alteration cannot merely be ephemeral; it must be long-lasting, if not permanent." The Festivals Committee cites no authority recognizing such conditions and section 30106 does not expressly or implicitly impose such limitations. To the contrary, the statutory scheme implies that permanent alteration to land or water is not a defining characteristic of development under the Act. Section 30610 provides in relevant part, "Notwithstanding any other provision of this division, no coastal development permit shall be required pursuant to this chapter for the following types of development . . . : [¶] . . . [¶] (i) [¶] (1) Any proposed development which the executive director finds to be a temporary event which does not have any significant adverse impact upon coastal resources within the meaning of guidelines adopted pursuant to this subdivision by the commission." Section 30624.7 authorizes the executive director to issue "waivers from coastal development permit requirements for any development that is de minimis" and defines "de minimis" as a development that "involves no potential for any adverse effect, either individually or cumulatively, on coastal resources." Thus, temporary or de minimis activity that does not adversely impact coastal resources is characterized in the statute as "development" but may be exempted from the permit requirement, These provisions necessarily imply that an activity need not be long-lasting to qualify as a development subject to the Commission's jurisdiction, although the Commission has the authority to exempt such development from the permit requirement. [3]

[3] The Festivals Committee offers the following hypothetical examples to demonstrate the "absurd results" that supposedly flow from a literal interpretation of the statute: "Even the mere act of breathing would be classified as a 'development' under the Commission's approach,

This interpretation is consistent with the purpose of the Act. The Act "was enacted by the Legislature as a comprehensive scheme to govern land use planning for the entire coastal zone of California. The Legislature found that 'the California coastal zone is a distinct and valuable natural resource of vital and enduring interest to all the people'; that 'the permanent protection of the state's natural and scenic resources is a paramount concern'; that 'it is necessary to protect the ecological balance of the coastal zone'[4] and that 'existing developed uses, and future developments that are carefully planned and developed consistent with the policies of this division, are essential to the economic and social well-being of the people of this state.' " (*Yost v. Thomas* (1984) 36 Cal.3d 561, 565; see § 30001.) One of the legislative goals of the Act is to "protect, maintain, and, where feasible, enhance and restore the overall quality of the coastal zone environment and its natural and manmade resources." (§ 30001.5, subd. (a).) Construing the Act to provide the Commission with both expansive jurisdiction to control even limited, temporary development and the authority to exempt from the permit process development that does not have "any *significant* adverse impact upon coastal resources" provides the Commission the necessary flexibility to manage the coastal zone environment so as to accomplish the statutory purposes. By recognizing the Commission's jurisdiction in this case, the Commission may protect not only natural and scenic coastal resources from litter and gaseous waste, but resident wildlife from adverse impacts. "The Act [] is to be

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because breathing literally involves the 'discharge . . . of . . . gaseous . . . waste' (carbon dioxide) into the air" and "everyday activities such as opening a beach umbrella or driving an automobile [could require] coastal permits." The exemption and waiver provisions, however, avoid the Festivals Committee's hypothetical absurdities. Presumably someone who breathes or opens an umbrella on the beach will not cause a "*significant* adverse impact upon coastal resources" and thus will not be subject to a permit requirement. The Commission has enacted regulations relating to de minimis developments (Cal. Code Regs., tit. 14, § 13238 et seq.) and in 1993 issued "Guidelines for the Exclusion of Temporary Events from Coastal Commission Permit Requirements."

[4] Section 30001, subdivision (c) reads in full: "That to promote the public safety, health, and welfare, and to protect public and private property, wildlife, marine fisheries, and other ocean resources, and the natural environment, it is necessary to protect the ecological balance of the coastal zone and prevent its deterioration and destruction."

liberally construed to accomplish its purposes and objectives." (*La Fe, Inc. v. County of Los Angeles, supra,* 73 Cal.App.4th at p. 235.) "Such a broad interpretation is consistent with the legislative policy of the Act found in section 30001.5 and the broad grant of power to the agency to adopt any regulations or take any action it deems reasonable and necessary to carry out its provisions. (§ 30333.)" (*Stanson v. San Diego Coast Regional Com., supra,* 101 Cal.App.3d at p. 47.)

The record contains evidence of the Commission's varying treatment of fireworks displays in other coastal locations, and this history demonstrates how the Commission's interpretation of its authority has been used to accomplish the purposes of the Act. For example, the Commission has previously required and approved a coastal development permit for the temporary closure of Seacliff State Beach for a fireworks display; the permit included provisions for wildlife monitoring and imposed clean-up requirements. With respect to a fireworks display in the City of Morro Bay, the city worked with the Commission to alleviate potential impacts to coastal resources so that the display would qualify for the permit exemption for temporary developments. The Festivals Committee reports that the Commission allowed Sea World to launch 150 fireworks displays a year over Mission Bay Park without a permit. The Festivals Committee acknowledges, however, that the Commission expressly indicated that the displays would be "re-evaluated" in five years "due to the potential, but undocumented adverse impacts to water quality, air quality and biological resources associated with the fireworks displays."

In this appeal, the Festivals Committee has not challenged the Commission's finding that the proposed fireworks display would cause significant adverse impacts upon coastal resources, nor does it contend that, assuming jurisdiction, the Commission abused its discretion in denying a permit exemption or waiver on this basis. Since we conclude that the Commission had jurisdiction over the proposed fireworks display and did not exceed its jurisdiction in requiring the Festivals Committee to obtain a permit to conduct such a display, the trial court properly denied the requested writ of mandate to set aside the Commission's cease-and-desist order.

Disposition

The judgment is affirmed.

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Pollak, Acting P.J.

We concur:

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Siggins, J.

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Jenkins, J.

Superior Court of Mendocino County, No. SCUKCVP008-51671, Leonard LaCasse, Judge.

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SHORE STEWARDS NEWS

June 2009 **Island County, Washington** **Issue No. 56**

This issue was written by Scott Chase, Shore Stewards Coordinator in Island County

"And the rockets' red glare, the bombs bursting in air…" Few can deny the beauty of a brilliant fireworks display on the 4th of July. Watching the colorful glitter and sparkles reflecting off the waters of a lake or Puget Sound is a perfect way to celebrate our country's birthday. Outside of the cardboard and plastic debris that need to be picked up off the beaches in the days following the event, there appears to be little damage done in shooting the fireworks out over our bodies of water. But there may be more left behind in the water than we realize.

A Kaleidoscope of Colors

Fireworks can be bought in a number of locations in the days before Independence Day, whether at "safe and sane" fireworks stands, or at the local Reservation. Wherever you buy them, you are probably looking at how much dazzle you can get for your money. This could include the colors the firework emits, the sparkle effect, how high it flies, whether it whistles or explodes, etc. Each of these effects is produced by a different chemical or metal, and many of these are toxic. Glittering greens are made by using barium nitrate, and blazing reds use lithium or strontium compounds. Brilliant whites result from use of aluminum, and blues are created by using copper compounds. Other ingredients may include nitric oxide, potassium nitrate, sulphur dioxide, nitrogen dioxide, antimony sulfide, and perchlorates. Unlike most other consumer products that are regulated by our government agencies, the ingredients of your typical firework are not listed on the package. Fireworks are generally produced in China, and those that are bought "illegally" on Reservations have little regulation as to what ingredients they contain. And there aren't many of us who ask the operator of the stand what chemicals are in their fireworks.

Our police and fire departments, as well as local governments, prefer "safe and sane" fireworks to be used in a location that will not create a fire hazard, and ask that the debris be cleaned up afterwards. The reality of the situation can be seen and heard by anyone: the use of fireworks begins at least a week before the 4th of July, with the majority used that evening. Those who live around lakes and the Sound typically shoot them off over the water, both to lessen the chance of starting a fire and to enjoy the extra color from the reflection off the water.

When these fireworks explode over a body of water, or fall into the water as duds or spent casings, some of these toxic chemicals and heavy metals are released into the water. Those fireworks that are ignited on roads further inland, a common location for many neighborhood displays, can also have their chemical residue carried into the water bodies through stormwater runoff. The rains following the 4th of July celebration wash the chemicals into the storm drains, which carry the contaminants into the streams and rivers, then into the Sound or ocean.

The Controversy over Contaminants

Other than small celebrations on New Years Eve, the majority of fireworks in our country are used during the 4th of July and to a lesser extent during the few days beforehand. Many feel that this short timeframe means that the amount of chemicals released into the water is insignificant. Others argue that the amount of toxins, particularly heavy metals like lead or mercury, can accumulate over a number of years. There have not been a large number of studies done regarding this, but some efforts are now being made to see if fireworks are indeed causing harmful pollution.

In 2006, SeaWorld in San Diego halted their nightly display of fireworks due to a threatened lawsuit by San Diego Coastkeeper, an environmental group. The displays had been held nightly during the summer since 1985, and Coastkeeper felt that the studies of water quality near SeaWorld were insufficient. Tests of the water were taken twice a year: in May, before the displays began, and in October, a month after the displays ended. Samples of bottom sediment in that location tested for 23 heavy metals. With one exception, testing revealed levels to be in the normal range. After halting the displays, SeaWorld officials stated they would apply for a discharge permit from the San Diego Regional Water Quality Control Board, part of the statewide regulatory system for water pollution. Aerial fireworks shows are not usually regulated by the various federal and state clean air and water quality agencies. The fireworks show resumed in 2007, after an agreement between SeaWorld and the Coastkeepers. Coastkeepers said they would not file suit if SeaWorld applied for a clean water permit, and cleaned up the debris left in the water after each show.

Perchlorates and Water Quality

One of the biggest concerns regarding potential firework contaminants is the harmful effect of perchlorates, which are largely used to propel the fireworks into the sky. Ammonium perchlorate is a chlorine compound used in the solid rocket boosters that lift the space shuttle into orbit, and in military rockets. Potassium perchlorate is often used in the production of aerial fireworks. The Environmental Protection Agency lists it as a contaminant of concern because it is very water soluable and can remain in water for a long period of time. Perchlorates can harmfully affect thyroid function in fish and animals, and elevated concentrations have been found in lakes and groundwater following fireworks demonstrations. An Oklahoma study of surface water locations near fireworks displays from 2004 – 2006 found that within 14 hours after the displays, concentrations of perchlorates spiked to values ranging from 24 to 1028 times the normal baseline value. These concentrations dropped to the baseline value within 20 – 80 days after the event, depending on water temperature. The Massachusetts Department of Environmental Protection did several studies of fireworks and perchlorates, and in 2006 became the first state to require most public water systems to test for perchlorates, with a standard of 2 parts per billion.

Fireworks displays are a very important event on many military bases during Independence Day. A memo dated May 21, 2009, from the Under Secretary of Defense to the Assistant Secretaries of the Army, Navy and Air Force, had the subject line heading: “Best Management Practices (BMPs) for Fireworks to Minimize Perchlorate Releases.” This 4 page memo detailed what perchlorates are, how they are found in aerial fireworks, and how fireworks should be displayed responsibly. From the report, it was advised that “The location of fireworks must be made to minimize risk to drinking water sources. Department of Defense personnel and the contractor should be aware of the existence of surrounding drinking water supplies and keep fireworks displays as far away from them as possible. Of particular concern are fireworks displays near surface waters used for drinking water supplies and within the recharge areas of public drinking water supply wells.”

The "Greening" of Fireworks

Scientists around the world are now looking into more environmentally-friendly alternatives to the traditional chemicals and metals used in fireworks, and are reformulating how they are used. One way in which they seek to reduce the use of perchlorates is to get that energy to propel the firework skywards from a higher nitrogen content in the formulation. By using less perchlorate, you produce less smoke, which also means less need for heavy metal coloring agents: barium, copper and strontium, to name a few. Use of nitrocellulose is another alternative to perchlorates. According to Darren Naud of DMD Systems, "Nitrocellulose is probably one of the best low-smoke ingredients. It burns with little smoke, and there's no fallout or residual combustion by-products that are nasty. There's just [carbon dioxide], water and nitrogen."

Many of the large amusement parks around the world use nightly fireworks displays as a big draw for customers. Few are as well known as Disneyland, who are on the forefront of clean fireworks technology. At Disneyland in California, they have eliminated the use of perchlorates altogether, relying instead on compressed air to propel the 361 colorful devices skyward. The system is not without problems, but over a 12 year period researchers obtained 7 patents in developing this system. Using "off-the-shelf" products that are easily obtained, Disney plans to donate the patents to a non-profit group that can license the technology for use worldwide. Other innovations being looked at by scientists include alternative compounds to the current chemicals and metals to provide the color and effects without the large quantities of smoke. Whether these technologies will make it down to the consumer level, where fireworks are mass produced cheaply in China and other Asian countries, remains to be seen. But even in China, where some of the world's largest fireworks displays are held, some concern about the pollution from fireworks is beginning to surface.

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Events/Activities

Digging for Dinner, June 20th - Learn how to do it the right way! Join WSU Island County Beach Watcher, Eugene Thrasher, to learn how to responsibly dig for clams. You'll gain an understanding of the importance of filling the holes you dig, how to determine the various limits, all about the Washington State rules and guidelines, and lots of clam lore. You'll need your own clamming license (sold at most hardware stores), and clothing/footwear that can get wet and sandy. Don't forget your shoveling tool and bucket! Demonstration begins at 8:30 am in Freeland. Cost: by donation. To register and receive driving directions/parking instructions, call 240-5558 or e-mail n.zaretzke@co.island.wa.us

Harvesting and Cooking Shellfish, June 24th - Learn techniques of how to properly harvest and cook the shellfish you collect on the beach. This includes a cooking demonstration of different shellfish. Presenters are WSU Beach Watchers: Bill Griffith, Tom Perry, Pete Domoto and Duane Hoekstra. This demonstration is free and will take place at 7:00 pm on Wednesday, June 24th, at the Camano Community Center, 141 N. E. Camano Drive, Camano Island. Questions or directions: (360) 387-3443, ext. 258

Composting 101, June 24th - Is your yard waste out of control? It must be time to start a compost pile or improve your existing one! Composting is a great way to dispose of your and kitchen wastes, lowering garbage bills and saving valuable landfill space while providing a wonderful mulch or soil amendment for your garden. This class will cover everything you need to know in order to begin or improve a functional composting system in your back yard. You'll also learn the safe way to use animal manures in your garden. This class is held 7:00 – 8:00 pm, Wednesday, June 24th, at the Good Cheer Community Garden in Bayview. Cost: by donation. To register and receive driving directions/parking instructions, call 240-5558 or e-mail n.zaretzke@co.island.wa.us

Rain Barrel Construction, June 25th - Save the rain for a sunny day! Come by the WSU Extension display at the Oak Harbor Outdoor Market and learn how you can construct your own rain barrel, which can be attached to your downspouts to capture runoff from the roof. You can provide your indoor and garden plants with naturally soft water they will love! Learn about the different methods of making your own rain barrel, what materials to buy, and where to get them. Making your own barrel is quick and easy, at a fraction of the cost of buying one already constructed. A rain barrel display and handout materials will be available, and demonstrations will be given at 4 pm, 5 pm, and 6 pm. Cost: by donation. Questions will also be answered by the presenter, Scott Chase, Shore Stewards coordinator for Island County. Questions or directions: (360) 387-3443, ext. 258

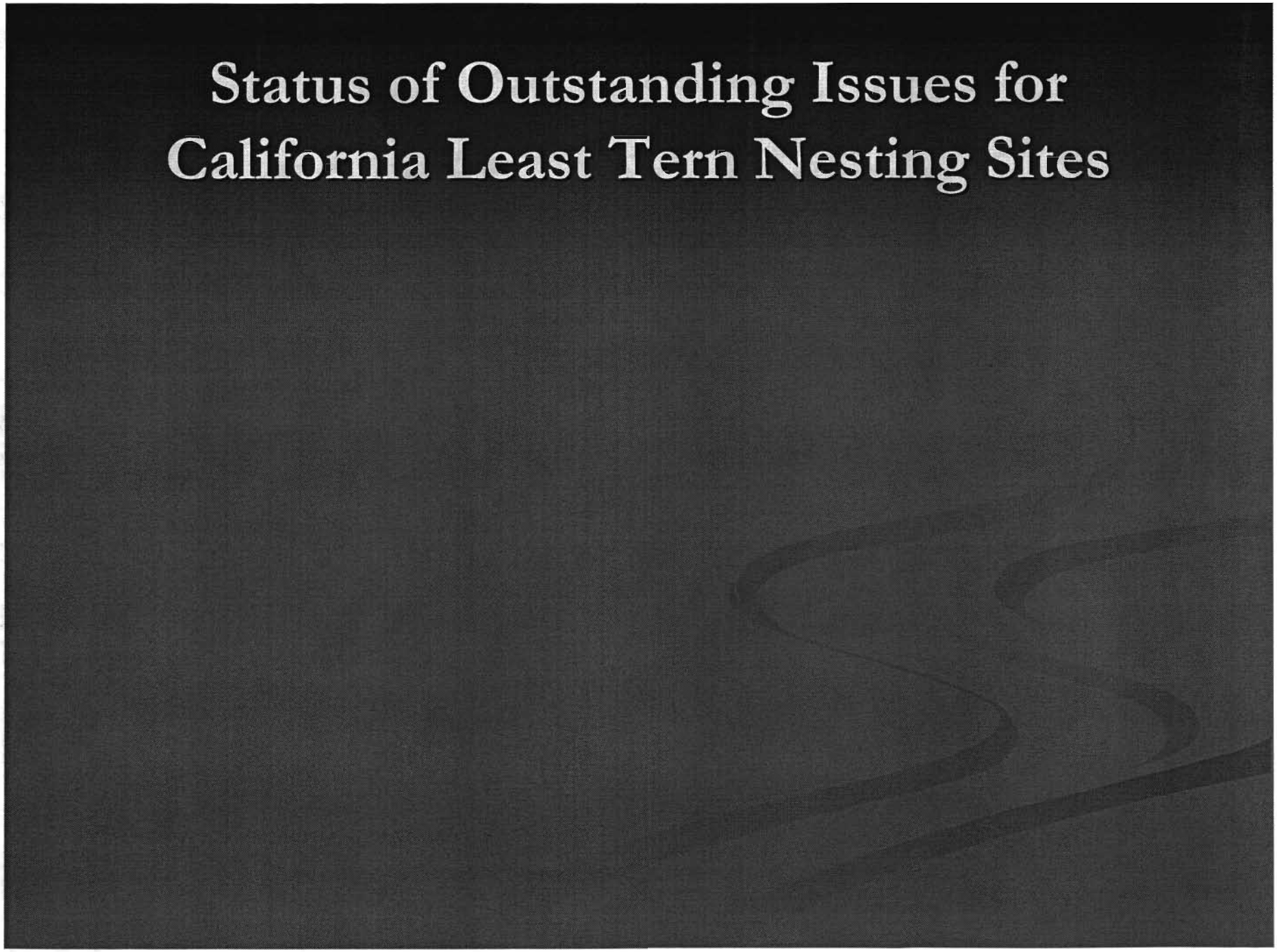
This product was funded through a grant from Washington State Department of Ecology. While these materials were reviewed for grant consistency, this does not necessarily constitute endorsement by the Department.

To view archived copies of past Island County Shore Stewards Newsletters, go to www.shorestewards.org/island/newsletter/

Island County Shore Stewards is a program of the Island County Marine Resources Committee, and is supported by WSU Extension in Island County and the Island County Marine Resources Committee, with grants from the Northwest Straits Foundation and the Washington State Department of Ecology.

Website: www.shorestewards.org/island/ email: shorestewards@wsu.edu
Contact: Shore Stewards Coordinator Scott Chase at schase@wsu.edu,
or phone 360-387-3443, ext 258

Status of Outstanding Issues for California Least Tern Nesting Sites



OVERVIEW – California Least Tern Nesting Islands

- Desirable as part of overall restoration of San Dieguito Lagoon.
- Restoration plan includes 11.83 acres of nesting habitat (NS 11, 12, 13 and 14)
- Location and design a culmination of years of planning and consultation with wildlife experts at USFWS and CDFG
- Not a permit requirement for SCE's SONGS Mitigation
- Responsibility of 22nd District Agricultural Association under a separate coastal development permit

BACKGROUND

- Coastal Development Permit issued in 1984 for construction of infield tunnel (CDP #6-84-525)
- To compensate for impacts to fairground habitat historically used by Least Terns
- District attempted unsuccessfully for years to acquire suitable property
- District worked with CDFG on an alternative site at the river mouth
- In the meantime, Coastal Commission imposed mitigation conditions on Edison's SONGS permit, including creation or restoration of 150 acres of wetland
  - Approved San Dieguito Lagoon as the restoration site in 1992
  - Commission staff asked District to defer further efforts on alternative site until restoration planning was completed

OUTSTANDING ISSUES

- Mitigation of impacts to existing wetlands from construction of new nesting sites
- Maintenance and monitoring responsibilities

Mitigation of wetland impacts from construction of new nesting sites

- 4 nesting sites designed to minimize impacts to existing wetlands
- However, about 2 wetland acres will be impacted from NS 12
- NS 12 cannot be reconfigured to avoid impacts
- Coastal Commission general policy requires mitigation a 4:1 mitigation ratio
- SONGS permit specifically requires a 4:1 mitigation ratio for unavoidable impacts to existing wetland
- Contribution to wetland functions and values by the nesting sites doesn't count toward mitigation of impacts caused by nest site construction
- Suitable acreage is available within the restoration area for such mitigation

Maintenance and monitoring responsibilities

- District responsibilities under its permit and agreements with CDFG include:
  - Site maintenance, including vegetation control, fence inspection and repair, and predator control
  - CDFG-conducted biological surveys consistent with available funds
- Based on results of previous restoration efforts Commission staff recommends:
  - Annual vegetation clearance and fence repair
  - Domestic and wild animal predator control and removal
  - Monitoring of nesting and rearing success (including time series data on number of breeding pairs and clutch size)
- Program adjustment in response to monitoring

STEPS TO RESOLUTION

- Representatives of the Attorney General's Office stepped in as facilitators to:
  1. Hear the issues from both parties
  2. Make site visits
  3. Suggest ways to resolve remaining differences
- Currently awaiting response from AGs office (step #3)

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UNITED STATES DISTRICT COURT
CENTRAL DISTRICT OF CALIFORNIA

CIVIL MINUTES - GENERAL

Case No.	CV 08-1467 AHM (PLAx)	Date	March 2, 2010
Title	NATURAL RESOURCES DEFENSE COUNCIL, INC., *et al.* v. COUNTY OF LOS ANGELES, *et al.*		

Present: The Honorable	A. HOWARD MATZ, U.S. DISTRICT JUDGE	
Stephen Montes	Not Reported	
Deputy Clerk	Court Reporter / Recorder	Tape No.

Attorneys **NOT** Present for Plaintiffs: Attorneys **NOT** Present for Defendants:

Proceedings: IN CHAMBERS (No Proceedings Held)

I. INTRODUCTION

On March 3, 2008, Plaintiffs Natural Resources Defense Council ("NRDC") and Santa Monica Baykeeper filed suit against Defendants the County of Los Angeles ("County"), the Los Angeles Flood Control District ("District"), and the individual County Supervisors and the Director of the Los Angeles County Department of Public Works in their official capacities, alleging that the County and the District violated several provisions in the National Pollutant Discharge Elimination System ("NPDES") Permit regulating municipal stormwater and urban runoff discharges within the County of Los Angeles (the "Permit"). In its June 20, 2008 Order, the Court denied Defendants' motion to stay the case and granted in part and denied in part Defendants' motion to dismiss, finding that Plaintiffs had failed to provide adequate notice to the District but allowing Plaintiffs to refile against the District after valid notice. On September 19, 2008, Plaintiffs filed their First Amended Complaint ("FAC").

The FAC alleges six causes of action under the Clean Water Act ("CWA"), 33 U.S.C. §§ 1251, *et seq.*, for: (1) causing and contributing to exceedances of water quality standards in the Santa Clara River watershed; (2) causing and contributing to exceedances of water quality standards in the Los Angeles River watershed; (3) causing and contributing to exceedances of water quality standards in the San Gabriel River watershed; (4) causing and contributing to exceedances of water quality standards and Total Maximum Daily Load ("TMDL") violations in the Malibu Creek watershed and at Surfrider Beach; (5) illegally discharging waste into the oceanic Area of Special

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Biological Significance ("ASBS") between Mugu Lagoon in Ventura County and Latigo Point in Los Angeles County; and (6) failing to submit adequate Receiving Water Limitations ("RWL") Compliance Reports.

On September 8, 2009, Plaintiffs moved for partial summary judgment as to liability as to claims two and three (as to the District); as to the Surfrider Beach violations in claim four; and as to all of claims five and six. On September 14, 2009, Defendants filed their motion for summary judgment as to all counts. Plaintiffs have also filed a Motion for Leave to File Surreply in Opposition to Defendants' Motion for Summary Judgment, which the Court DENIES.[1] The Court held a hearing on the summary judgment motions on February 8, 2010. For the following reasons, the Court GRANTS IN PART AND DENIES IN PART Plaintiffs' motion.[2] The Court also GRANTS IN PART AND DENIES IN PART Defendants' motion.[3]

Specifically, the Court DENIES summary judgment for both parties as to the watershed claims (claims one, two, three, and the Malibu Creek portion of claim four). The Court GRANTS summary judgment for the Plaintiffs on claim five and on the Surfrider Beach portion of claim four.[4] The Court GRANTS summary judgment for the

[1]Docket No. 173.

[2]Docket No. 87.

[3]Docket No. 113.

[4]The parties have notified the Court that Judge Yaffe of the Superior Court for the County of Los Angeles has stayed the operation of Los Angeles Regional Water Quality Control Board Order No. R4-2006-0074 (the "Regional Board Order"). Defendants' Notice of Los Angeles Superior Court Order, Ex. 1. This Regional Board Order amended the Permit to establish the TMDL limits at Surfrider Beach. Plaintiffs' Response to Defendants' Notice of Los Angeles Superior Court Order at 1. With this Regional Board Order stayed, the TMDL limits at Surfrider Beach at issue in claim four are not currently operational. The state court stay does not affect the Court's analysis as to liability on claim four. However, the state court proceeding may affect the remedies stage of this case. In addition, should Judge Yaffe invalidate the Regional Board Order, Defendants

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Defendants on all portions of claim six except for the adequacy of the 2008 Compliance Reports' treatment of Surfrider Beach. The Court DENIES summary judgment for both parties as to the adequacy of the 2008 Compliance Reports' treatment of Surfrider Beach.

II. SUMMARY OF UNDISPUTED FACTS

The municipal separate storm sewer system ("MS4") in the Los Angeles County basin carries urban runoff from local storm drains to inland rivers and eventually to ocean waters. Plaintiffs' SUF ¶ 3. No treatment plant cleans the runoff before it enters the so-called receiving waters of the region, so the runoff can contain a number of untreated pollutants it acquires as it flows over streets, parking lots, commercial sites, and residential areas. Plaintiffs' SUF ¶ 2. The MS4 is a complicated web, with thousands of miles of storm drains, hundreds of miles of open channels, and hundreds of thousands of connections. Plaintiffs' SUF ¶¶ 4-5. The MS4 includes storm drains operated by—and runoff coming from—84 incorporated cities, in addition to that from the County and District. Defendants' SUF ¶ 8. The District owns, operates, and maintains approximately 500 miles of open channel and 2,800 miles of storm drains, which is more of the MS4 than all 84 co-permittee cities combined. Plaintiffs' SUF ¶¶ 20-21. The County owns and operates additional storm drains, separate from the District, that connect to the MS4. *Id.* at ¶ 22. The County has no central record of these storm drains and does not know their complete extent. *Id.*

The Regional Water Quality Control Board ("Regional Board"), an entity of the State of California, issued collectively to the County, the District, and these 84 cities a National Pollutant Discharge Elimination System ("NPDES") Permit required under the Clean Water Act. This Permit allows the Permittees to discharge stormwater runoff from the MS4, contingent on meeting a number of conditions. Defendants' SUF ¶ 7-9. Most notably, Part 2.1 of the Permit provides that "discharges from the MS4 that cause or contribute to the violation of Water Quality Standards or water quality objectives are prohibited." Defendants' SUF ¶ 12. The Permit incorporates water quality standards from the Los Angeles Region Basin Plain for the Coastal Watersheds of Los Angeles and Ventura Counties ("Basin Plan") and the California Ocean Plan ("Ocean Plan"). *See* Cal.

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may move to vacate the judgment on this claim.

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Water Code §§ 13170.2 & 13240.

The Permit sets forth a monitoring program, which includes a requirement for the Principal Permittee (the District) to monitor the runoff flowing past seven specific mass emissions stations. Plaintiffs' SUF ¶¶ 23-24; Defendants' SUF ¶ 10. These mass emissions stations include the Malibu Creek, Los Angeles River, San Gabriel River, and Santa Clara River monitoring stations at issue in this case. The Los Angeles River and San Gabriel River mass emissions monitoring stations are located within the portion of the MS4 owned and operated by the Flood Control District. Plaintiffs' SUF ¶ 24. Monitoring data from the Los Angeles River and San Gabriel River mass emissions stations indicate that water quality standards have repeatedly been exceeded for a number of pollutants, including aluminum, copper, cyanide, fecal coliform bacteria, and zinc (the "standards-exceeding pollutants").[5] Plaintiffs' SUF ¶¶ 33-37.

The Permit's monitoring program also includes a requirement that water quality samples be taken five times per week at Surfrider Beach, a beach within the Santa Monica Bay. Plaintiffs' SUF ¶ 25. This monitoring shows that the water at Surfrider Beach has exceeded bacterial limits (including limits on total coliform, fecal coliform, and enterococcus) on dozens of occasions during summer dry weather seasons. Plaintiffs' SUF ¶¶ 25-28. The Regional Board has issued Notices of Violation to the County and the District (and the 84 cities that discharge to the MS4) indicating that discharges from the MS4 are causing or contributing to bacterial exceedances at Surfrider Beach. Colangelo Decl. Exs. G & H.

The California Ocean Plan prohibits the discharge of waste into the Malibu Area of Special Biological Significance ("ASBS"), which covers the 4-mile coastline from Latigo Point in Malibu to Laguna Point in Ventura. Plaintiffs' SUF ¶¶ 17, 42. Plaintiffs assert that this prohibition has been incorporated into the Permit. The District and the County own and operate drains (at least 13 District drains and 8 County drains) that discharge to the Malibu ASBS. Plaintiffs' SUF 45. County sampling of 11 of these drains in 2004 indicated that every single wet-weather event (rainstorm) sampled had discharges

[5]Neither party has provided the Court with monitoring data from the other mass emissions monitoring stations.

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exceeding bacteria limits. Plaintiffs' SUF ¶ 46. Sampling data collected by Santa Monica Baykeeper covering at least 2004-2006 show numerous instances of discharge from these drains exceeding applicable water quality standards. Plaintiffs' SUF ¶ 47.

The Permit also requires Permittees to submit to the Regional Board annual Receiving Water Limitations ("RWL") Compliance Reports describing the Permittee's plan to remedy violations of the permit "[u]pon a determination by either the Permittee or the Regional Board that discharges are causing or contributing to an exceedance of an applicable Water Quality Standard." Defendants' SUF ¶¶ 13-14. Defendants did not submit any Compliance Reports in 2003, 2004, or 2005. Plaintiffs' SUF ¶ 53. Defendants submitted Compliance Reports in 2006, 2007, and 2008, but the parties disagree as to whether these Reports satisfied the requirements under the Permit. Plaintiffs' SUF ¶¶ 54-62.

III. LEGAL STANDARD FOR A MOTION FOR SUMMARY JUDGMENT

Federal Rule of Civil Procedure 56(c) provides for summary judgment when "the pleadings, the discovery and disclosure materials on file, and any affidavits show that there is no genuine issue as to any material fact and that the movant is entitled to judgment as a matter of law." The moving party bears the initial burden of demonstrating the absence of a "genuine issue of material fact for trial." *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 256 (1986). A fact is material if it could affect the outcome of the suit under the governing substantive law. *Id.* at 248. The burden then shifts to the nonmoving party to establish, beyond the pleadings, that there is a genuine issue for trial. *Celotex Corp. v. Catrett*, 477 U.S. 317, 324 (1986).

"When the party moving for summary judgment would bear the burden of proof at trial, it must come forward with evidence which would entitle it to a directed verdict if the evidence went uncontroverted at trial. In such a case, the moving party has the initial burden of establishing the absence of a genuine issue of fact on each issue material to its case." *C.A.R. Transp. Brokerage Co., Inc. v. Darden Rests., Inc.*, 213 F.3d 474, 480 (9th Cir. 2000) (citations omitted). In contrast, when the non-moving party bears the burden of proving the claim or defense, the moving party can meet its burden by pointing out the absence of evidence from the non-moving party. The moving party need not disprove the

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other party's case. *See Celotex*, 477 U.S. at 325. Thus, "[s]ummary judgment for a defendant is appropriate when the plaintiff 'fails to make a showing sufficient to establish the existence of an element essential to [his] case, and on which [he] will bear the burden of proof at trial.'" *Cleveland v. Policy Mgmt. Sys. Corp.*, 526 U.S. 795, 805-06 (1999) (citing *Celotex*, 477 U.S. at 322).

When the moving party meets its burden, the "opposing party may not rely merely on allegations or denials in its own pleading; rather, its response must—by affidavits or as otherwise provided in this rule—set out specific facts showing a genuine issue for trial." Fed. R. Civ. P. 56(e). Summary judgment will be entered against the opposing party if that party does not present such specific facts. *Id*. Only admissible evidence may be considered in deciding a motion for summary judgment. *Id*.; *Beyene v. Coleman Sec. Servs., Inc.*, 854 F.2d 1179, 1181 (9th Cir. 1988).

"[I]n ruling on a motion for summary judgment, the nonmoving party's evidence 'is to be believed, and all justifiable inferences are to be drawn in [that party's] favor.'" *Hunt v. Cromartie*, 526 U.S. 541, 552 (1999) (quoting *Anderson*, 477 U.S. at 255). But the non-moving party must come forward with more than "the mere existence of a scintilla of evidence." *Anderson*, 477 U.S. at 252. Thus, "[w]here the record taken as a whole could not lead a rational trier of fact to find for the nonmoving party, there is no genuine issue for trial." *Matsushita Elec. Indus. Co., Ltd. v. Zenith Radio Corp.*, 475 U.S. 574, 587 (1986) (citation omitted).

Simply because the facts are undisputed does not make summary judgment appropriate. Instead, where divergent ultimate inferences may reasonably be drawn from the undisputed facts, summary judgment is improper. *Braxton-Secret v. A.H. Robins Co.*, 769 F.2d 528, 531 (9th Cir. 1985).

IV. ANALYSIS

A. Threshold Issues: Standing and Notice

1. Plaintiffs have standing to sue.

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Plaintiffs NRDC and Santa Monica Baykeeper have demonstrated that they have associational standing in this suit.

> [A]n association has standing to bring suit on behalf of its members when: (a) its members would otherwise have standing to sue in their own right; (b) the interests it seeks to protect are germane to the organization's purpose; and (c) neither the claim asserted nor the relief requested requires the participation of individual members in the lawsuit.

Hunt v. Washington State Apple Advertising Commission, 432 U.S. 333, 343 (1977).

In order for Plaintiffs' members to have standing to sue on their own, they would have to show: (1) they have "suffered an 'injury in fact' that is (a) concrete and particularized and (b) actual or imminent, not conjectural or hypothetical; (2) the injury is fairly traceable to the challenged action of the defendant; and 3) it is likely, as opposed to merely speculative, that the injury will be redressed by a favorable decision." *Friends of the Earth, Inc. v. Laidlaw Environmental Services*, 528 U.S. 167, 180-81 (2000) (citing *Lujan v. Defenders of Wildlife*, 504 U.S. 555, 560-61 (1992)). Plaintiffs' members use and enjoy the water bodies involved in this case for recreational and professional reasons, and their reasonable concern about exposure to pollutants has caused them to curtail their use of the Los Angeles River, San Gabriel River, Surfrider Beach, and the Malibu ASBS. Plaintiffs' SUF ¶¶ 68-70. The impairment of aesthetic, recreational, and professional interests is an injury in fact. *Laidlaw*, 504 U.S. at 184-85. Moreover, the Plaintiffs' members' injuries are caused in part by Defendants' MS4 discharges, and reducing those discharges would help to redress their injuries. Plaintiffs' SUF ¶ 71.

In addition, the lawsuit is germane to each Plaintiff's organizational purpose, as they are environmental organizations, and Santa Monica Baykeeper has a specific focus on protecting Santa Monica Bay. Plaintiffs' SUF ¶ 72. If, as here, associational plaintiffs do not seek individualized relief for their members that would require individualized proof, the participation of individual members is not required. *Hunt*, 432 U.S. at 343-44. Thus, Plaintiffs have standing to sue.

2. Plaintiffs have provided sufficient notice.

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Defendants ask the Court to reconsider its decision in its June 20, 2008 Order in light of a recent Ninth Circuit case addressing the notice requirement in CWA citizen suits and find that the Court has no jurisdiction to hear the claims against any defendant. *See Center for Biological Diversity v. Marina Point Development Co.*, 566 F.3d 794 (9th Cir. 2009); Motion at 10. *Marina Point Development* did not alter the standard for CWA notice in this Circuit; it merely reiterated the need for the notice to, at a minimum, tell a potential defendant "precisely what it allegedly did wrong, and when." *Id*. at 801.[6] Plaintiffs' notice to the Defendants here does precisely that. It lists with detailed specificity the exact portions of the permits and statutes allegedly violated and the exact date, location, and nature of each alleged violation. Colangelo Decl., Ex. RR at 570-83. The Court finds this notice adequate for the case to proceed against Defendants and will not reconsider the ruling in its June 20, 2008 Order.

B. Neither Plaintiffs Nor Defendants Are Entitled to Summary Judgment on the Watershed Claims Because Genuine Issues of Material Fact Remain.

The "Watershed Claims" encompass those claims that involve the rivers and creeks into which the MS4 flows (claims one through three and the Malibu Creek portion of claim four). With respect to these claims, Defendants do not dispute Plaintiffs' monitoring data.[7] Defendants argue, however, that because the monitoring stations are located downstream of where their own storm drains connect with the larger MS4, the discharges for which they are responsible will be commingled with those of other MS4 users. Defendants argue, therefore, that they cannot be found to be "causing or

[6]The court cited, discussed, and did not reject, much less overrule, the case that this Court cited in its June 20, 2008 Order, *Community Ass'n for Restoration of the Environment v. Henry Bosma Dairy*, 305 F.3d 943, 953 (9th Cir. 2002).

[7]Plaintiffs move for summary judgment only on the claims involving the Los Angeles River and San Gabriel River watersheds, and so the Court has been presented with data of permit exceedances only in these locations.

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contributing" to the permit exceedances.[8]

The Permit clearly prohibits "discharges from the MS4 that cause or contribute to the violation of Water Quality Standards or water quality objectives." Permit, Burhenn Decl. Ex. 1, Part 2.1 at 23. The Permit designates the mass emissions monitoring stations as the locations where monitoring shall take place to "[d]etermine if the MS4 is contributing to exceedances of Water Quality Standards . . . ." *Id.* at T-6. Because the permit specifies that these stations are the proper monitoring locations to determine if the MS4 is contributing to exceedances, Defendants' argument that these locations cannot be the basis for determining whether there were exceedances fails.

Defendants also assert that because the monitoring stations are located downstream of where their own storm drains join these water bodies, the monitoring data cannot possibly pinpoint that their discharges—as opposed to those of the other entities using the

[8]As a preliminary matter, Defendants argue that in order for a permittee to be in violation of the permit, either the permittee or the Regional Board must have made a determination that the permittee is causing or contributing to exceedances of water quality standards, and the permittee must also fail to participate in good faith in the iterative process to remedy the exceedances. Opp'n at 17-19. A state court has already ruled on these arguments in Defendants' challenge to the validity of the Permit and found that, based on the regulatory history underlying the creation of the Permit, there is no safe harbor for a Permittee who complies with the iterative process. *In re Los Angeles County Municipal Storm Water Permit Litigation*, No. BS 080548, at 6-7 (Los Angeles Super. Ct. Mar. 24, 2005) (Colangelo Decl., Ex. D at 166-67). This Court agrees with that analysis. At the hearing, Defendants directed the Court's attention to a January 22, 2001 letter from the then-chair of the Regional Board, Francine Diamond, which the Superior Court cited in its opinion. *See* Gest Decl. Ex. D at 8-9. The Diamond letter suggests that as long as a Permittee is engaged in a good faith effort in the iterative process to remedy exceedances, it is in compliance with the Permit. However, as an informal mailing to Permittees, this letter does not have the force of law, and the Court need not defer to it, especially when it runs counter to the language of the Permit.

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MS4—caused the exceedances.[9] However, in order for the Permit to be violated, it is not necessary to pinpoint the source of pollutants. The Permittees, collectively, are violating the permit if "discharges from the MS4" are "caus[ing] or contribut[ing] to the violation of Water Quality Standards or water quality objectives." Permit Part 2.1 at 23; *see also In re Los Angeles County Municipal Storm Water Permit Litigation*, No. BS 080548, at 6 (Los Angeles Super. Ct. Mar. 24, 2005) (Colangelo Decl., Ex. D at 166) (explaining that subparts 2.1 and 2.2 of the Permit set forth the "basic receiving water requirements for Los Angeles area waters" and acknowledging that a permittee could be in "violation" of these requirements).

According to the Permit, monitoring at the mass emissions stations shall be used to determine if the MS4 is causing or contributing to exceedances. Permit at T-6. Here, Plaintiffs have alleged that water quality standards were exceeded at the monitoring stations on each of the four rivers (Los Angeles River, San Gabriel River, Santa Clara River, and Malibu Creek) on multiple occasions. FAC ¶¶ 79-229. Defendants have offered no facts to dispute these allegations. Moreover, Defendants have even acknowledged that their MS4 is conveying the specified pollutants to the water bodies in question. *See* Colangelo Decl., Ex. N at 291-93, 295, 298. Thus, Defendants are not entitled to judgment as a matter of law that they are in compliance with the Permit.

With respect to the Los Angeles River and San Gabriel River, Plaintiffs have moved for partial summary judgment as to liability for the District. Plaintiffs offer data showing the exceedances at the monitoring stations for these bodies of water. Plaintiff's SUF ¶¶ 33-37. In addition, Plaintiffs argue that because the mass emissions monitoring stations for these bodies of water are located in the portion of the MS4 owned and operated by the District, the District is responsible for the pollutants in the MS4 at this point. *See* Plaintiffs' SUF ¶ 24. The Court agrees with this proposition. As a Permittee,

[9]At the hearing, Defendants cited to numerous portions of the Permit and witness depositions and declarations that demonstrate that there were other sources of the pollutants at issue here. This is undoubtedly true, but irrelevant to liability under the Permit. "[D]ischarges from the MS4 that cause *or contribute* to the violation of Water Quality Standard . . . are prohibited." Permit Part 2.1 at 23. The MS4 or a particular Permittee need not be the sole source of the pollutant to be in violation of the Permit.

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the District is "required to comply with the requirements of this Order applicable to discharges within its boundaries . . . ." Permit Part 3.E at 26; *see also* Part 3.D.8 at 25 (explaining that as Principal Permittee, the District must comply with the requirements of general Permittees, as well). However, that does not necessarily determine the question of whether the water passing by these points is a "discharge" within the meaning of the Permit and the Clean Water Act.

Indeed, Defendants argue that the water sampled at these monitoring stations does not constitute a discharge from the MS4, but merely reflects water passing by the stations. Defendants insist that no liability can attach to the District because it is merely allowing water to move within the same waterbody and, thus, no discharge occurs at the monitoring stations. The Act defines "discharge of a pollutant" to mean "any addition of any pollutant to navigable waters from any point source." 33 U.S.C. § 1362(12). A "point source" is "any discernible, confined and discrete conveyance, including . . . any pipe, ditch, channel, tunnel, conduit, . . . [or other examples], from which pollutants are or may be discharged." 33 U.S.C. § 1362(14). A point source can include objects "that do not themselves generate pollutants." *South Florida Water Management District v. Miccosukee Tribe of Indians*, 541 U.S. 95, 105 (2004). In *Miccosukee*, the Supreme Court held that if two portions of a water body are part of the same water body, moving "water from one into the other cannot constitute an 'addition' of pollutants" so as to constitute a discharge under the Act. *Id.* at 109.[10] Plaintiffs have not provided the Court with the necessary evidence to establish that the Los Angeles River and the San Gabriel River below the mass emissions monitoring stations are bodies of water that are distinct from the MS4 above these monitoring stations. In other words, the record before the Court does not show where the MS4 ends and either River begins. In order for the District's actions to violate Part 2.1 of the Permit, it must be discharging pollutants from a point source. The Court has been presented with no evidence clearly establishing that

[10]At the hearing, Plaintiffs took issue with the Court's citation to *Miccosukee* since that case addressed whether a permit could be issued under the Clean Water Act, and a permit already regulates the MS4 here. However, the Court is not relying on *Miccosukee* to invalidate the Permit, but rather to clarify the meaning of the terms "discharge" and "point source" under the Clean Water Act, in order to help understand how those terms in the Permit should be interpreted .

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the District is discharging pollutants from any given point source at or near the monitoring stations.

Plaintiffs pointed out during their oral argument that the District releases runoff through outlets that are *upstream* of the mass emissions stations on the San Gabriel and Los Angeles Rivers. Ex. UU to Second Colangelo Decl. at 283:4-17 (Pestrella D. Tr.). Outflow from these upstream outlets would be considered discharges under the Permit and the Clean Water Act. However, there is no data showing that any of these upstream discharges by the District are causing or contributing to the violations of the Water Quality Standards.

At the hearing, Plaintiffs also argued that exceedances at the mass emissions stations establish a violation of the Permit as a matter of law. They cited to 40 C.F.R. § 122.26(d)(ii)(3)(D), which requires a stormwater permit application to include

> A proposed monitoring program for representative data collection for the term of the permit that describes the location of outfalls or field screening points to be sampled (or the location of instream stations), why the location is representative, the frequency of sampling, parameters to be sampled, and a description of sampling equipment.

Plaintiffs assert that this regulation means that MS4 sampling need not be conducted at a point of discharge, but instead may be conducted at a "representative" location elsewhere, and that such a representative location may be used to determine the MS4's compliance with the Permit. *See* Permit at T-6 (stating that the monitoring from the mass emissions stations shall be used to "determine if the MS4 is contributing to exceedances of Water Quality Standards").

Where Plaintiff's argument runs into trouble, however, is the fact that although the mass emissions station data may be the appropriate way to determine whether the MS4 in its entirety is in compliance with the Permit or not, that data is not sufficient to enable the Court to determine that the District is responsible for "discharges from the MS4 that cause or contribute to the violation" of standards under Part 2.1 of the Permit, since a co-permittee is responsible "only for a *discharge* for which it is the operator." Permit ¶ G.4

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at 20 (emphasis added). *See also* 40 C.F.R. § 122.26(b)(1) ("*Co-permittee* means a permittee to a NPDES permit that is only responsible for permit conditions relating to the discharge for which it is operator."). There is no evidence showing that *discharges* from the District portions of the MS4 are contributing to the exceedances at the mass emissions stations. Plaintiffs would need to present some evidence (monitoring data or an admission) that some amount of a standards-exceeding pollutant is being discharged through at least one District outlet.[11] They have not done so. Consequently, the Court cannot grant summary judgment for the Plaintiffs on any of the watershed claims.

In their Motion for Summary Judgment, Defendants do not assert that, as a factual matter, the runoff they are discharging from their MS4 outlets is devoid of the observed pollutants. They instead make the legal arguments that the Court has already rejected above—that the flow from their MS4 outlets does not constitute discharge, that the monitoring data cannot be used to establish Permit noncompliance, that the presence of other sources of the pollutants absolves them of responsibility, and that the Permit provides a "safe harbor" for Permittees that participate in the iterative process. If the Court had an evidentiary basis to find that the standards-exceeding pollutants did not pass through the Defendants' MS4 outflows at or near the time the exceedances were observed, then Defendants could be entitled to summary judgment on these claims. However, neither side has introduced evidence of whether the standards-exceeding pollutants passed through the Defendants' outflows.

The Court therefore ORDERS each side to specify whether there is any basis in the record or in other facts currently in their possession establishing that the standards-exceeding pollutants identified at page 4 passed through the Defendants' MS4 outflows at or near the time the exceedances were observed. In addition, both sides must disclose whether any facts in the record or already in their possession support a finding that Water Quality Standards were exceeded at the monitoring stations in Santa Clara River and

[11]In support of their position, Plaintiffs invoke the specter of being forced to sample every single outflow in Los Angeles County, which would be impossible. This ruling would not require that result. It would require sampling from at least one outflow that included a standards-exceeding pollutant, in order to show that a discharge from a particular permittee is contributing to an exceedance downstream.

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Malibu Creek. Each side's response to these inquiries must not exceed five pages and must be filed by March 10, 2010. No response to the other side's filing will be permitted.

C. Plaintiffs Are Entitled to Summary Judgment on the Surfrider Beach Claim.

The Permit prohibits discharges from the MS4 that cause or contribute to violations of bacterial limits during the dry summer months at beaches in the Santa Monica Bay, including at a designated monitoring location at Surfrider Beach. Permit at 17, 22 (Part 1.B), 24 n.4, 24 (Part 2.5), & Att. U-2. Defendants do not dispute that exceedances of bacterial limits at Surfrider Beach have occurred dozens of times in the summer months since 2006. Defendants also do not dispute that the Regional Board has expressly identified MS4 discharges as one of the sources of fecal bacteria at the beach, in Notices of Violations that it issued to the County and the Flood District in March 2008. Plaintiffs' SUF ¶¶ 26-28, 31-32.

Defendants make two arguments that they are not liable for the exceedances at Surfrider Beach. First, they argue that because there are many other potential sources of bacteria at Surfrider Beach, the exceedances cannot be attributed to them. However, the existence of other potential sources is irrelevant to determining whether there has been a violation under the Permit. With respect to Surfrider Beach, the Permit specifies that all permittees are "jointly responsible for compliance" with the requirements prohibiting discharges that cause or contribute to bacterial exceedances. Permit at 22 n.3. Thus, Defendants are liable for the exceedances so long as they contributed to them.

Defendants next argue that there is no evidence that they contribute to the exceedances at Surfrider Beach, in part because none of their storm drains discharge directly to Surfrider Beach. Nowhere does the Permit require that a permitee discharge directly to a monitoring site to "cause or contribute" to exceedances in violation of the Permit. *See* Permit at 17, 22 (Part 1.B), 24 n.4, 24 (Part 2.5), & Att. U-2. Indeed, the shoreline monitoring at Surfrider Beach itself is dispositive evidence of Permit violations. Permit at 16-17 (¶ 36). The Permit specifies that if bacterial limits are exceeded at a compliance monitoring site, the Regional Board will issue an appropriate investigative order. Only if the Regional Board thereafter determines that a permittee is not

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responsible for the exceedances will the permittee be absolved of responsibility. Permit at 17-18 (¶¶ 37-38). Here, the Regional Board has issued Notices of Violation to the County and the District (and the other entities that discharge to the MS4) indicating that the discharges from the MS4 are causing or contributing to bacterial exceedances at Surfrider Beach. Colangelo Decl. Exs. G & H. As Defendants conceded at oral argument, the Regional Board has not yet made a finding that discharges from the MS4 are not contributing to the documented bacterial violations at Surfrider Beach. *See* Plaintiff's SUF ¶ 29. Thus, until the Regional Board decides otherwise, the Defendants are jointly responsible (along with the other permitees) for the bacterial exceedances at Surfrider Beach. Consequently, summary judgment as to liability for the Plaintiffs on this claim is warranted.

D. Plaintiffs Are Entitled to Summary Judgment on Their Claim of Illegal Discharge into the ASBS.

Defendants make two arguments for why they should not be held liable for discharging waste into the protected coastal Malibu ASBS. First, they assert that the prohibition on waste discharge has not properly been incorporated into the Permit and so Plaintiffs cannot enforce it under the citizen suit provision of the Clean Water Act. Next, they argue that even if the prohibition is considered part of the Permit, Defendants cannot be held liable for its violation because they are awaiting the outcome of their application for an exemption from the prohibition from the State Water Resources Control Board ("State Board").

1. Incorporation of the ASBS discharge prohibition into the Permit

Contrary to Defendants' arguments, the prohibition on discharging waste into the ASBS is incorporated into the Permit. The Permit prohibits "discharges from the MS4 that cause or contribute to the violation of Water Quality Standards or water quality objectives." Permit at 23. The Permit defines "water quality standard" to include "water quality criteria contained in . . . the California Ocean Plan . . . ." Permit at 70. The California Ocean Plan ("Ocean Plan"), in turn, prohibits the discharge of waste (defined as "a discharger's total discharge, of whatever origin") into any ASBS. Colangelo Decl. Ex. C at 150, 154.

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Defendants argue that this prohibition of discharge of waste is not a "water quality standard" because it is located in Part III of the Ocean Plan—the program of implementation—rather than in Part I or Part II—the beneficial uses and water quality criteria sections. However, the State Board found, in a precedential order issued before the current Permit was adopted, that the "Ocean Plan discharge is a water quality standard" that is enforceable in an NPDES Permit. *In re California Department of Transporation*, Order WQ 2001-08 at 8-9 (Apr. 26, 2001) (Colangelo Decl. Ex. I at 243-44). Because this was a precedential order, the Regional Board was bound to follow it when issuing the Permit, and therefore, the prohibition on waste discharge in an ASBS is a water quality standard for purposes of the Permit. *See* State Board Order WR 96-01 at 17 n.11 (Jan. 18, 1996) (designating all water quality decisions and orders as precedential decisions) (Second Colangelo Decl. Ex. XX at 45); Cal. Gov. Code § 11425.60 (authorizing precedential decisions by state agencies).

Moreover, this decision by the State Board, that the Ocean Plan's prohibition on the discharge of waste is a water quality standard, is supported by the Clean Water Act's regulations. The regulations specify, "Water quality standards are provisions of State or Federal law which consist of a designated use or uses for the waters of the United States and water quality criteria for such waters based upon such uses." 40 C.F.R. § 131.3(i). Water quality criteria are, in turn, defined as "elements of State water quality standards, expressed as constituent concentrations, levels, or narrative statements, representing a quality of water that supports a particular use." 40 C.F.R. § 131.3(b). The prohibition on the discharge of waste into an ASBS is a classic example of the type of narrative statement that would qualify as a water quality criterion under this definition. *See, e.g.*, *PUD No. 1 of Jefferson County v. Washington Dept. of Ecology*, 511 U.S. 700, 715 (1994) ("'[C]riteria' are often expressed in broad, narrative terms, such as 'there shall be no discharge of toxic pollutants in toxic amounts.'" (citation omitted)).

Based on this analysis, the Ocean Plan's prohibition on discharge into an ASBS is a water quality standard that is covered by the Permit's prohibition on "discharges from the MS4 that cause or contribute to the violation of Water Quality Standards or water quality objectives." Permit at 23.

2. Defendants' exemption application

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Defendants also argue that they need not comply with the prohibition on discharging waste in the ASBS because they have applied for an exception from that prohibition with the State Board and their application is still pending. Opp'n at 23-24. This Court has already ruled on this issue in its June 20, 2008 Order on Defendants' motion to dismiss. In that Order, the Court found that the "State Water Resources Control Board has explicitly stated that the provisions of the 'Ocean plan' remain fully enforceable while it reviews the administrative applications on which Defendants rely." Order at 2. Defendants' attempt to relitigate this issue is improper.

Moreover, the cases which Defendants rely upon are inapposite, as they involve situations where no permit had yet been issued to regulate the defendant's discharge, not a situation like this one, where a valid permit limits the defendants' discharge, but an application for an exception is pending. *See Hughey v. JMS Development Corp.*, 78 F.3d 1523 (11th Cir. 1996); *Mississippi River Revival v. City of Minneapolis*, 319 F.3d 1013 (8th Cir. 2003). The Defendants will not be permitted to avoid responsibility for their conduct currently regulated under the Permit, simply because a discretionary exception application is still pending with the State Board.

E. Defendants Are Entitled to Summary Judgment as to Part of the Compliance Reports Claim.

Defendants assert that they are not required to submit Receiving Water Limitations ("RWL") Compliance Reports under the Permit until and unless the Permittee or the Regional Board determines that MS4 discharges are causing or contributing to an exceedance of an applicable water quality standard. The Court agrees. The Permit clearly states, "Upon a determination by either the Permittee or the Regional Board that discharges are causing or contributing to an exceedance of an applicable Water Quality Standard, the Permittee shall promptly notify and thereafter submit a [RWL Compliance Report] to the Regional Board . . . ." Permit Part 2.3.a, at 23. In order for a Permittee to be required to submit RWL Reports, either the Permittee or the Regional Board must determine that discharges from the MS4 are causing or contributing to exceedances of a Water Quality Standard.

At oral argument, Plaintiffs directed the Court's attention to another provision of

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the Permit, which states (with respect to the Principal Permittee—the District), "When *data indicate* that discharges are causing or contributing to exceedances of applicable Water Quality Standards . . . a RWL Compliance Report . . . shall be submitted with the subsequent Unified Annual Report." Permit ¶ I.C.6 at T-4 (emphasis added). Plaintiffs would have the Court read this language to find that anytime the monitoring data at the mass emissions stations exceeds water quality limits, an RWL Report is automatically required. However, as the Court found in Part IV.B of this Order, discussing the Watershed claims, data showing exceedances at the mass emissions stations does not necessarily show that *discharges* from the MS4 are causing or contributing to those exceedances, because the Court has been presented with no evidence establishing that standards-exceeding pollutants are passing through an outflow of either Defendant. Thus, even though Plaintiffs have presented evidence of exceedances at the monitoring stations since 2003, Fernandez Decl., Exs. A-G, this monitoring data did not automatically invoke the RWL requirements. Because neither the Regional Board nor the Defendants has formally determined that the MS4 is causing or contributing to exceedances of water quality standards in the four watersheds, Defendants are not required to submit RWL reports with respect to these water bodies. Thus, the Court grants summary judgment to Defendants with respect to the claim of inadequate Compliance Reports for the rivers and creeks.

In contrast, the Regional Board has made a determination, and notified Defendants through formal Notices of Violation, that the MS4 is causing or contributing to exceedances of bacterial limits at Santa Monica Bay beaches, including Surfrider Beach. Colangelo Decl., Exs. G & H. These Notices of Violation were sent to Defendants on March 4, 2008, so they were required to submit RWL Compliance Reports addressing the violations at Surfrider Beach beginning on this date. Defendants have each submitted one RWL Compliance Report since receiving a Notice of Violation—their 2008 Compliance Reports. Colangelo Decl. Exs. NN & OO. These Reports do address the bacterial exceedances in Santa Monica Bay, but there is a genuine issue of material fact as to whether their discussion of the proposed changes to the monitoring program is adequate to meet the requirements of the Permit. SGI ¶ 62. Thus, the Court cannot grant either Defendants or Plaintiffs summary judgment as to the adequacy of the 2008 Compliance Reports.

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V. CONCLUSION

For the foregoing reasons, the Court DENIES summary adjudication for the Plaintiffs as to the Watershed Claims (claims one, two, three, and the Malibu Creek portion of claim four). The Court reserves its ruling on the Defendants' Motion for Summary Judgment on the Watershed Claims pending the receipt of the briefing requested in Part IV.B of this Order. The Court GRANTS summary adjudication for the Plaintiffs on claim five and on the Surfrider Beach portion of claim four. The Court GRANTS summary adjudication for the Defendants on all portions of claim six except for the adequacy of the 2008 Compliance Reports' treatment of Surfrider Beach. The Court DENIES summary adjudication for both parties as to the adequacy of the 2008 Compliance Reports' treatment of Surfrider Beach.

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ENVIRONMENTAL ASSESSMENT OF THE ISSUANCE OF A SMALL TAKE REGULATIONS AND LETTERS OF AUTHORIZATION AND THE ISSUANCE OF NATIONAL MARINE SANCTUARY AUTHORIZATIONS FOR COASTAL COMMERCIAL FIREWORKS DISPLAYS WITHIN THE MONTEREY BAY NATIONAL MARINE SANCTUARY, CALIFORNIA

National Oceanic and Atmospheric Administration
National Marine Fisheries Service and Monterey Bay National Marine Sanctuary

June, 2006

INTRODUCTION

A. Summary

On May 10, 2002, the National Marine Fisheries Service (NMFS) received an application from the Monterey Bay National Marine Sanctuary (MBNMS or the Sanctuary) requesting an Incidental Harassment Authorization (IHA) under section 101 (a)(5)(D) and a Letter of Authorization (LOA) under section 101 (a)(5)(A) of the Marine Mammal Protection Act (MMPA), for the possible harassment of small numbers of several species of marine mammals incidental to coastal commercial fireworks displays approved by MBNMS and occurring along the coastline within the Sanctuary, over California waters. Under the preferred alternative for this action, the LOA would be issued annually under 5-year regulations, which would take effect upon expiration of the one-year IHA. This Environmental Assessment (EA) is intended to jointly address impacts on the environment that would result from the issuance of the 5-year incidental take regulations (under the MMPA) and subsequent issuance of National Marine Sanctuary Authorizations for fireworks displays in the MBNMS (under the National Marine Sanctuaries Act (NMSA)).

B. Background

The MBNMS was designated as the ninth national marine sanctuary in the United States on September 18, 1992. Managed by the National Marine Sanctuary Program (NMSP) within the National Oceanic and Atmospheric and Atmospheric Administration (NOAA), the MBNMS adjoins 276 miles (444 km) of central California's outer coastline (overlaying 25 percent of state coastal waters), and encompasses 5,300 square miles of ocean waters from mean high tide to an average of 25 miles (40 km) offshore between Rocky Point in Marin County and Cambria in San Luis Obispo County.

Federal regulations governing activities within the MBNMS became effective on January 1, 1993. The MBNMS was the first national marine sanctuary to be designated along urban shorelines and, when first designated, became the largest marine sanctuary in the United States, equal in area to 77 percent of all other Federal marine sanctuaries in existence at the time. As a result of its large size and near proximity to urban areas, the MBNMS has addressed many regulatory issues not previously encountered by the NMSP. Authorization of professional fireworks displays is one such issue that has required a steady refinement of policies and procedures to limit the location, timing, and composition of professional fireworks events as more has been learned about its impacts to the Sanctuary and effects on the environment. The Sanctuary has monitored individual displays over the years to improve its understanding of their characteristics and potential impacts to Sanctuary resources.

Fireworks displays have been conducted over current Sanctuary waters for many years as part of national and community celebrations (such as Independence Day and municipal anniversaries) and to foster public use and enjoyment of the marine environment. The marine venue for this activity is the preferred setting for fireworks in central California in order to optimize public access and avoid the fire hazard associated with terrestrial display sites. Many

fireworks displays occur at the height of the dry season in central California, when area vegetation is particularly prone to ignition from sparks or embers. The MBNMS has worked diligently to balance these needs with its primary mandate for marine resource protection.

II. PURPOSE AND NEED FOR THE ACTIONS

A. Request for Incidental Take under the MMPA

Section 101(a)(5)(A) of the MMPA (16 U.S.C. 1361 et seq.) directs the Secretary of Commerce (the Secretary) to allow, upon request, the incidental, but not intentional taking of marine mammals by U.S. citizens who engage in a specified activity (other than commercial fishing) within a specified geographical region if certain findings are made and regulations are issued.

Authorization for incidental takings may be granted if the Secretary finds that the taking will have a negligible impact on the species or stock(s); will not have an unmitigable adverse impact on the availability of the species or stock(s) for subsistence uses; and the permissible methods of taking and requirements pertaining to the monitoring and reporting of such taking are set forth. NMFS has defined "negligible impact" in 50 CFR 216.103 as "...an impact resulting from the specified activity that cannot be reasonably expected to, and is not reasonably likely to, adversely affect the species or stock through effects on annual rates of recruitment or survival."

Except with respect to certain activities not relevant here, the MMPA, as amended, now defines "harassment" as "...any act of pursuit, torment, or annoyance which (a) has the potential to injure a marine mammal or marine mammal stock in the wild [Level A harassment]; or (b) has the potential to disturb a marine mammal or marine mammal stock in the wild by causing disruption of behavioral patterns, including, but not limited to, migration, breathing, nursing, breeding, feeding, or sheltering [Level B harassment]."

The MBNMS determined that authorizing fireworks displays above the MBNMS might potentially disturb marine mammals and, accordingly, submitted an application in 2002 for a 5-year rule, authorizing take, by harassment, of a small number of California sea lions and Pacific harbor seals incidental to fireworks displays. If the action proposed in the small take application will have no more than a negligible impact on the species or stock, will not have an unmitigable adverse impact on the availability of the species or stock for subsistence uses, and the permissible methods of taking and required monitoring are set forth, then the NMFS shall issue the regulations. NMFS would then issue an LOA to the MBNMS each year that the rule is in effect, provided MBNMS complied with the previous LOA’s mitigation, monitoring, and reporting requirements and no unauthorized take occurred during the previous year. The purpose of the 5-year rule and LOAs is to investigate the status of the marine mammals that may be impacted by the action, set forth the types and amount of take that may occur, and list the mitigation and monitoring required to ensure the least practicable impact to marine mammal species.

B. Issuance of Marine Sanctuary Authorizations for Fireworks under the NMSA

Section 308 of the NMSA authorizes the Secretary of Commerce to issue such regulations as may be necessary to protect National Marine Sanctuary resources and qualities, among other purposes. Accordingly, the Secretary promulgated regulations in Title 15 of the Code of Federal Regulations (15 CFR), section 922.132(a) prohibiting several activities within the MBNMS as environmental protection measures, including unauthorized discharges into Sanctuary waters and harassment of marine mammals, seabirds, and sea turtles. The Secretary may grant specific exceptions to otherwise prohibited activities under special circumstances. Sections 922.49 and 922.132(e) of Title 15 CFR allow the Secretary to authorize any valid Federal, State, or local lease, permit, license, approval, or other authorization for activities within the MBNMS that would otherwise be prohibited under Sanctuary regulations, provided the applicant complies with any terms and conditions to protect Sanctuary resources and qualities.

Coastal fireworks displays within the MBNMS result in discharges of debris into Sanctuary waters, incidental harassment of wildlife, and potential negative impacts to habitat; such incidental impacts are prohibited by MBNMS regulations. The MBNMS has developed an extensive list of terms and conditions designed to minimize the impacts of fireworks displays within the Sanctuary. Coastal fireworks displays over the MBNMS generally require Federal, state, and or local permits that address public safety and coastal access. The Secretary of Commerce has delegated authority to the MBNMS Superintendent to authorize such permits (i.e. approve the activity if the Superintendent determines that terms and conditions may be applied to the activity that adequately protect Sanctuary resources and qualities.

This EA, in addition to assessing impacts of coastal fireworks displays upon marine mammals pursuant to the MMPA, analyzes impacts of fireworks displays upon the broader resources and qualities of the MBNMS. If it is determined that coastal fireworks displays can be conducted in a manner that safeguards Sanctuary resources and qualities, then the MBNMS may issue authorizations of other valid Federal, State, and local fireworks approvals for up to 5-year periods, with terms and conditions that mitigate negative impacts.

III. DESCRIPTION OF ACTIVITY TO BE COVERED BY PROPOSED MMPA LOAs AND MBNMS AUTHORIZATIONS

A. Description of Fireworks Displays Authorized by MBNMS

The activity to be conducted is the display of commercial-grade fireworks in the atmosphere and at ground or sea level. Since 1993, the MBNMS, a component of NOAA, has processed requests for the professional display of fireworks that affect the Sanctuary and its resources. The MBNMS has determined that debris fallout (spent pyrotechnic materials) from fireworks events constitute a discharge into the Sanctuary and thus a violation of Sanctuary regulations, unless written authorization is secured from the Sanctuary. Therefore, sponsors of fireworks displays conducted in the MBNMS are required to obtain Sanctuary authorization prior to conducting such displays.

Since 1993, the MBNMS has received a total of 79 requests for professional fireworks displays and has issued 67 Authorizations, the majority of which have been associated with large community events such as Independence Day and municipal festivals. The Sanctuary redirected at least 4 displays away from the Sanctuary and 2 applications are currently (as of March 2006) being processed. However, the Sanctuary projects that as many as 20 coastal displays per year may be conducted in, or adjacent to, the MBNMS boundaries in the future. The number of "public" fireworks displays within the Sanctuary has remained relatively constant over time. "Private" fireworks displays averaged one per year from 1993 to 2000. But within a six-month period from October 2000 to March 2001, the MBNMS received four requests for private displays in the Sanctuary, and information suggests that such requests could increase in the future. Table 1 presents a relative comparison of the types of fireworks events authorized by the MBNMS between 1993 and 2005.

Fireworks Event Category	**Percentage of Total Fireworks Permits Issued**
Independence Day Festivals	45%
City Festivals	28%
Private Events	27%

Table 1. Percentage of total fireworks Authorizations issued by event.

In considering requests to conduct fireworks displays, the MBNMS has consulted biologists from state and federal agencies and universities, local property managers and residents, environmental sensitivity index (ESI) maps prepared for the California Department of Fish and Game (CDFG) and NOAA, other environmental maps, and both published and unpublished resources. As a result, the MBNMS has added special conditions to fireworks Authorizations that are designed to minimize fireworks impacts upon resources and qualities. Jointly developed by the MBNMS, NMFS Southwest Region, and the U.S. Fish and Wildlife Service (USFWS), the special Authorization conditions help assure that protected species and habitats are not jeopardized by this activity.

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However, the application of individual Authorization conditions alone are not sufficient to assure that protected species will be adequately safeguarded from potential cumulative impacts of fireworks activity within the Sanctuary. NMFS and the USFWS thus support additional conservation measures described in sections (VI)(A)(4) and (VII)(A).

B. Description of Pyrotechnic Devices

Professional pyrotechnic devices used in firework displays can be grouped into three general categories: aerial shells (paper and cardboard spheres or cylinders ranging from 2 inches to 12 inches in diameter and filled with incendiary materials), low-level comet and multi-shot devices similar to over-the-counter fireworks such as roman candles, and set piece displays that are mostly static in nature and are mounted on the ground.

Aerial shells are launched from tubes (called mortars), using black powder charges, to altitudes of 200 to 1000 feet where they explode and ignite internal burst charges and incendiary chemicals. Most of the incendiary elements and shell casings burn up in the atmosphere; however, portions of the casings and some internal structural components and chemical residue fall back to the ground or water, depending on prevailing winds. An aerial shell casing is constructed of paper/cardboard or plastic and may include some plastic or paper internal components used to compartmentalize chemicals within the shell. Within the shell casing is a burst charge (usually black powder) and a recipe of various chemical pellets (stars) that emit prescribed colors when ignited. Table 2 describes a list of chemicals that are commonly used in the manufacturing of pyrotechnic devices. Manufacturers consider the amount and composition of chemicals within a given shell to be proprietary information and only release aggregate descriptions of internal shell components. The arrangement and packing of stars and burst charges within the shell determine the type of effect produced upon detonation.

Common Contents of Pyrotechnic Devices		
Potassium Chlorate	Strontium Nitrate	Iron
Potassium Perchlorate	Strontium Carbonate	Titanium
Potassium Nitrate	Sulfur	Shellac
Sodium Benzoate	Charcoal	Dextrine
Sodium Oxalate	Copper Oxide	Phenolic Resin
Ammonium Perchlorate	Polyvinyl Chloride	Aluminum

Table 2. List of chemicals commonly used in manufacture of polytechnic devices.

Attached to the bottom of an aerial shell is a lift charge of black powder. The lift charge and shell are placed at the bottom of a mortar that has been buried in earth/sand or affixed to a wooden rack. A fuse attached to the lift charge is ignited with an electric charge or heat source, the lift charge explodes, and propels the shell through the mortar tube and into the air to a height determined by the amount of powder in the lift charge and the weight of the shell. As the shell travels skyward, a time-delay secondary fuse is burning that eventually ignites the burst charge within the shell at peak altitude. The burst charge detonates, igniting and scattering the stars, which may, in turn, possess small secondary explosions. Shells can be launched one at a time or in a barrage of simultaneous or quick succession launches. They are designed to detonate between 200 and 1000 feet above ground level (AGL).

In addition to color shells (also known as designer or starburst shells), a typical fireworks show will usually include a number of aerial “salute” shells. The primary purpose of salute shells is to announce the beginning and end of the show and produce a loud percussive audible effect. These shells are typically two to three inches in diameter and packed with black powder to produce a punctuated explosive burst at high altitude. From a distance, these shells sound similar to cannon fire when detonated.

Low-level devices consist of stars packed linearly within a tube, and when ignited, the stars exit the tube in succession producing a fountain effect of single or multi-colored light as the stars incinerate through the course of their flight. Typically, the stars burn rather than explode, thus producing a ball or trail of sparkling light to a prescribed altitude where they simply extinguish. Sometimes they may terminate with a small explosion similar to a firecracker. Other low-level devices emit a projected hail of colored sparks or perform erratic low-level flight while emitting a high-pitched whistle. Some emit a pulsing light pattern or crackling or popping sound effects. In general, low-level launch devices and encasements remain on the ground or attached to a fixed structure and can be removed upon completion of the display. Common low-level devices are multi-shot devices, mines, comets, meteors, candles, strobe pots and gerbs. They are designed to produce effects between 0 and 200 feet AGL.

Set piece or *ground level* fireworks are primarily static in nature and remain close to the ground. They are usually attached to a framework that may be crafted in the design of a logo or familiar shape, illuminated by pyrotechnic devices such as flares, sparklers and strobes. These fireworks typically employ bright flares and sparkling effects that may also emit limited sound effects such as cracking, popping, or whistling. Set pieces are usually used in concert with low-level effects or an aerial show and sometimes act as a centerpiece for the display. It may have some moving parts, but typically does not launch devices into the air. Set piece displays are designed to produce effects between 0 and 50 feet AGL.

Each display is unique according to the type and number of shells, the pace of the show, the length of the show, the acoustic qualities of the display site, and even the weather and time of day. The vast majority (97 percent) of fireworks displays authorized in the Sanctuary between 1993 and 2005 were aerial displays that usually include simultaneous low-level displays. An average large display will last 20 minutes and include 700 aerial shells and 750 low-level effects. An average smaller display lasts approximately 7 minutes and includes 300 aerial shells and 550 low-level effects. There seems to be a declining trend in the total number of shells used in aerial displays, due to increasing shell costs and/or fixed entertainment budgets. Low-level displays sometimes compensate for the absence of an aerial show by squeezing a larger number of effects into a shorter timeframe. This results in a dramatic and rapid burst of light and sound effects at low level. A large low-level display may expend 4,900 effects within a seven-minute period, and a small display will use an average of 1,800 effects within the same timeframe. Some fireworks displays are synchronized with musical broadcasts over loudspeakers and may incorporate other non-pyrotechnic sound and visual effects. Table 3 provides a comparison of fireworks displays performed within the Sanctuary in the past.

Display Types	Duration of Display	Number of Aerial Effects	Number of Low-level Effects	Number of Set-Piece Devices
Aerial, Small	5 Minutes	300	550	0
Aerial, Large	20 Minutes	700	750	1
Aerial, Largest to Date	25 Minutes	1700	1800	0
Low-level, Small	7 Minutes	0	1800	0
Low-level, Large	7 Minutes	0	4900	1

Table 3. Comparison of fireworks displays performed within MBNMS in the past (as of 2005).

IV. ALTERNATIVES INCLUDING THE PROPOSED ACTION

A. Issuance of LOAs and Sanctuary Authorizations for 20 Fireworks Displays Annually (Preferred Alternative)

The preferred alternative is for NMFS to issue annual LOAs to MBNMS for up to five years, authorizing the incidental take, by Level B harassment, of a small number of California sea lions and Pacific harbor seals for up to 20 fireworks displays per year within the MBNMS boundaries. The MBNMS would then exercise its regulatory authority to issue Authorizations to applicants seeking permission to conduct fireworks displays within the MBNMS. The potential impacts to marine mammals from a LOA would be as described in section (VI)(A) of this document. Potential impacts to other Sanctuary resources from issuance of Sanctuary Authorizations are also described in section (VI)(A). Under this alternative, the mitigation measures and reporting requirements described in section (VII) will be incorporated into the LOAs and Sanctuary Authorizations. NMFS has determined that the fireworks displays MBNMS proposes to authorize would result in the taking by Level B harassment of only small numbers of marine mammals and have no more than a negligible impact on affected stocks. The MBNMS has determined that issuance of Sanctuary Authorizations for a limited number of fireworks displays under certain conditions and terms will not exceed negligible short-term impacts upon Sanctuary resources and qualities.

A description of the activity to be covered by the proposed LOAs and Sanctuary Authorizations was provided above. A further-detailed description of the fireworks displays authorized at MBNMS may be found in the application and the 2001 Assessment of Pyrotechnic Displays and Impacts within the MBNMS.

B. Issuance of LOAs and Sanctuary Authorizations for 7 Fireworks Displays Annually

Another alternative is for NMFS to issue annual LOAs to MBNMS for up to five years authorizing the incidental take, by Level B harassment of a small number of California sea lions and Pacific harbor seals over the course of 7 fireworks displays per year authorized by MBNMS that occur within the MBNMS boundaries. The potential impacts to marine mammals would be

as described in section (VI)(B). Under this alternative, the mitigation measures and reporting requirements described in Section (VII) would be incorporated into the LOAs and Sanctuary Authorizations. NMFS has determined that the fireworks displays MBNMS proposes to authorize would result in the harassment taking of only small numbers of marine mammals. The MBNMS has determined that issuance of Sanctuary Authorizations for a limited number of fireworks displays under certain conditions and terms will not exceed negligible short-term impacts upon Sanctuary resources and qualities.

C. Issuance of LOAs to Individual Fireworks Sponsors

A third alternative is for NMFS to issue annual LOAs to individual sponsors (e.g. municipalities, civic organizations, commercial companies) of fireworks displays within the coastal area of the MBNMS. The potential impacts to marine mammals would be as described in section (VI)(B). Under this alternative, many of the mitigation measures and reporting requirements described in Section (VII) would be incorporated into LOAs, except that MBNMS Authorization provisions would not apply. This alternative would require submission of multiple application requests and a case-by-case assessment of proposed fireworks displays by NMFS, since the MBNMS will not be serving in a coordinating role regarding MMPA requirements. This alternative would also necessitate monitoring and individual reporting by fireworks sponsors instead of consolidated reporting by the MBNMS on their behalf. Individual fireworks sponsors will be fully responsible for compliance with the terms and conditions of LOAs issued for displays conducted under their supervision.

D. No Action Alternative

The No Action Alternative would not involve the issuance of LOAs and Sanctuary Authorizations for fireworks displays within the MBNMS. The MMPA prohibits all takings of marine mammals unless authorized by a permit or exempted under the MMPA. If an authorization to incidentally take California sea lions and Pacific harbor seals were denied, the applicant could choose to amend the project to avoid harassing marine mammals or choose not to pursue the project at that location. Execution of the project without a take authorization could result in the incidental take of marine mammals in violation of the MMPA. Impacts to marine mammals would vary between no takes if fireworks are not conducted to impacts similar to those assessed for 20 displays.

If no Sanctuary Authorizations were issued for coastal fireworks displays, such displays would have to be cancelled or moved to inland sites. Execution of such displays without the issuance of Sanctuary Authorizations would likely result in the discharge of debris into Sanctuary waters and the disturbance of wildlife in violation of Sanctuary regulations.

V. DESCRIPTION OF THE AFFECTED ENVIRONMENT

A. Display Areas

The Monterey Bay area is located in the Oregonian province subdivision of the Eastern Pacific Boreal Region. The six types of habitats found in the bay area are: (1) submarine canyon habitat, (2) nearshore sublittoral habitat, (3) rocky intertidal habitat, (4) sandy beach intertidal habitat, (5) kelp forest habitat, and (6) estuarine/slough habitat. Pyrotechnic displays within the Sanctuary are conducted from a variety of coastal launch sites - beaches, bluff tops, piers, offshore barges, and golf course sand traps and tee boxes. In the past, authorized displays have been confined to eight general locations in the Sanctuary. However, these regulations authorize displays in only four prescribed areas within the Sanctuary. These sites are approved for fireworks events based on their proximity to urban areas and pre-existent high human use patterns, seasonal considerations such as the abundance and distribution of marine wildlife, and the acclimation of wildlife to human activities and elevated ambient noise levels in the area.

The four "conditional" display areas (areas authorized for displays under the NMFS regulation subject to terms and conditions imposed by MBNMS) are located at Half Moon Bay, the Santa Cruz/Soquel area, the northeastern Monterey Peninsula, and Cambria (Santa Rosa Creek). Under the preferred alternative, no more than 20 events per year may be authorized within these four specific areas of the Sanctuary's 276 mi (444 km) of coastline are authorized by this regulation.

The conditional display areas for fireworks displays must first be described in order to understand which marine mammals in the area may be affected by the activity. Monterey Bay supports a wide array of temperate cold-water species with occasional influxes of warm-water species, and this species diversity is directly related to the diversity of habitats.

1. Half Moon Bay

Site Description – The site has been used annually for a medium-sized Independence Day fireworks display on July 4, which lasts about 20 minutes. The launch site is on a sandy beach inside and adjacent to the east outer breakwater, upon which the aerial shells are launched and aimed to the southwest. The site is often fogged in during summer months. The marine venue adjacent to Pillar Point Harbor is preferred for optimal public access and to avoid the fire hazard associated with terrestrial display sites. The fireworks display occurs at the height of the dry season in central California, when area vegetation is particularly prone to ignition from sparks or embers.

Human Use Patterns – The harbor immediately adjacent to the impact area is home to a major commercial fishing fleet that operates at all times of the day and night throughout the year. The harbor also supports a considerable volume of recreational boat traffic. Half Moon Bay Airport (HAF) is located adjacent to the harbor, and approach and departure routes pass directly over the impact area. The airport is commonly used by general aviation pilots for training, with an annual average attendance of approximately 15 flights per day. On clear sunny weekends, the

airport may accommodate as many as 50 flights in a single day. Beachgoers and water sport enthusiasts use the beaches to the south of the launch site. The impact area is also used by recreational fishermen, surfers, swimmers, boaters, and personal watercraft operators. To the north, around Pillar Point is an area known as "Mavericks" considered a world-class surfing destination. Periodically, surfing contests are held at Mavericks. The impact area is also subjected to daily traffic noise from California Highway 1, which runs along the coast and is the primary travel route through the area.

Marine Mammals – A considerable concentration of harbor seals are present to the north around Pillar Point and on the coast to the south of the launch site. Within the Half Moon Bay area, depending on time of year and local environmental factors, MBNMS has estimated that an average of 20 sea lions (100 maximum) and an average of 15 harbor seals (65 maximum) may be present during a fireworks display. Sea otters are not concentrated in the impact area, though some individuals may be present. It is possible that individual elephant seals may enter the area from breeding sites at Año Nuevo Island and the Farallon Islands, but breeding occurs in the winter and displays in Half Moon Bay are limited to summer. Gray whales typically migrate west of the reefs extending south from Pillar Point.

Other Marine Wildlife – Resource information and discussions with area biologists indicate that snowy plover are present within 2 statute miles to the south of the launch site. Brown pelicans, gulls, cormorants, and other marine birds are present in the harbor where they roost on piers and other structures or rest on the calm waters within the breakwater.

2. Santa Cruz/Soquel

Site Description – Three separate fireworks display sites (Santa Cruz, Capitola, and Aptos) are located within the Santa Cruz/Soquel area. The Santa Cruz launch site has been used annually for City anniversary fireworks displays in early October. The launch site is on a sandy beach, adjacent to the Santa Cruz Boardwalk and the San Lorenzo River and along the west bank. The aerial shells are aimed to the south. The site is sometimes fogged in during summer months.

The Capitola launch site has been used only once since 1993 for a 50-year City anniversary fireworks display on May 23, 1999. This display was the largest volume fireworks display conducted in the MBNMS to date, incorporating 1700 aerial shells and 1800 low-level effects and lasting 25 minutes. The launch site was on the Capitola Municipal Pier, adjacent to the City of Capitola. The aerial shells were aimed above the pier. The site is sometimes fogged in during summer months.

The Aptos site has been used annually for a large fundraiser for Aptos area schools in October. The launch site is on the Aptos Pier and part of a grounded cement barge at Seacliff State Beach. The aerial shells are aimed above and to the south of the pier. The site is sometimes fogged in during summer months. The large aerial show lasts for approximately 20 minutes.

Human Use Patterns – The harbor immediately adjacent to the Santa Cruz impact area is home to a commercial fishing fleet that operates at all times of the day throughout the year. The harbor primarily supports a large volume of recreational boater traffic. The launch site is in the center of the shoreline of a major urban coastal city. The beaches to the west of the launch site are adjacent to a large coastal amusement park complex and are used extensively by beachgoers and water sport enthusiasts from the local area as well as San Jose and San Francisco. The impact area is used by boaters, recreational fishermen, swimmers, surfers, and other recreational users. Immediately southwest of the launch site is a mooring field and the Santa Cruz Municipal Pier which is lined with retail shops, restaurants, and offices. To the west of the pier is a popular local surfing destination known as "Steamer Lane." Surfing contests are routinely held at the site. During the period from sunset through the duration of the fireworks display, 40-70 vessels anchor within the impact area to view the fireworks. Vessels criss-cross through the waters south of the launch site to take up position. In addition, U. S. Coast Guard and harbor patrol vessels motor through the impact area to maintain a safety zone around the launch site.

The Capitola impact area is immediately adjacent to a small urban community. The beaches to the east and west of the launch site are used daily by beachgoers and water sport enthusiasts from the regional area. The impact area is used by boaters, recreational fishermen, swimmers, surfers, and other recreational users. To the east of the Pier is a mooring field and popular public beach.

The Aptos impact area is immediately adjacent to a recreational beach. The beaches to the east and west of the launch site are used daily by beachgoers and water sport enthusiasts from the regional area. The impact area is used by boaters, recreational fishermen, swimmers, surfers, and other recreational users, but typically at moderate to light levels of activity. To the east and west of the Pier are public use beach areas and private homes at the top of steep coastal bluffs. During the period from sunset through the duration of the fireworks display, 30-40 vessels anchor within the impact area to view the fireworks. Vessels criss-cross through the waters seaward of the cement barge to take up position. In addition, U. S. Coast Guard and State Park Lifeguard vessels motor through the impact area to maintain a safety zone around the launch site.

Marine Mammals – California sea lions routinely use the Santa Cruz Municipal Pier as a haulout and resting site. Sea otters are moderately concentrated in the impact area, primarily around the nearshore kelp forests. Within the Santa Cruz/Soquel area, depending on time of year, specific launch site, and local environmental factors, MBNMS has estimated that an average of 0-100 sea lions (5-190 maximum) and an average of 0-15 harbor seals (5-50 maximum) may be present during a fireworks display. Gray whales typically migrate along a southerly course, west of Point Santa Cruz and away from the pier. Sea otters are moderately concentrated in the impact areas near the Capitola Municipal Pier and Aptos Pier, primarily in and around the nearshore kelp forests. At the seaward end of the Aptos Pier is a 400-foot grounded cement barge. The barge was set in position as an extension of the pier, but has since been secured against public access. The exposed interior decks of the barge have created convenient haulout surfaces for harbor seals. In a 2000 survey, the MBNMS recorded as many as 45 harbor seals hauled out on the barge in the month of October.

Other Marine Wildlife – The Santa Cruz Municipal Pier is a roost for a large number of gulls, Brown pelicans, and other marine birds. Brown pelicans, cormorants, gulls, and other marine birds routinely use the Capitola Municipal Pier as a roosting site. Seabirds also often gather on the sand beach at the mouth of Soquel Creek where a lagoon forms in the summer. The creek empties into the ocean immediately east of the Municipal Pier. Brown pelicans, cormorants, gulls, and other marine birds routinely use the Aptos cement barge (described above) as a roosting site. The barge has broken into two parts isolating the bow section from the rest of the vessel. The isolated bow section is particularly favored by pelicans and cormorants, and contains the bulk of roosting seabirds. Black turnstones seem to favor the interior spaces of the vessel along the aft section, and gulls attend the upper portions of the aft superstructure. Approximately 1/2 statute miles to the east of the pier is the mouth of Aptos Creek where shorebirds congregate.

3. Monterey Peninsula

Site Description – Two separate fireworks display sites (City of Monterey and Pacific Grove) are located within the Monterey Peninsula Area. Each Independence Day, the City of Monterey launches approximately 750 shells and an equal number of low-level effects from a barge anchored approximately 1000 feet east of Municipal Wharf II and 1000 feet north of Del Monte Beach. The aerial shells are aimed above and to the northeast. The site is often fogged in during summer months. The City's display lasts approximately 20 minutes and is accompanied by music broadcasted from speakers on Wharf II. The marine venue adjacent to Monterey Harbor is preferred for optimal public access and to avoid the fire hazard associated with terrestrial display sites. The fireworks display occurs at the height of the dry season in central California, when area vegetation is particularly prone to ignition from sparks or embers. Since 1999, a Monterey New Year's festival has used the City's launch barge for an annual fireworks display. The medium-size aerial display lasts approximately 8 minutes. In addition, three private displays (1993, 1998, and 2000) have been authorized from a launch site on Del Monte Beach. The 1993 display was an aerial display. Subsequent displays have been low-level displays, lasting approximately 7 minutes.

The Pacific Grove site has been used annually for a "Feast of Lanterns" fireworks display in late July. The Feast of Lanterns is a community event that has been celebrated in the City of Pacific Grove for over 95 years. The fireworks launch site is at the top of a rocky coastal bluff adjacent to an urban recreation trail and public road. The aerial shells are aimed to the northeast. The site is often fogged in during summer months. The small aerial display lasts approximately twenty minutes and is accompanied by music broadcasted from speakers at Lover's Cove. The fireworks are part of a traditional outdoor play that concludes the festival. The marine venue is preferred for optimal public access and to avoid the fire hazard associated with terrestrial display sites. The fireworks display occurs at the height of the dry season in central California, when area vegetation is particularly prone to ignition from sparks or embers.

Human Use Patterns – The Monterey fireworks impact area lies directly under the approach/departure flight path for Monterey Peninsula Airport (MRY) and is commonly exposed to noise and exhaust from general aviation, commercial, and military aircraft at approximately

500 feet altitude. The airport supports approximately 280 landings/takeoffs per day in addition to touch-and-goes (landing and takeoff training). Commercial and recreational vessels operate in the area during day and night hours from the adjacent harbor. A 30-station mooring field lies within the impact area between the launch barge and Municipal Wharf II. The moorings are completely occupied during the annual fireworks event. Auto traffic and emergency vehicles are audible from Lighthouse and Del Monte Avenues, main transportation arteries along the adjacent shoreline. The impact area is utilized by thousands of people each week for boating, kayaking, scuba diving, fishing, swimming, and harbor operations. During the period from sunset through the duration of the fireworks display, 20-30 vessels anchor within the impact area to view the fireworks. Vessels criss-cross through the waters south of the launch site to take up position. In addition, U. S. Coast Guard and harbor patrol vessels motor through the impact area to maintain a safety zone around the launch site.

The Pacific Grove launch site is in the center of an urban shoreline, adjacent to a primary public beach in Pacific Grove. The shoreline to the east and west of the launch site is lined with residences and a public road and pedestrian trail. The impact area is used by boaters, recreational fishermen, swimmers, surfers, divers, beachgoers, tidepoolers, and others. The center of the impact area is in a cove with 30-40 foot coastal bluffs. Immediately north of the launch site is a popular day use beach area. On a clear summer day, the beach may support up to 500 visitors at any given time. Surfing activity is common immediately north of the site. During the period from sunset through the duration of the fireworks display, 10-20 vessels anchor within the impact area to view the fireworks. A U. S. Coast Guard vessel motors through the impact area to maintain a safety zone seaward of the launch site.

Marine Mammals – The largest concentration of wildlife near the Monterey impact area are California sea lions and marine birds resting at the Monterey breakwater approximately 700 yards northwest of the center of the impact area. Within the Monterey Bay area, depending on time of year, specific launch site, and local environmental factors, MBNMS has estimated that an average of 0-700 sea lions (150-1500 maximum) and an average of 7-50 harbor seals (60-100 maximum) may be present during a fireworks display. Several sea otters are present within Monterey Harbor and the impact area during the time of the fireworks display. Otters outside the harbor are most concentrated to the northwest of the Monterey breakwater, however, otters routinely forage and loiter within the impact area and along the shoreline to the north.

Sea otters and pups routinely forage and loiter within the Pacific Grove impact area in moderate numbers. Harbor seals routinely use offshore rocks and wash rocks for haulout and also forage in the area.

Other Marine Wildlife - Non-breeding California brown pelicans appear in greatest number in central California during the late summer and fall. Within the Monterey harbor area, pelicans roost on the Monterey breakwater; on wharfs, piers, and structures; on exposed rocks in the harbor; and on the barge used to launch pyrotechnics during the fireworks display. The southernmost documented plover nest site (no longer active) near east Monterey was located approximately 1000 yards north of the launch site. The public beaches where spectators gather for City fireworks displays are routinely groomed by municipal public works department staff

and frequented daily by beachgoers and their domestic pets. These beaches are high human use areas, and therefore, do not present optimal nesting habitat. The likelihood of successful nesting and nest survival in these high-use beach areas is low. The greatest nesting density for snowy plover in the local region is centered 6-10 statute miles to the north.

Individual cormorants and gulls often roost on offshore rocks adjacent to the Pacific Grove launch site, but there are no large concentrations of marine birds due to the high volume of human activity and lack of significant roosting habitat. A small roost site exists at Point Cabrillo, approximately 3/4 miles southeast of the launch site, and hosts aggregations of gulls, cormorants, pelicans, and other marine birds. Extensive kelp beds cover much of the impact area. The Hopkins Marine Reserve boundary is approximately 1/2 statute mile southeast of the launch site.

4. Cambria

Site Description – The site has been used annually for a small Independence Day fireworks display on July 4, which lasts approximately 20 minutes. The launch site is on a sandy beach at Shamel County Park, and the aerial shells are aimed to the west. Immediately north of the launch site is the mouth of Santa Rosa Creek and Lagoon. The marine venue is preferred for optimal public access and to avoid the fire hazard associated with terrestrial display sites. The fireworks display occurs at the height of the dry season in central California, when area vegetation is particularly prone to ignition from sparks or embers.

Human Use Patterns – The impact area is immediately adjacent to a county park and recreational beach. The impact area is used by boaters, recreational fishermen, swimmers, surfers, and beachgoers. The shoreline south of the launch site is lined with hotels, abuts a residential neighborhood, and is part of San Simeon State Beach.

Marine Mammals – The impact area includes low concentrations of harbor seals. Sea otters and sea lions are present in the impact area in moderate numbers. Within the Cambria area, depending on time of year, specific launch site, and local environmental factors, MBNMS has estimated that an average of 0 sea lions (25-50 maximum) and an average of 20 harbor seals (60 maximum) may be present during a fireworks display. It is possible that individual elephant seals may enter the area from breeding sites to the north at Point Piedras Blancas, but breeding occurs in the winter and displays at Cambria are limited to the summer. Gray whales migrate along the coast in this area and may pass through the impact area, but July is not peak gray whale migration period.

Other Marine Wildlife - Immediately north of the launch site is the mouth of Santa Rosa Creek and Lagoon. Gulls, shorebirds, and waterfowl are commonly found in the lagoon. Snowy plover habitat is located 1 1/2 miles to the north of the launch site.

B. Marine Mammals Potentially Found in the Area

Twenty-six species of marine mammals have been observed in the Monterey Bay area, including five species of the sub-order pinnipeds (seals and sea lions), one species from the sub-order fissipeds (sea otter), and twenty species of the order cetaceans (whales and dolphins). Of these, the species of marine mammals that are likely to be present in any of the four fireworks display impact zones at the time of fireworks displays include the California sea lion *(Zalophuus californianus)*, Pacific harbor seal *(Phoca vitulina richardsi)*, bottlenose dolphin *(Tursiops truncatus)*, harbor porpoise *(Phocena phocena)*, California gray whale *(Eschrichtius robustus)*, and Southern sea otters *(Enhydra lutris neries)*. One additional species that would be found only rarely within fireworks impact zones at the time of display is the northern elephant seal *(Mirounga angustirostris)*. General information on these species can be found in Folkens' *Guide to the Marine Mammals of the World* (2002). Information relevant to the distribution, abundance and behavior of the species that are most likely to be impacted by fireworks displays within the MBNMS is provided below. Additional information regarding these species may be found the FR Notice for the IHA (68 FR 28810, May 27, 2003) and in the NMFS stock assessments on the NMFS website: http://www.nmfs.noaa.gov/pr/PR2/Stock_Assessment_Program/individual_sars.html. Relevant information from these sources on these species is incorporated by reference.

1. California Sea Lions *(Zalophuus californianus)*

The population of California sea lions ranges from southern Mexico to southwestern Canada (Caretta et al., 2004). In the U.S., they breed during July after pupping in late May to June, primarily in the Channel Islands of California. Most individuals of this species breed on the Channel Islands off southern California (100 miles south of the MBNMS) and off Baja and mainland Mexico (Odell 1981), although a few pups have been born on Año Nuevo Island (Keith et al., 1984). Following the breeding season on the Channel Islands, most adult and sub-adult males migrate northward to central and northern California and to the Pacific Northwest, while most females and young animals either remain on or near the breeding grounds throughout the year or move southward or northward, as far as Monterey Bay.

Since nearing extinction in the early 1900's, the California sea lion population has increased and is now robust and growing at a current rate of 5.4 to 6.1 percent per year (based on pup counts) with an estimated "minimum" population (U.S. West Coast) of 138,881 animals. Actual population level may be as high as 237,000 to 244,000 animals. The population is not listed as "endangered" or "threatened" under the Endangered Species Act (ESA); nor is this species designated as "depleted" or classified as a "strategic stock" under the MMPA.

In any season, California sea lions are the most abundant pinniped in the area (Bonnell et al., 1983), primarily using the central California area to feed during the non-breeding season. After breeding farther south along the coast and migrating northward, populations peak in the Monterey Bay area in fall and winter and are at their lowest numbers in spring and early summer. A minimum of 12,000 California sea lions is probably present at any given time in the MBNMS

region. Año Nuevo Island is the largest single haul-out site in the Sanctuary, hosting as many as 9,000 California sea lions at times (Weise, 2000 and Lowry, 2001).

2. Harbor Seal *(Phoca vitulina richardsi)*

Harbor seals are distributed throughout the west coast of the U.S., inhabiting near-shore coastal and estuarine areas from Baja California, Mexico, to the Pribilof Islands in Alaska. They generally do not migrate, but have been known to travel extensive distances to find food or suitable breeding areas (Caretta et al., 2004). In California, approximately 400-500 harbor seal haulout sites are widely distributed along the mainland and on offshore islands (Caretta et al., 2004).

The harbor seal population in California is healthy and growing at a current rate of 3.5 percent per year with an estimated "minimum" population (California) of 25,720 animals (Caretta et al., 2004). The California population is estimated at 27,863 animals. The population is not listed as "endangered" or "threatened" under the ESA; nor is this species designated as "depleted" or classified as a "strategic stock" under the MMPA.

Harbor seals are residents in the MBNMS throughout the year, occurring mainly near the coast. They haul out at dozens of sites along the coast from Point Sur to Año Nuevo. Within MBNMS, tagged harbor seals have been documented to move substantial distances (10-20 km) to foraging areas each night (Oxman 1995, Trumble 1995). The species does breed in the Sanctuary, and pupping within the Sanctuary occurs primarily during March and April followed by a molt during May and June. Peak abundance on land within the Sanctuary is reached in late spring and early summer when they haul out to breed, give birth to pups, and molt (MBNMS Final Environmental Impact Statement (FEIS), 1992).

3. Southern Sea Otters *(Enhydra lutris neries)*

The southern sea otter population presently contains about 2,150 animals, and can be found along the coast of central and southern California from Half Moon Bay to Point Conception (USFWS, 2003). They can be found throughout the shallow waters of Monterey Bay from Pismo Beach to Año Nuevo Island. Approximately 31 percent of this population is currently found in the area from Point Sur north to Año Nuevo/Pigeon Point. Southern sea otters breed and give birth year round, however the seasonality is not highly synchronous and the birth peak may extend over several months.

Range-wide population counts declined at a rate of approximately 5 percent per year between 1995 and 1999, although the population's range expanded both to the north and the south. The current population status is less certain, with recent counts being relatively stable (USFWS, 2003). The southern sea otter is listed as "threatened" under the ESA, and is therefore also designated as "depleted" under the MMPA. Take of southern sea otters is regulated by the USFWS.

Within the MBNMS, sea otters inhabit a narrow zone of coastal waters, normally staying within one mile from shore (MBNMS FEIS, 1992). They forage in both rocky and soft-sediment communities as well as in the kelp understory and canopy. They seldom are found in open waters deeper than 30 m, preferring instead the kelp beds, which serve as vital resting, foraging, and nursery sites. An official state-designated Sea Otter Game Refuge extends from Carmel south to Santa Rosa Creek near Cambria, encompassing about half the otter's established range.

4. California Gray Whale *(Eschrichtius robustus)*

The latest abundance estimate is 26,635, based on counts made during the 1997/1998 southbound migration; however, the population size of this species has increased slightly over the past few decades (Caretta et al., 2002). Because of these increases, in 1994 the gray whale was de-listed from its "endangered" under the ESA, and was also undesignated as "depleted" under the MMPA.

Gray whales are seasonal migrants, traveling close to shore, and are the object of most of the whale watching in the area. They pass through the area of the Sanctuary twice during their yearly migrations. The peak northward migration of male gray whales occurs in mid-March, followed two months later by the second migration wave, which is composed of cows and calves. These whales migrate from wintering grounds in Baja California, Mexico, northward to Alaska. The southbound migration occurs in late December and January, from their breeding grounds in the north back down to the south. The species does not breed in the Sanctuary.

No California gray whales have ever been sighted in fireworks impact areas during displays. Display locations within Monterey Bay are not immediately adjacent to the prime coastal migration route, since most gray whales bypass the inner shorelines of the bay, instead transiting between Point Piños and Point Santa Cruz. Likewise, the Half Moon Bay display occurs east of the natural reef barrier between the migration route and the shoreline. The only remaining display site that might impact gray whales is at Cambria, but the current display authorized for the area occurs in July, outside of the prime migration seasons.

5. Bottlenose dolphin *(Tursiops truncatus)*

Bottlenose dolphins are distributed world-wide in tropical and warm-temperate waters, including California where separate coastal and offshore populations are known to exist (Caretta et al., 2004). Relative to the location of the MBNMS, California coastal bottlenose dolphins are found within about 1 kilometer of shore primarily from Point Conception south into Mexican waters. Bottlenose dolphins are found in small numbers (12-18) within the bay seemingly on a year-round basis (MBNMS FEIS, 1992). The best current estimate of the average number of coastal bottlenose dolphins from this stock in this area is 206 animals (Caretta et al., 2004). This species is not listed under the ESA or listed as depleted under the MMPA.

6. Harbor porpoise *(Phocena phocena)*

In the Pacific Ocean, harbor porpoises are found in coastal and inland waters from Point Conception, CA to Alaska and across the Pacific to Kamchatka and Japan (Barlow et al., 1995, Gaskin 1984). This species appears to have more restricted movements along the west coast of the continental U.S. than along the eastern coast. Harbor porpoises prefer shallow waters, and can usually be found over sandy bottoms just off the surf in the north central part of the bay.

Based on aerial surveys from 1997-1999 under good survey conditions, the estimate of abundance for the Monterey Bay stock of this species is 1,603 animals with a minimum abundance estimate of 1,143 animals (Caretta et al., 2002). Population growth has not been measured for any harbor porpoise population (Caretta et al., 2002). This species is not listed under the ESA or listed as depleted under the MMPA.

7. Northern elephant seal *(Mirounga angustirostris)*

Northern elephant seals breed and give birth in California (U.S.) and Baja California (Mexico), primarily on offshore islands (Stewart et al.,1994), in the winter months from December to March (Stewart and Huber, 1993). They then disperse to feed in pelagic waters throughout the eastern North Pacific. Adults return to land between March and August to molt, with males returning later than females (Caretta et al., 2002).

Elephant seals nearly became extinct in the past century, but have undergone a remarkable sustained population growth, and colonies continue to grow. Based on an estimated 28,845 pups born in California in 2001, the California stock was estimated to be 101,000 in 2001, while the minimum population size was estimated conservatively to be 60,547 Caretta et al., 2004). They are not listed under the ESA or listed as depleted under the MMPA.

Peak abundances on land within the MBNMS occur in the spring when juvenile males and females haulout to molt. The breeding population at these locations presently numbers about 3,500 animals, and the spring population on land exceeds 4,000 animals (MBNMS FEIS, 1992). The largest populations are on Año Nuevo Island and the adjacent mainland point. Estimates based on population structure indicate that elephant seals of the Año Nuevo colony account for about 4% of the entire world population of this species (MBNMS FEIS, 1992). The elephant seal would only rarely be found within the fireworks areas of the MBNMS.

C. Other Protected Marine Wildlife Potentially Found in the Area

1. Brown Pelican (*Pelecanus occidentalis*)

The brown pelican was federally listed as endangered in 1970 (35 *Federal Register* 16047). The recovery plan for the brown pelican describes the biology, reasons for decline, and actions needed for recovery of the species (USFWS, 1983). Critical habitat for the brown pelican has not been designated.

The California brown pelican is one of six recognized subspecies of the brown pelican. The brown pelican is a large bird recognized by the long, pouched bill that is used to catch surface-schooling fishes. The California brown pelican weighs up to ten pounds and has a wingspan of up to eight feet.

The brown pelican is a conspicuous resident along the coasts of California and Baja California. Brown pelicans nest in colonies on small coastal islands that are free of mammalian predators and human disturbance. They are associated with an adequate and consistent food supply and areas with appropriate roosting sites for both resident and migrant pelicans (USFWS 1983). During the non-breeding season, brown pelicans roost communally in areas that are near adequate food supplies, have some type of physical barrier to predation and disturbance, and that provide some protection from environmental stresses such as wind and high surf. Offshore rocks, breakwaters, and jetties are often used for roosting.

The breeding distribution of the California brown pelican ranges from the Channel Islands of southern California southward to the islands off Nayarit, Mexico. When not breeding, pelicans may range as far north as Vancouver Island, British Columbia, Canada, and south to Colima, Mexico. The maximum breeding population of the California brown pelican throughout its range may number about 55,000 to 60,000 pairs. The largest breeding group is located on the Gulf of California, comprising approximately 68 percent of the total breeding population. Only two breeding colonies exist in the United States. These are located on Anacapa and Santa Barbara Islands. In the past, breeding occurred as far north as Point Lobos near Monterey.

Brown pelicans are seasonally present at all general fireworks display locations within the MBNMS and react to fireworks in the same general manner as other marine birds. Pelicans do not nest or breed in the Sanctuary.

2. Western Snowy Plover (*Charadrius alexandrinus nivosus*)

The Pacific coast population of the western snowy plover was federally listed as threatened on March 5, 1993 (58 *Federal Register* 12864). A draft recovery plan for the western snowy plover has been completed (USFWS, 2001).

Critical habitat for this taxa was designated for 28 units along the coasts of Washington, Oregon, and California on December 7, 1999 (64 *Federal Register* 68508). The primary constituent elements for western snowy plover critical habitat include space for individual and population growth, and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, and rearing of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species. The primary constituent elements are found in areas that support or have the potential to support intertidal beaches (between mean low water and mean high tide), associated dune systems, and river estuaries. Important components of the beach/dune/estuarine ecosystem include surf-cast kelp, sparsely vegetated foredunes (beach area immediately in front of a sand dune), interdunal flats (flat land between dunes), spits, washover areas, blowouts (a hole or cut in a dune caused by storm action), intertidal flats (flat

land between low and high tides), salt flats, flat rocky outcrops, and gravel bars. Several of these components (sparse vegetation, salt flats) are mimicked in artificial habitat types used less commonly by snowy plovers (*i.e.*, dredge spoil sites and salt ponds and adjoining levees).

The western snowy plover is one of 12 subspecies of the snowy plover (*Charadrius alexandrinus*). The species occurs within the family Charadriidae. The western snowy plover is a small, pale-colored shorebird with dark patches on either side of the upper breast.

Western snowy plovers prefer coastal beaches that are relatively free from human disturbance and predation. Sand spits, dune-backed beaches, beaches at creek and river mouths, and salt pans at lagoons and estuaries are the preferred habitats for nesting plovers. Several of these components (*e.g.*, sparse vegetation, salt flats) are mimicked in artificial habitat types used less commonly by western snowy plovers.

Western snowy plovers tend to be gregarious during the winter months. Western snowy plovers are primarily visual foragers, feeding on invertebrates in the wet sand and surf-cast kelp within the intertidal zone, in dry, sandy areas above the high tide, on salt pans, on spoil sites, and along the edges of salt marshes, salt ponds, and lagoons.

The Pacific coast population of the western snowy plover breeds primarily on coastal beaches from southern Washington to southern Baja California, Mexico. Historically, western snowy plovers bred or wintered at 157 locations on the Pacific coast, including 133 sites in California. Larger numbers of birds are found in southern and central California, in Monterey Bay (estimated 200 to 250 breeding adults), Morro Bay (estimated 85 to 93 breeding adults), Pismo Beach to Point Sal (estimated 130 to 246 breeding adults), Vandenberg Air Force Base (estimated 130 to 240 breeding adults), and the Oxnard Lowland (estimated 69 to105 breeding adults).

During the non-breeding season, western snowy plovers may remain at breeding sites or may migrate to other locations. Most winter south of Bodega Bay, California. Many birds from the interior population winter on the central and southern coast of California.

Western snowy plovers bred at 53 coastal locations in California prior to 1970. Between 1970 and 1981, western snowy plovers stopped breeding in parts of San Diego, Ventura, and Santa Barbara counties, most of Orange County, and all of Los Angeles County (Page and Stenzel 1981). By 1991, 78 percent of the remaining breeding population in coastal California nested at only eight sites: San Francisco Bay, Monterey Bay, Morro Bay, Callendar-Mussel Rock dunes area, the Point Sal to Point Conception area (Vandenberg Air Force Base), Oxnard lowlands, Santa Rosa Island, and San Nicolas Island (Page et al., 1991).

Five critical habitat units for the Pacific coast population of the western snowy plover have been designated within the area where fireworks events may be authorized. Some of these units are subdivided into one or more subunits. These areas include the Half Moon Bay Beaches (one subunit), the Santa Cruz Coast Beaches (four subunits), Monterey Beaches (five subunits), Point Sur Beach (one subunit), and Arroyo Hondo Creek Beach (one subunit).

3. Other Marine Birds

Cormorants and gulls commonly forage, roost, and nest near most fireworks launch sites. These species are common throughout the MBNMS and nest in the spring and early summer months on piles, dolphins, piers, buildings, and coastal rocks and structures. Their population numbers are healthy and growing, and birds inhabiting urban areas have adapted to increased noise levels caused by various human activities.

Other marine birds occasionally found near fireworks sites on a seasonal basis are sooty shearwaters, western grebes, common loons and surf scoters. None of these birds nest within the MBNMS nor roost onshore. All enter the Sanctuary to forage during non-breeding seasons. Loons, grebes, and scoters appear in the Sanctuary in modest numbers during late fall and winter months. Shearwaters are true pelagic seabirds that appear throughout the Sanctuary in large aggregations totaling tens of thousands from spring until early fall.

The USFWS has determined that the protected marine bird species marbled murrelet, California condor, California clapper rail, California least tern do not occur in assigned fireworks display areas and are thus not likely to be impacted by authorized fireworks activity.

VI. ENVIRONMENTAL CONSEQUENCES

A. Issuance of LOAs and Sanctuary Authorizations For 20 Fireworks Displays (Preferred Alternative)

1. Potential Direct Effects on Marine Mammals and Other Sanctuary Resources – Sound and Light

Marine mammals can be impacted by fireworks displays in three ways: light, sound, and debris. The primary causes of disturbance are light flashes and sound effects from exploding fireworks. Pyrotechnic devices that operate at higher altitudes are more likely to have a larger impact area (such as aerial shells), while ground and low-level devices have more confined effects. The impact area is defined as the area where sound, light, and debris effects have direct impacts on marine organisms and habitats. Direct impacts include, but are not limited to, immediate physical and physiological impacts such as abrupt changes in behavior, flight response, diving, evading, flushing, cessation of feeding, and physical impairment or mortality.

The largest commercial aerial shells used within the Sanctuary are 10-12 inches in diameter and reach a maximum altitude of 1000 feet AGL. The bursting radius of the largest shells is approximately 850 feet. The impact area can extend from 1 to 2 statute miles from the center of the detonation point depending on the size of the shell, height of the explosions, type of explosions, wind direction, atmospheric conditions, and local topography.

Aerial shells produce flashes of light that can be brilliant (exceeding 30,000 candela[8]) and can occur in rapid succession. Loud explosive and crackling sound effects stem primarily from salutes (described earlier) and bursting charges at altitude. People and wildlife on the ground

and on the surface of the water can feel the sound waves and the accompanying rapid shift of ambient atmospheric pressure. This pressure wave has been known to activate car alarms that detect vibration. Sounds attenuate farther from high altitude shells than low altitude shells since they are not as easily masked by buildings and landforms, allowing the sound envelope to ensonify more surface area on the ground and water. The sound from the lifting charge detonation is vectored upward through the mortar tube opening and reports as a dull thump to bystanders on the ground, far less conspicuous than the high-level aerial bursts. The intensity of an aerial show can be amplified by increasing the number of shells used, the pace of the barrage, and the length of the display.

Low-level devices reach a maximum altitude of 200 feet AGL. The impact area can extend to 1 statute mile from the center of the ignition point depending on the size and flight patterns of projectiles, maximum altitude of projectiles, the type of special effects, wind direction, atmospheric conditions, and local structures and topography. Low-level devices also produce brilliant flashes and fountains of light and sparks accompanied by small explosions, popping, and crackling sounds. Since they are lower in altitude than aerial shells, sound and light effects impact a smaller area. Low-level devices do not typically employ large black powder charges like aerial shells, but are often used in large numbers in concert with one another and in rapid succession, producing very intense localized effects.

Set Pieces are stationary, do not launch any encased effects into the air, and produce effects between 0 and 50 ft AGL. Small pellets of a pyrotechnic composition, such as those from sparklers or roman candles may be expelled a short distance into the air. Loud, but not explosive, noises, such as crackling, popping, or whistling may emanate from a set piece, though they are usually used in concert with low-level effects and aerial displays. Depending on the size and height of the structure, the number and type of effects, wind direction, and local topography, the impact area can extend up to 0.5 mile from the center of the ignition point, though fallout is generally confined within a 100 yard radius. Residue may include smoke, airborne particulates, fine solids, and slag.

The primary impact to wildlife noted in past observation reports by Sanctuary staff is the disturbance of marine mammals and seabirds from the light and sound effects of the exploding aerial shells. The loud sound bursts and pressure waves created by the exploding shells appear to cause more wildlife disturbance than the illumination effects. In particular, the percussive aerial salute shells have been observed to elicit a strong flight response in California sea lions and marine birds in the vicinity of the impact area (within 800 yards of the launch site).

a. Physical Impairment

In 2001, the MBNMS and USFWS monitored the July 4 City of Monterey fireworks display with the most thorough effort to date. Monitors recorded species abundance before, during, and after the event and measured the decibel level of exploding fireworks. A hand-held decibel meter was located aboard a vessel adjacent to the Monterey Breakwater, approximately one half mile from the fireworks launch site. The highest sound pressure level (SPL) reading observed on the decibel meter during the fireworks display (which did not include aerial salutes)

was 82 decibels. In the Vandenberg Air Force Base (VAFB) studies (described in sub-section b. below), some harbor seals remained at their haul-out during a space rocket launch until the sound exposure level (SEL) was 100 decibels or above (which, in the case of the VAFB launch locations and durations, is equivalent to an SPL of 89 to 95 decibels), and only short-term effects were detected. The typical decibel levels for the display ranged from 70 to 78 decibels, and no salute effects were used in the display. An ambient noise level of 58 decibels was recorded at the survey site 30 minutes following the conclusion of the fireworks. The final regulations for incidental take of marine mammals during fireworks displays include an acoustic monitoring requirement to measure sound levels at the Monterey Breakwater (where sea lions typically haul out) during the 2006 City of Monterey Fourth of July fireworks display (which will include aerial salutes).

Permanent (auditory) threshold shift (PTS) occurs when there is physical damage to the sound receptors in the ear. In some cases there can be total or partial deafness, while in other cases the animal has an impaired ability to hear sounds in specific frequency ranges. Although there is no specific evidence that exposure to fireworks can cause PTS in any marine mammals, physical damage to a mammal's ears can potentially occur if it is exposed to sound impulses that have very high peak pressures, especially if they have very short rise times (time required for sound pulse to reach peak pressure from the baseline pressure). Such damage can result in a permanent decrease in functional sensitivity of the hearing system at some or all frequencies.

Temporary (auditory) threshold shift (TTS) is the mildest form of hearing impairment that can occur during exposure to a strong sound (Kryter, 1985). When an animal experiences TTS, its hearing threshold rises and a sound must be stronger in order to be heard. TTS can last from minutes or hours to (in cases of strong TTS) days. Richardson et al. (1995) note that the magnitude of TTS depends on the level and duration of noise exposure, among other considerations. For sound exposures at or somewhat above the TTS threshold, hearing sensitivity recovers rapidly after exposure to the noise ends.

Temporary or permanent hearing impairment is a possibility when marine mammals are exposed to very strong sounds, but there has been no specific documentation of this for marine mammals exposed to fireworks. Based on current information, NMFS precautionarily sets impulsive sounds equal to or greater than 190 dB re 1 microPa (rms) as the exposure thresholds for onset of Level A harassment (injury or mortality) for pinnipeds, *in water* (NMFS, 2000). If measured by an inanimate receiver 190 dB re 1 microPa (rms) would equal an A-weighted sound intensity level of 128 dB re 20 microPa, which are the units used for airborne sound. However, environmental conditions and the ear of the receiving animal may alter how the sound is received in air versus water, and precise exposure thresholds for airborne sounds have not been agreed upon.

Some factors that contribute to onset of PTS are as follows: (1) exposure to single very intense noises, (2) repetitive exposure to intense sounds that individually cause TTS but not PTS, and (3) recurrent ear infections or (in captive animals) exposure to certain drugs.

Given the frequency, duration, and intensity of sounds (maximum measured 82 dB for larger aerial shells) that marine mammals may be exposed to, it is unlikely that they would sustain temporary, much less permanent, hearing impairment during fireworks displays.

In order to determine if harbor seals experience any change in their hearing sensitivity as a result of launch noise, researchers at VAFB conducted Auditory Brainstem Response (ABR) testing on 10 harbor seals prior to, and after, the launches of 3 Titan IV rockets (one of the loudest launch vehicles at the south VAFB haul-out site). Detailed analysis of the changes in waveform latency and waveform replication of the ABR measurements showed that there were no detectable changes in the seals' hearing sensitivity as a result of the launch noise, which ranged from an A-weighted SPL Lmax of 111.4 to 111.2 dB and an A-weighted SEL from 96.6 to 103.6 (SEL is an energy metric that takes duration of the sound into account, and since the rocket sounds last more than one second, SEL is higher than SPL) (SRS Technologies, 2001).

b. Behavioral Response

In some display locations, marine mammals and other wildlife may avoid or temporarily depart the impact area during the hours immediately prior to the beginning of the fireworks display due to increased human recreational activities associated with the overall celebration event (noise, boating, kayaking, fishing, diving, swimming, surfing, picnicking, beach combing, tidepooling, etc.), and as a fireworks presentation progresses, most marine mammals and birds generally evacuate the impact area. In particular, a flotilla of recreational and commercial boats usually gathers in a semi circle within the impact area to view the fireworks display from the water. From sunset until the start of the display, security vessels of the U.S. Coast Guard and/or other government agencies often patrol throughout the waters of the impact area to keep vessels a safe distance from the launch site.

Non-nesting marine birds (especially pelicans, cormorants, and gulls) are among the first wildlife to evacuate the area at the start of fireworks displays. Past observations by the MBNMS indicate that virtually all birds within the impact area depart in a burst of flight within one minute of the start of a fireworks display, including low-level displays. However, staff have also repeatedly observed that Brandt's cormorants nesting at the Monterey Breakwater remain on their nests (over 200 nests) throughout the large July 4th aerial display that is launched each year from a barge approximately 900 yards away. Most non-nesting marine birds on the breakwater evacuate the area until the conclusion of the display. Their numbers return to normal levels by the following morning. During a 1998 display in Monterey, MBNMS staff observed a marine bird swim within 70 yards of the launch site during the fireworks display. The bird remained on the water as the pyrotechnic effects were ignited aboard the barge and made no effort to swim away from the launch site. No injuries, fatalities, or negative impacts to marine birds have been detected during several years of monitoring and observations by the MBNMS.

Sea lions have been observed evacuating haul-out areas upon initial detonation of fireworks, and then returning to the haul-out sites within 4 to 15 hours following the end of the fireworks display. Harbor seals have been seen to remain in the water after initial fireworks detonation around the haul-out site. Sea lions in general are more tolerant of noise and visual

disturbances than harbor seals - adult sea lions have likely habituated to many sources of disturbance and are therefore much more tolerant to nearby human activities. For both pinniped species, pups and juveniles are more likely to be harassed when exposed to disturbance than older animals. In general, marine wildlife depart or avoid surface waters and haul-out sites within a 1000-yard radius of the center of the impact area during fireworks displays. Even short, low-level displays can cause a flight response in wildlife within the impact area (fireworks report).

NMFS and MBNMS found no peer-reviewed literature that specifically investigates the response of California sea lions and harbor seals to commercial fireworks displays. Similarly, general harassment or injury thresholds for exposure to airborne sounds have not been set. However, extensive studies have been conducted at VAFB to determine responses by California pinnipeds to the effects of periodic rocket launches, the light and sound effects of which would be roughly similar to the effects of pyrotechnic displays, but of greater intensity. This ongoing scientific research program has been conducted since 1997 to determine the long-term cumulative impacts of space vehicle launches on the haul-out behavior, population dynamics and hearing acuity of harbor seals at VAFB. In addition, when prediction models projected that a sonic boom from the rocket launches would hit one of the northern Channel Islands, pinniped populations were studied at identified haul-out sites in order to determine the impact of the sound wave on pinniped behavior.

The response of harbor seals to rocket launch noise at VAFB depended on the intensity of the noise (dependent on the size of the vehicle and its proximity) and the age of the seal (SRS Technologies 2001). Not surprisingly, the highest noise levels are typically from launch vehicles with launch pads closest to the haul-out sites. The percentage of seals leaving the haul-out increases with noise level up to approximately 100 decibels (dB) A-weighted SEL, after which almost all seals leave, although recent data has shown that an increasing percentage of seals have remained on shore, and those that remain are adults. Given the high degree of site fidelity among harbor seals, it is likely that those seals that remained on the haul-out site during rocket launches had previously been exposed to launches; that is, it is possible that adult seals have become acclimated to the launch noise and react differently than the younger inexperienced seals. Of the 20 seals tagged at VAFB, 8 (40 percent) were exposed to at least 1 launch disturbance but continued to return to the same haul-out site. Three of those seals were exposed to 2 or more launch disturbances. Most of the seals exposed to launch noise (n=6, 75 percent) appeared to remain in the water adjacent to the haul-out site and then returned to shore within 2 to 22 minutes after the launch disturbance. Of the 2 remaining seals that left the haul-out after the launch disturbance, both had been on shore for at least 6 hours and returned to the haul-out site on the following day (SRS Technologies 2001).

The launches at VAFB do not appear to have had long-term effects on the harbor seal population in this area. The total population of harbor seals at VAFB is estimated to be 1,040 animals and has been increasing at an annual rate of 12.6 percent. Since 1997, there have been 5 to 7 space vehicle launches per year and there appears to be only short-term disturbance effects to harbor seals as a result of launch noise (SRS Technologies, 2001). Harbor seals will

temporarily leave their haul-out when exposed to launch noise; however they generally return to the haul-out within one hour.

On San Miguel Island, when California sea lions and elephant seals were exposed to sonic booms from vehicles launched on VAFB, sea lion pups were observed to enter the water, but usually remained playing in the water for a considerable period of time. Some adults approached the water, while elephant seals showed little to no reaction. This short-term disturbance to sea lion pups does not appear to have caused any long-term effects to the population.

The conclusions of the five-year VAFB study are almost identical to the MBNMS observations of pinniped response to commercial fireworks displays. Observed impacts have been limited to short-term disturbance only and NMFS believes that the fireworks activities would have a negligible impact on the affected pinniped species and stocks.

c. *Sea Otters*

Past Sanctuary observations have not detected any disturbance to California sea otters as a result of the fireworks displays; however, past observations have not included specific surveys for this species. Sea otters do frequent all general display areas. Sea otters and other species may temporarily depart the area prior to the beginning of the fireworks display due to increased human activities.

Some sea otters in Monterey harbor have become quite acclimated to very intense human activity, often continuing to feed undisturbed as boats pass simultaneously on either side and within 20 feet of the otters. It is therefore possible that select individual otters may have a higher tolerance level than others to fireworks displays. Otters in residence within the Monterey harbor display a greater tolerance for intensive human activity than their counterparts in more remote locations.

The USFWS is responsible for regulating the take of southern sea otters. The USFWS issued a biological opinion on June 22, 2005, which concluded that the authorization of fireworks displays, as proposed in the preferred alternative, is not likely to jeopardize the continued existence of endangered and threatened species within the Sanctuary or to destroy or adversely modify any listed critical habitat. The USFWS further found that MBNMS would be unlikely to take any southern sea otters, and therefore issued neither an incidental take statement under the ESA nor an IHA. Further information may be found in the USFWS' Biological Opinion for the Authorization of Fireworks Displays Within the Monterey Bay National Marine Sanctuary, San Mateo, Santa Cruz, Monterey, and San Luis Obispo Counties, California (1-8-02-F-33).

d. Cetaceans

Though the aforementioned species are known to frequent nearshore areas within the Sanctuary, they have never been reported in the vicinity of a fireworks display, nor have there

been any reports to the MBNMS of strandings or injured/dead animals discovered after any display. Since sound does not transmit well between air and water, these animals would likely not encounter the effects of fireworks except when surfacing for air. NMFS does not anticipate any impacts to cetaceans and they are not addressed further in this document.

e. Pinnipeds

The northern elephant seal is seen infrequently in the areas with fireworks displays and NMFS believes that they are not likely to be impacted by fireworks displays. Therefore, the only pinniped species likely to be harassed by the fireworks displays, and further addressed in this document, are the California sea lion and the Pacific harbor seal.

Past monitoring by the MBNMS has identified only a short-term harassment of animals by fireworks displays, with the primary causes of disturbance being sound effects and light flashes from exploding fireworks. Additionally, the VAFB study of the effects of rocket-launch noise, which is more intense than fireworks noise, on California sea lions and Pacific harbor seals indicated only short-term behavioral impacts. With the mitigation measures proposed below, takes will be limited to the temporary incidental harassment of California sea lions and Pacific harbor seals due to evacuation of usual and accustomed haul-out sites for as little as 15 minutes and as much as 15 hours following any fireworks event. Most animals depart affected haul-out areas at the beginning of the display and return to previous levels of abundance within 4 to 15 hours following the event. This information is based on observations made by Sanctuary staff over an eight-year period (1993-2001) and a quantitative survey made in 2001. Empirical observations have focused on impacts to water quality and selected marine mammals and birds in the vicinity of the displays. No observations were made in upland areas (beyond the jurisdiction of the Sanctuary) due to limited staff resources.

California Sea Lions

Sea lions in general are more tolerant to noise and visual disturbances than harbor seals. In addition, pups and juveniles are more likely to be harassed when exposed to disturbance than the older animals. Adult sea lions have likely habituated to many sources of disturbance and are therefore much more tolerant of human activities nearby. Of all the display sites in the Sanctuary, California sea lions are only present in significant concentrations at Monterey. The following is an excerpt from a 1998 MBNMS staff report on the reaction of sea lions to a large aerial fireworks display in Monterey:

> In the first seconds of the display, the sea lion colony becomes very quiet, vocalizations cease, and younger sea lions and all marine birds evacuate the breakwater. The departing sea lions swim quickly toward the open sea. Most of the colony remains intact until the older bulls evacuate, usually after a salvo of overhead bursts in short succession. Once the bulls depart, the entire colony follows suit, swimming rapidly in large groups toward the open sea. A select few of the largest bulls may sometimes remain on the breakwater. Sea lions have

been observed attempting to haul out onto the breakwater during the fireworks display, but most are frightened away by the continuing aerial bursts.

Sea lions begin returning to the breakwater within 30 minutes following the conclusion of the display but have been observed to remain quiet for some time. The colony usually reestablishes itself on the breakwater within 2-3 hours following the conclusion of the display, during which vocalization activity returns. Typically, the older bulls are the first to renew vocalization behavior (within the first hour), followed by the younger animals. By the next morning, the entire colony seems to be intact and functioning with no visible sign of abnormal behavior.

In the 2001 Monterey survey (discussed earlier), most animals were observed to evacuate haul-out areas upon the initial report from detonated fireworks. Surveys continued for 4.5 hours after the initial disturbance and numbers of returning California sea lions remained at less than 1% of pre-fireworks numbers. When surveys resumed the next morning (13 hours after the initial disturbance), sea lion numbers on the breakwater equaled or exceeded pre-fireworks levels. MBNMS staff have been opportunistically monitoring sea lions at the City of Monterey's Fouth of July celebration for more than 10 years. The following is a summary of their general observations: sea lions begin leaving the breakwater as soon as the fireworks begin, evacuate completely after an aerial salute or quick succession of loud effects, usually begin returning within a few hours of the end of the display, and are present on the breakwater at pre-firework numbers by the following morning.

Pacific Harbor Seals

Up to 15 harbor seals may typically be present on rocks in the outer Monterey harbor in early July. The seal haulout area is approximately 2,100 ft (640 m horizontal distance) from the impact zone for the aerial pyrotechnic display. Only two harbor seals were observed on and near the rocks adjacent to Fisherman's Wharf prior to the 2001 display. Neither were observed to haul out after the initial fireworks detonation, but remained in the water around the haul-out. The haul-out site was only surveyed until the conclusion of the fireworks display, therefore, no animal return data is available. However, the behavior of the seals after the initial disturbance and during the fireworks display is similar to the response behavior of seals during the VAFB rocket launches, where they loitered in the water adjacent to their haul-out site during the launch and returned to shore within 2 to 22 minutes after the launch disturbance.

MBNMS staff monitored harbor seal reactions to a coastal fireworks display at Aptos in October 2000. The staff report made the following finding:

Harbor seals could not be seen during and immediately after the event. It's likely, based on the reaction of the birds and the noise of the display, that the seals evacuated the area on and around the cement ship. Harbor seals were sighted hauled out on the ship and in the water the following morning.

A private environmental consultant has monitored the Aptos fireworks display each October from 2001 through 2005 (per California Coastal Commission permit conditions) and concluded that harbor seal activity returns to normal at the site by the day following the display. Surveys have detected no evidence of injury or mortality in harbor seals as a result of the annual 30-minute fireworks display at the site.

Since harbor seals have a smaller profile than sea lions and are less vocal, their movements and behavior are often more difficult to observe at night. In general, harbor seals are more timid and easily disturbed than California sea lions. Thus, based on past observations of sea lion disturbance thresholds and behavior, it is very likely that harbor seals evacuate exposed haul outs in the impact area during fireworks displays, though they may loiter in adjacent surface waters until the fireworks have concluded.

f. Estimated levels of incidental take of marine mammals

As discussed above, the two marine mammals NMFS believes likely to be taken by Level B harassment incidental to fireworks displays authorized within the Sanctuary are the California sea lion (*Zalophuus californianus*) and the harbor seal (*Phoca vitulina richardsi*), due to the temporary evacuation of usual and accustomed haul-out sites. Both of these species are protected under the MMPA, and neither is listed under the ESA. Numbers of animals taken by Level B harassment are expected to vary due to factors such as tidal state, seasonality, shifting prey stocks, climatic phenomenon (such as El Nino events), and the number, timing, and location of future displays. The take of sea lions and harbor seals was estimated using a synthesis of information, including data gathered by MBNMS biologists at the specific display sites, results of independent surveys conducted in the MBNMS, and population estimates from government wildlife surveys covering larger geographic areas. More detailed information regarding the estimates of take of sea lions and harbor seals may be found in the application at: http://www.nmfs.noaa.gov/pr/permits/incidental.htm.

With the incorporation of mitigation measures proposed below, NMFS expects that only Level B incidental harassment of a small number of pinnipeds may occur as a result of the proposed authorized coastal fireworks displays. NMFS further believes that the fireworks displays will have a negligible impact on the affected species and stocks and will not have an unmitigable adverse impact on the availability of such species or stocks for subsistence uses.

California Sea Lions

Stage structure of California sea lions within the Sanctuary varies by location, but generally, the majority are adult and sub-adult males. Weise (2000) reported on the stage structure of California sea lions at two historic fireworks display areas within the MBNMS, and speculated that juveniles may haul out at the Monterey jetty in large numbers due to a need for a more protected haul-out location. He also reported that most animals on Año Nuevo Island appeared to be adult males and suggested that the stage structure may vary between mainland haul-out sites and offshore islands and rocks. At all four designated display areas combined, twenty fireworks events per year could disturb an average total of 2,630 California sea lions,

with the maximum being 6,170 animals, out of a total estimated population of 237,000-244,000. These numbers are small relative to the population size (1.1-2.6 percent).

Harbor Seals

At all four designated display areas combined, twenty fireworks events per year could disturb an average of 302 harbor seals and a maximum of 1,065 harbor seals within the Sanctuary out of a total estimated population of 27,836. These numbers are small relative to the population size (1.1-3.8 percent). Nicholson (2000) studied the stage structure of harbor seals on the northeast Monterey Peninsula (an area with the largest single concentration of animals within the Sanctuary) for two years. For the final spring season of the study, survey numbers equate to a stage structure comprising 38% adult females, 15% adult males, 34% sub-adults, and 13% yearlings or juveniles.

2. Potential Indirect Effects on Marine Mammals and Other Sanctuary Resources

a. Chemical Residue

Possible indirect impacts to marine mammals and other marine organisms include those resulting from chemical residue or physical debris emitted into the water. When an aerial shell detonates, its chemical components burn at high temperatures, which usually promotes efficient incineration. Pyrotechnic vendors have stated that the chemical components are incinerated upon successful detonation of the shell. However, by design, the chemical components within a shell are scattered by the burst charge, separating them from the casing and internal shell compartments.

Chemical residue is produced in the form of smoke, airborne particulates, fine solids, and slag (spent chemical waste material that drips from the deployment canister/launcher and cools to a solid form). The fallout area for chemical residue is unknown, but is probably similar to that for solid debris. Similar to aerial shells, the chemical components of low-level devices produce chemical residue that can migrate to ocean waters as a result of fallout. The point of entry would likely be within a small radius (about 100 yards) of the launch site.

The MBNMS has found only one scientific study directed specifically at the potential impacts of chemical residue from fireworks upon the environment. A 1992 Florida study (DeBusk et al, 1992) indicates that chemical residues (fireworks decomposition products) do result from fireworks displays and can be measured under certain circumstances. The report, prepared for the Walt Disney Corporation in 1992, presented the results of a 10-year study of the impacts of fireworks decomposition products (chemical residue) upon an aquatic environment. Researchers studied a small lake in Florida subjected to two thousand fireworks shows over a ten-year period to measure key chemical levels in the lake. The report concluded that detectable amounts of barium, strontium, and antimony had increased in the lake but not to levels considered harmful to aquatic biota. The report further suggested that “environmental impacts from fireworks decomposition products typically will be negligible in locations that conduct fireworks displays infrequently“. Based on the findings of this report, the lack of any evidence

that fireworks displays within the Sanctuary have degraded water quality, and the fact that the chemical byproducts of less frequent fireworks displays in an open marine system are even less likely to accumulate to a harmful level than those described in the report, NMFS and the MBNMS believe that chemical residue from fireworks does not pose a significant risk to the marine environment. No negative impacts to water quality have been detected.

b. Debris

The fallout area for the aerial debris is determined by local wind conditions. In coastal regions with prevailing winds, the fallout area can often be projected in advance. This information is calculated by pyrotechnicians and fire department personnel in selection of the launch site to abate fire and public safety hazards. Mortar tubes are often angled to direct shells over a prescribed fallout area, away from spectators and property. Generally, the bulk of the debris will fall to the surface within a 1/2 statute mile radius of the launch site. In addition, the tops of the mortars and other devices are usually covered with household aluminum foil to prevent premature ignition from sparks during the display and to protect them from moisture. The shells and stars easily punch through the thin aluminum foil when ignited, scattering pieces of aluminum in the vicinity of the launch site. Through various means, the aluminum debris and garbage generated during preparation of the display may be swept into ocean waters.

Some low-level devices may project small casings into the air (such as small cardboard tubes used to house flaming whistle and firecracker type devices). These casings will generally fall to earth within a two hundred yard radius of the launch site, since they do not attain altitudes sufficient for significant lateral transport by winds. Though typically within 300 ft (91 m), the impact area for set piece devices can extend to 1/2 statute mile from the center of the ignition point depending on the size and height of the fixed structure, the number and type of special effects, wind direction, atmospheric conditions, and local structures and topography. Like aerial shells, low-level pyrotechnics and mortars are often covered with aluminum foil to protect them from weather and errant sparks, pieces of which are shredded during the course of the show and initially deposited near the launch site.

The explosion in a firework separates the cardboard and paper casing and compartments, scattering some of the shell's structural pieces clear of the blast and burning others. Some pieces are immediately incinerated, while others burn up or partially burn on their way to the ground. Many shell casings simply part into two halves or into quarters when the burst charge detonates and are projected clear of the explosion. However, during the course of a display, some devices will fail to detonate after launch (duds) and fall back to earth/sea as an intact sphere or cylinder. Aside from post display surveys and recovery, there is no way to account for these misfires. The freefalling projectile could pose a physical risk to any wildlife within the fallout area, but the general avoidance of the area by wildlife during the display and the low odds for such a strike probably present a negligible potential for harm. Whether such duds pose a threat to wildlife (such as curious sea otters) once adrift is unknown. After soaking in the sea for a period of time, the likelihood of detonation rapidly declines. Even curious otters are unlikely to attempt to consume such a device. At times, some shells explode in the mortar tube (referred to as a flower pot) or far below their designed detonation altitude. It is highly unlikely that mobile organisms

would remain close enough to the launch site during a fireworks display to be within the effective danger zone for such an explosion.

The MBNMS has conducted surveys of solid debris on surface waters, beaches, and subtidal habitat and has discovered no visual evidence of or chronic impacts to the environment or wildlife. Aerial displays generally produce a larger volume of solid debris than low-level displays. Past MBNMS fireworks Authorizations (discussed later) require the fireworks sponsor to clean area beaches of fireworks debris for up to two days following the display. In some cases, debris has been found in considerable quantity on beaches the morning following the display. The MBNMS staff have recovered many substantial uncharred casing remnants on ocean waters immediately after marine displays. Other items found in the impact area are cardboard cylinders, disks, and shell case fragments; paper strips and wading; plastic wading, disks, and tubes; aluminum foil; cotton string; and even whole unexploded shells (duds or misfires). In other cases, virtually no fireworks debris was detected. This variance is likely due to several factors, such as type of display, tide state, sea state, and currents. In either case, due to the requirement for the fireworks sponsor to clean up following the displays, NMFS and the MBNMS do not believe the small amount of remaining debris is likely to significantly impact the environment, including marine mammals or their habitat.

c. Increased Boat Traffic

Increased boat traffic is often an indirect effect of fireworks displays as boaters move in to observe the event. The more boats there are in the area, the larger the chance that a boat could potentially collide with a marine mammal or other marine wildlife. The number of boats present at any one event is largely dependent upon weather, sea state, distance of the display from safe harbors, and season. At the MBNMS, some events have virtually no boat traffic, while others may have as many as 40 boats ranging in size from 10 to 65 feet in length.

Prior to and during fireworks displays at the MBNMS, boats typically enter the observation area at slow speed (less than 8 kts) due to the other vessels present and limited visibility (i.e., most fireworks displays occur at night). The U.S. Coast Guard and/or other federal agency vessels are on site to enforce safe boating laws and keep vessels out of the debris fallout area during the display. Most boaters anchor prior to the display, while others drift with engines in neutral for convenient repositioning.

MBNMS staff have observed boat traffic during several fireworks displays and generally found that boaters are using good boating and safety practices. They have also never witnessed the harassment, injury, or death of marine mammals or other wildlife as a result of vessels making way at these events. In general, as human activity increases and concentrates in the viewing areas leading up to the display, wildlife avoid or gradually evacuate the area. As noted before, the fireworks venues are marine areas with some of the highest ambient levels of human activity in the MBNMS. Many resident animals are accustomed to stimuli such as emergency sirens, vehicle noise, boating, kayaking, swimming, tidepooling, crowd noise, etc. Due to the gradual nature of the increase in boat traffic, it’s infrequent occurrence and short duration, and

the slow speed of the boats, NMFS does not believe the increased boat traffic is likely to significantly impact the human environment, including marine mammals.

d. Fire

The marine venue is the preferred site for fireworks displays in coastal areas, in part, due to the considerable reduction of fire hazard by siting the aerial debris fallout zone over ocean waters. While there is no guarantee that all airborne embers will fall into the water, siting is managed for that intent. The coastal areas of California generally receive more moisture than the interior areas and are inherently less prone to wildfire than the drier upland regions. Authorized fireworks launch sites within the MBNMS are primarily located on sand beaches or steel/concrete offshore barges, minimizing fire hazard at a launch site, even if devices explode prematurely on the surface.

All coastal fireworks displays within the MBNMS must be authorized by a fire marshal permit in accordance with California state law and local ordinances. In issuing such permits, a local or state fire marshal establishes terms and conditions to protect spectators and property from potential fire hazards associated with fireworks displays. The terms and conditions govern the siting of the launch site away from flammable materials and environments and establish viewing areas a prescribed safe distance from the launch site in the event of misfires or premature detonations. These permits typically require that fire fighting equipment (e.g. fire engines and trucks) be on-scene during the display to respond to any fire emergency. The permits also govern the unloading, handling, and preparation of pyrotechnics for the display.

Display preparation requires the placement of racks of mortar tubes on a flat surface (usually a sand beach or barge) distant from vegetation, structures, and overhangs. The racks may be partially buried on a sand beach or in long, narrow boxes filled with sand. Ground displays are usually affixed to wooden frameworks staked into the ground or fixed to a sturdy base. Fireworks devices are detonated electrically from a central control box connected to the launch tubes and other devices by wire. Preparation of the launch site involves no more than short-term negligible impacts to the surrounding environment. Sanctuary Authorizations require fireworks sponsors to collect all debris at and near a fireworks launch site following each display, including mortars, racks, frameworks, stands, undetonated devices, wrappers, paper debris, etc.

Where boat traffic is expected to attend a coastal fireworks display, the U.S. Coast Guard issues a marine event permit and establishes a safety zone over the waters below the impact zone. Coast Guard and/or other public safety vessels patrol the zone during the fireworks display to assure that spectator vessels remain out of the area where airborne fireworks debris and embers are likely to fall. In Monterey, the fire department deploys its fire boat to augment the Coast Guard patrol. At Aptos, State Parks deploys an enforcement vessel to assist the Coast Guard. At Half Moon Bay, the harbor authorities provide a safety patrol during the event.

The culmination of the above measures considerably minimize the risk of fire resulting from coastal fireworks displays within the MBNMS. Since the MBNMS began authorizing

coastal fireworks displays in 1993, no uncontrolled fires have occurred, and no property or marine resources have been damaged due to fire.

3. Impact on Marine Wildlife Habitat (Habitat Exclusion)

Impacts on marine mammal habitat are part of the consideration in making a finding of negligible impact on the species and stocks of marine mammals. Impacts upon Sanctuary habitat are also considered for any activity reviewed for a Sanctuary Authorization. Habitat includes, but is not necessarily limited to, rookeries, mating grounds, feeding areas, roosting areas, nest sites, and areas of similar significance. The amount of debris and chemical residue resulting from fireworks displays authorized in the MBNMS is determined by wind conditions, weather, and other local variations. LOAs and Sanctuary Authorizations will require fireworks sponsors to clean up affected areas following approved fireworks displays. No evidence of water quality deterioration has been found in relation to prior MBNMS fireworks displays and Section (VI)(A)(2) of this document discusses the 1992 Walt Disney report, which found that environmental impacts from fireworks decomposition products typically will be negligible in locations that conduct fireworks displays infrequently. Because of the aforementioned mitigation measure and report, NMFS does not expect the debris and residue resulting from authorized fireworks displays to significantly impact marine mammals or marine mammal habitat in the MBNMS. Likewise, the MBNMS has determined that fireworks debris has only negligible short-term effects upon Sanctuary resources and qualities.

4. Potential Cumulative Effects

Cumulative effects are defined as “the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (Federal or non-federal) or person undertakes such other actions” (40 CFR §1508.7).

With the exception of regular ongoing boat and aircraft traffic and urban background noise levels at some sites, NMFS and MBNMS are aware of no other human activities occurring in the action area that may affect marine mammals. NMFS notes here that stress from long-term and continuous cumulative sound exposures can result in physiological effects on reproduction, metabolism, and general health, or on marine mammals’ resistance to disease. However, because of the infrequent nature and short duration of the noise generated from the fireworks, and adaptation of urban marine mammal populations to elevated sound levels, NMFS does not believe that cumulative impacts are likely to occur at MBNMS as a result of the issuance of LOAs for the permitting of limited fireworks displays by the MBNMS. We anticipate impacts to be limited to temporary behavioral disturbance and displacement of marine mammals from their accustomed haulouts during the actual fireworks.

Since 1993, 67 fireworks displays have been conducted within the Sanctuary. MBNMS staff have been opportunistically monitoring sea lions at the City of Monterey's Fouth of July celebration for more than 10 years. Their general observations may be summarized as follows: sea lions begin leaving the breakwater as soon as the fireworks begin, clear completely off after

an aerial salute or quick succession of loud effects, usually begin returning within a few hours of the end of the display, and are present on the breakwater at pre-firework numbers by the following morning. No long term effects on the population of either species of pinniped have been noted, and, in fact, the California sea lion population has increased and is growing at a current rate of 5.4 to 6.1 percent per year and the harbor seal population in California is healthy and growing at a current rate of 3.5 percent per year.

In upcoming years (during the five-year duration of the regulations), the number of fireworks displays in the Sanctuary throughout a given year may increase by two and a half times (up to 20 authorized per year versus the average 7 per year previously). However, LOAs and the USFWS Biological Opinion will limit fireworks displays by number of displays, geographical area, display duration, temporal interval, and seasonal restrictions for the express purpose of minimizing cumulative impacts to wildlife and habitat. Due to these measures and additional terms and conditions applied by the Sanctuary, NMFS and the MBNMS do not believe that authorization of fireworks displays within the Sanctuary, including an increase in number up to the maximum authorized under the regulations, will produce measurable cumulative impacts.

5. Impacts on Endangered Species

As mentioned earlier in this document, the Steller sea lion and several species of federally listed cetaceans may be present at MBNMS at different times of the year and could potentially swim through the fireworks impact area during a display. In a 2001 consultation with MBNMS, the Southwest Region, NMFS, concluded that the proposed fireworks displays is not likely to adversely affect federally listed species under NMFS' jurisdiction.

The MBNMS has not observed sea otter responses to fireworks events; however, sea otters do frequent all general display areas. As noted under Environmental Impacts above, otters and other species may temporarily depart the area prior to the beginning of the fireworks display due to increased human activities. Some otters in Monterey harbor have become quite acclimated to very intense human activity, often continuing to feed undisturbed, as boats pass simultaneously on either side and within 20 feet of the otters. It is therefore possible that select individual otters may have a higher tolerance level than others to fireworks displays. Sea otters in residence within the Monterey harbor display a greater tolerance for intensive human activity than their counterparts in more remote locations. Past Sanctuary observations have not detected any disturbance to California sea otters as a result of the fireworks displays; however, past observations have not included specific surveys for this species.

Within the scope of the potential effects of the MBNMS fireworks displays, the USFWS is responsible for regulating take of the southern sea otter and any terrestrial plants or animals. MBNMS consulted with the USFWS pursuant to Section 7 of the ESA regarding impacts to these species from fireworks displays. The USFWS issued a Biological Opinion (BiOp) on June 22, 2005, which concluded that the authorization of fireworks displays, as described in the preferred alternative, is not likely to jeopardize the continued existence of the southern sea otter, brown pelican, western snowy plover, San Francisco garter snake, California red-legged frog, Smith's blue butterfly, Monterey gilia, Menzie's wallflower, Monterey spineflower, or

Tidestrom's lupine and is not likely to destroy or adversely modify the critical habitat of the western snowy plover or Monterey spineflower.

More specifically, the USFWS further concluded that no southern sea otters would be taken as a result of the proposed fireworks events, and therefore issued neither an incidental take statement under the ESA nor an IHA. The USFWS found that an incidental take of brown pelicans in the form of harassment, injury, or mortality could occur as a result of pelicans flushing quickly in response to the visual or acoustic stimuli and subsequently colliding with boats, wires, or other objects in the area. The USFWS issued an incidental take statement for the brown pelican, but because they considered the chance of take resulting to be "remote and unpredictable", they did not exempt a specific number of birds, but instead included two terms and conditions that require MBNMS notify the USFWS if a dead pelican is found, and notify the USFWS if more than one dead pelican is found to discuss re-initiation of formal consultation. The Sanctuary authorization incorporates these terms and conditions by requiring that the entity authorized to conduct fireworks look for dead or injured wildlife during their debris cleanup the day after the fireworks display and that they report any dead or injured animals found immediately to the Sanctuary.

The BiOp did not include incidental take statements for any of the other species analyzed and did not include any other terms and conditions. The BiOp does, however, contain non-mandatory conservation recommendations for some of the other species, and the Sanctuary provides these conservation measures to authorized entities that will be conducting fireworks in areas to which the recommendations apply.

B. Issuance of LOAs and Sanctuary Authorizations for 7 Fireworks Displays

If LOAs and Sanctuary Authorizations for 7 fireworks displays per year were issued to the MBNMS, the nature of the effects on the marine environment and marine mammals (Level B harassment in the form of temporary abandonment of haulout sites) would be the same as those described above for 20 fireworks displays per year, however, the estimated numbers of pinnipeds taken by the activity would be smaller, or, potentially the number of times a single pinniped were exposed to fireworks in one year could be smaller. The number of marine mammals taken by Level B harassment is expected to vary due to factors such as tidal state, seasonality, shifting prey stocks, climatic phenomenon (such as El Nino events), and the number, timing, and location of future displays. If the 7 fireworks events per year continued at their historic locations, NMFS estimates they could disturb an average total of 1,070 California sea lions (2,795 maximum) out of a total estimated population of 237,000-244,000 (0.4-1.2 %) and an average total of 122 harbor seals (400 maximum) out of a total estimated population of 27,836 (0.5-1.4 %) within the Sanctuary. These numbers are small relative to the population size.

Limiting Sanctuary Authorizations for fireworks to 7 events per year would reduce overall disturbance to wildlife at fireworks launch sites within the Sanctuary, but it would have little measurable effect on species abundance or distribution within the Sanctuary due to the negligible short-term nature of the disturbance. Under this alternative, the same mitigation and

monitoring measures would be required as are required under the preferred alternative, which would further reduce the adverse effects to wildlife.

C. Issuance of LOAs to Individual Fireworks Sponsors

If LOAs were issued to individual fireworks sponsors, the activities would be the same, the same mitigation and monitoring would be required as in the two previous alternatives, the nature and extent of the effects on the marine environment would be the same as those described in (VI)(A) and (VI)(B) above, and the effects would similarly have a negligible impact on the affected species or stocks. This alternative primarily relates to administrative matters and has no direct bearing upon environmental consequences. By requiring multiple permits in lieu of one consolidated permit through the MBNMS, this alternative would increase administrative costs by NMFS and fireworks sponsors in order to comply with incidental take provisions of the MMPA.

D. No Action Alternative

If LOAs and Sanctuary Authorizations were not issued, any takes resulting from fireworks displays would be unauthorized, and a violation of the MMPA and NMSA would occur. If the MBNMS were to stop authorizing fireworks displays, the previously described risks to marine mammals and other marine wildlife would be eliminated; however, applicants could potentially consider alternate terrestrial venues, which are dangerous, as many fireworks displays occur at the height of the dry season, when area vegetation is particularly prone to ignition from sparks or embers. The central California region is a semi-arid environment with elevated fire hazards throughout the year. The relocation of fireworks displays inland would shift, and could significantly increase, environmental hazards to upland habitats. Such action would also pose increased hazards to public health and safety and property.

VII. MITIGATION AND MONITORING

In order to ensure that fireworks displays within the MBNMS will have the least practicable impact on marine mammals and their habitat under both the 20 displays per year (preferred) and the 7 displays per year alternatives, the MBNMS would adopt the following mitigation and monitoring requirements as part of an approved 5-year incidental take regulation (under the MMPA) and subsequent LOAs. Furthermore, the MBNMS would implement the mitigation measures as part of its fireworks Authorization process (under the NMSA) to protect overall Sanctuary resources and qualities.

A. Mitigation

NMFS has collaborated with the MBNMS and USFWS since 2001 to develop conservation measures that minimize fireworks impacts on protected species and the marine environment within the MBNMS by defining the locations, frequency, and conditions under which the MBNMS can authorize marine fireworks displays.

The mitigation measures can be grouped into five broad approaches for managing fireworks displays and will be implemented under alternatives 1 and 2 by the MBNMS:

(1) *Limit displays to certain seasons to safeguard reproductive periods*: This regulation does not authorize fireworks events between March 1 and June 30 of any year, since this period is the primary reproductive season for many marine species.

(2) *Establish four conditional display areas*: Traditional fireworks display areas within the MBNMS are located adjacent to urban centers where wildlife has often acclimated to human disturbances, such as low-flying aircraft, emergency vehicles, unleashed pets, beach combing, recreational and commercial fishing, surfing, swimming, boating, and personal watercraft operations. This regulation only authorizes fireworks displays in four prescribed areas of the Sanctuary. The conditional display areas (described earlier in detail) are located at Half Moon Bay, the Santa Cruz/Soquel area, the northeastern Monterey Peninsula, and Cambria (Santa Rosa Creek).

(3) *Create a per-annum limit on the number of displays allowed in each display area*: If properly managed, a limited number of fireworks displays conducted in areas already heavily impacted by human activity can occur with sufficient safeguards to prevent any long-term or chronic impacts upon local natural resources. This regulation authorizes no more than 20 displays along the entire Sanctuary coastline in order to prevent cumulative negative environmental effects from fireworks proliferation. Additionally, displays will be authorized at an average frequency equal to or less than 1 every 2 months in each conditional display area. Fireworks displays shall not exceed 30 minutes with the exception of two longer displays per year that shall not exceed 1 hour.

(4) *Retain Authorization requirements and general and special restrictions for each event:* The Sanctuary will continue to assess displays on a case-by-case basis, using specially developed terms and conditions to address concerns unique to fireworks displays (e.g. restricting the number of aerial "salute" effects used; requiring the removal of plastic and aluminum labels and wrappings; and requiring post-show reporting and cleanup). Such terms and conditions have evolved over twelve years, as the Sanctuary has sought to improve its understanding of the potential impacts that fireworks displays have upon marine wildlife and the environment. The MBNMS will implement general and special restrictions unique to each fireworks event as necessary.

(5) *Institute a 5-year Authorization system for annual displays:* The Sanctuary intends to institute a 5-year Authorization system for fireworks displays that occur annually at fixed locations in a consistent manner, such as municipal Independence Day shows. Authorizations will include special conditions that mitigate negative impacts upon species and habitat from fireworks displays, such as the requirement for Authorization holders to clean up debris following each event. Authorizations for fireworks displays will not be valid unless current LOAs have been issued by NMFS for unintentional harassment incidental to the displays.

The above conservation measures are designed to prevent an incremental proliferation of fireworks displays and disturbance throughout the Sanctuary and minimize area of impact by

authorizing displays in primary traditional use areas. They also place multiple special conditions on the displays and allow fireworks displays only during seasons that avoid sensitive wildlife breeding cycles. These measures and MBNMS Authorization conditions assure that protected species and habitats are not jeopardized by fireworks activities. They have been well received by local fireworks sponsors who have pledged their cooperation in protecting Sanctuary resources.

B. Monitoring and Reporting

The MBNMS has monitored commercial fireworks displays for potential impacts to marine life and habitats for 12 years. In July 1993, the MBNMS performed its initial field observations of professional fireworks at the annual Independence Day fireworks display conducted by the City of Monterey. Subsequent field observations were conducted in Monterey by the MBNMS staff in July 1994, July 1995, July 1998, March 1998 (private display), October 2000 (private display), July 2001, and July 2002. Documented field observations have also been made at Aptos each October from 2000 to 2005. The MBNMS staff have observed additional displays at Monterey, Pacific Grove, Capitola, and Santa Cruz, but those observations were primarily for permit compliance purposes, and written assessments of environmental impacts were not generated. Though monitoring techniques and intensity have varied over the years and visual monitoring of wildlife abundance and behavioral responses to nighttime displays is challenging, observed impacts have been consistent. Wildlife activity nearest to disturbance areas returns to normal (pre-display species distribution, abundance, and activity patterns) within 12 hours, and no signs of wildlife injury or mortality have ever been discovered as a result of managed fireworks displays.

Of all the past authorized fireworks display sites within the Sanctuary, the City of Monterey site has received the highest level of Sanctuary monitoring effort. The City of Monterey has hosted a marine fireworks display each July 4th since 1988 (five years prior to designation of the MBNMS). The display is the longest running and largest annual commercial fireworks display within the Sanctuary. The Monterey Breakwater (approximately one half statute mile from the pyrotechnic launch site) was constructed in the 1930s and, along with other natural rock formations, has been a regular haul-out site for California sea lions and harbor seals for many decades. For this reason, the Monterey site has been studied and surveyed by government and academic researchers for over 20 years. Consequently, the Monterey site has the best background data available for assessing status and trends of key marine mammal populations relative to annual fireworks displays. Therefore, the MBNMS proposes that Monterey be monitored as necessary to assess how local California sea lion and harbor seal distribution and abundance are affected by an annual fireworks display.

The Sanctuary proposes conducting a visual census of the Monterey Breakwater and Harbor Rocks on July 4-5, 2006 to update annual abundance, behavioral response patterns, and departure and return rates for California sea lions and harbor seals relative to the July 4 fireworks display. Data will be collected by an observer aboard a kayak or small boat and from ground stations (where appropriate). The observer will use binoculars, counters, and data sheets to census animals. The pre and post fireworks census data will be analyzed to identify any significant temporal changes in abundance and distribution that might be attributed to impacts

from the annual fireworks display. The data will also be added to past research statistics on the abundance and distribution of stocks at Monterey Harbor.

It should be noted however that annual population trends at any given pinniped haul-out site can be influenced by a myriad of environmental and biological factors, ranging from predation upon pups at distant breeding colonies to fluctuating prey stocks due to El Nino events. These many variables make it difficult to measure and differentiate the potential impact of a single stimulus on long-term population trends.

The Sanctuary also proposes to conduct one-time acoustic monitoring at a future City of Monterey Fourth of July fireworks display. The procedures and equipment for this monitoring will be outlined and described in the proposed rule, the regulations, and appropriate LOA.

In addition to the comprehensive behavioral monitoring to be conducted at the Monterey Bay Breakwater in 2006, under alternatives 1 and 2 MBNMS will require its applicants to conduct a pre-event census of local marine mammal populations within the fireworks impact area each year. Each applicant will also be required to conduct post-event monitoring in the fireworks impact area to record injured or dead marine mammals brown pelicans, and other wildlife.

Under a NMFS LOA (alternatives 1 and 2) a draft final report must be submitted to NMFS within 60 days after the conclusion of each calendar year. A final report must be submitted to the Regional Administrator within 30 days after receiving comments from NMFS on the draft final report. If no comments are received from NMFS, the draft final report will be considered to be the final report. In addition, the MBNMS will continue to incorporate updated census data from government and academic surveys into its analysis and will make its information available to other marine mammal researchers upon request.

Last, a comprehensive draft final report must be submitted to NMFS 120 days prior to the expiration of the regulations, and a final report submitted within 30 days after receiving comments from NMFS on the draft final comprehensive report.

As stated previously, NMFS and MBNMS have identified no other directed research or monitoring efforts (within California or elsewhere) that specifically address the impacts of fireworks on pinnipeds. The Sanctuary coordinates a Research Activities Panel comprised of 21 marine research institutions and organizations adjacent to the Sanctuary and receives constant updates of ongoing research within the Sanctuary that might be related to this issue. The MBNMS is coordinating with researchers at the NMFS, the USFWS, the California Department of Fish and Game, and various specific research institutions concerning the status and local trends of wildlife stocks in the Sanctuary.

VIII. CONCLUSION

As a result of this environmental review, NMFS and the National Marine Sanctuary Program have determined that the implementation of any of the four alternatives (the issuance of

LOAs and Sanctuary Authorizations for 20 displays, the issuance of LOAs and Sanctuary Authorizations for 7 displays, the issuance of LOAs to individual fireworks sponsors, or the denial of the permit and MBNMS Authorizations) will not significantly affect the quality of the human environment. Additionally, the issuance of these Authorizations is not controversial (one general comment of opposition was received during the 30-day comment period) and will not set a precedent for future actions with significant effects. Accordingly, an environmental impact statement is not required.

IX. REFERENCES

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Short communication

Effect of fireworks events on urban background trace metal aerosol concentrations: Is the cocktail worth the show?

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ABSTRACT

We report on the effect of a major firework event on urban background atmospheric $PM_{2.5}$ chemistry, using 24-h data collected over 8 weeks at two sites in Girona, Spain. The firework pollution episode (*Sant Joan* fiesta on 23rd June 2008) measured in city centre parkland increased local background $PM_{2.5}$ concentrations as follows: Sr (x86), K (x26), Ba (x11), Co (x9), Pb (x7), Cu (x5), Zn (x4), Bi (x4), Mg (x4), Rb (x4), Sb (x3), P (x3), Ga (x2), Mn (x2), As (x2), Ti (x2) and SO_4^{2-} (x2). Marked increases in these elements were also measured outside the park as the pollution cloud drifted over the city centre, and levels of some metals remained elevated above background for days after the event as a reservoir of metalliferous dust persisted within the urban area. Transient high-PM pollution episodes are a proven health hazard, made worse in the case of firework combustion because many of the elements released are both toxic and finely respirable, and because displays commonly take place in an already polluted urban atmosphere.

1. Introduction

The polluting nature of the smoke plumes arising from firework displays has recently received considerable scientific attention, with published studies reporting mainly on specific events such as Independence Day in the USA [1], European World Cup football celebrations [2], Lantern festival in China [3], Diwali in India [4], Las Fallas in Spain [5], and New Year's Day [e.g. [6]]. The smoke plumes arising from such events can raise atmospheric particulate matter (PM) levels from tens to thousands of $\mu g\,m^{-3}$ [7], with most particles being fine (1–2 μm) and therefore potentially respirable [1]. The chemistry of these plumes is complex, but is always characterised by a high metal content due to presence of K in the black powder propellent and a range of other metals/metalloids used as oxidisers, stabilisers, and to add colour and other special effects.

The inhalation of smoke loaded with metalliferous particles small enough to enter the lung alveoli causes negative health effects in humans [e.g. [8]], especially among vulnerable individuals such as asthmatics [9]. However, demonstrating toxicological responses to the inhalation of fireworks smoke is hampered by a continuing lack of detail about the exact nature of the inorganic chemical cocktail being inhaled. Most publications to date have published only partial chemical analyses, and/or have measured materials also contaminated by sources other than fireworks. Furthermore, focussing only on the specific fireworks event fails to provide the longer term context of urban atmospheric PM chemistry normally present in a given urban area. In this short communication we summarise new chemical data from filters collected daily over a 7-week period prior to a major summer fireworks festival in Mediterranean Spain (*Sant Joan*), and compare them with data collected during and 1 week after the event. The primary aim of the study was to characterise the concentrations and chemistry of urban background levels of $PM_{2.5}$ in Girona, compare these with more traffic-polluted sites within the city, and to identify unusual spikes in air pollution such as, in this case, a fireworks festival. Our data include analyses of trace elements (using ICP-AES and ICP-MS) in 107 24 h filter samples: such a comprehensive database on the effect of fireworks emissions on urban background atmospheric chemistry has not previously been published.

2. Methodology

Filter samples for this study were collected during a monitoring campaign in May–June 2008 from two locations in the city of Girona (population 96,000) in NE Spain, including an urban background and a more traffic-polluted site in the city centre. The traffic site lies

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Table 1
Concentrations, detection limits and uncertainty of selected elements at the *Parc Migdia* and *Escola Musica* monitoring sites before, during and after the *Sant Joan* fireworks event. See text for details.

	Parc Migdia			*Escola Musica*			DL ($\mu g\,m^{-3}$)	Uncertainty (%)
	Before 05/05–22/06	During 23/06	After 24/06–30/06	Before 05/05–22/06	During 23/06	After 24/06–30/06		
$\mu g\,m^{-3}$								
$PM_{2.5}$	16.3	25.3	22.1	22.1	30.8	20.1		
OM + EC	5.7	7.9	7.3	13.6	11.9	14.4	0.58558	15–20
CO_3^{2-}	0.3	0.3	0.5	0.5	0.4	0.5	0.06008	4–5
SiO_2	2.1	2.7	2.1	1.9	2.1	1.9	0.11739	3–4
Al_2O_3	0.7	0.9	0.7	0.6	0.7	0.6	0.03913	3–4
Ca	0.2	0.2	0.3	0.3	0.3	0.3	0.04005	4–5
Fe	0.1	0.1	0.1	0.1	0.1	0.2	0.02017	3–4
K	0.1	2.6	0.2	0.1	1.3	0.2	0.02075	3–4
Na	0.2	0.2	0.2	0.3	0.3	0.4	0.10293	4–6
Mg	<0.1	0.2	0.1	0.1	0.2	0.1	0.01697	3–5
SO_4^{2-}	2.5	5.7	3.7	2.8	5.5	4.0	0.12572	5
NO_3-	0.4	0.2	0.1	0.5	0.1	0.1	0.07476	6–15
Cl^-	0.3	0.3	0.4	0.3	0.4	0.4	0.18700	15–28
NH_4^+	1.0	0.8	1.3	1.0	1.5	1.4	0.01793	14
$ng\,m^{-3}$								
P	10.3	26.9	25.7	15.2	15.8	23.8	0.00579	3–4
Ti	10.3	15.5	16.0	8.6	14.0	13.0	0.00218	3–4
V	4.1	4.5	3.7	3.8	4.6	3.8	0.00020	4
Mn	3.9	7.2	5.3	4.3	5.4	5.3	0.00079	4–6
Co	0.1	0.9	0.1	0.2	0.2	0.1	0.00004	5–6
Ni	2.9	3.2	3.1	3.6	4.5	3.6	0.00118	5
Cu	4.0	20.2	5.1	12.8	17.5	11.5	0.00278	4
Zn	18.3	71.3	64.3	39.8	74.6	86.8	0.03073	7–10
Ga	0.1	0.2	0.1	0.1	0.1	0.1	0.00004	8–10
Ge	1.1	1.1	0.9	0.8	0.9	0.7	0.00057	9–12
As	0.3	0.6	0.3	0.3	0.6	0.4	0.00007	4–5
Se	0.3	0.2	0.3	0.2	0.1	0.3	0.00008	4–5
Rb	0.2	0.8	0.4	0.3	0.6	0.4	0.00005	5–7
Sr	1.4	120.5	2.1	1.5	106.8	2.1	0.00043	6–8
Cd	0.1	0.2	0.1	0.1	0.2	0.2	0.00009	7–9
Sn	0.9	1.2	1.1	1.4	1.4	1.6	0.00201	4–5
Sb	0.4	1.4	0.7	0.9	1.7	1.0	0.00010	4–5
Ba	29.4	321.7	131.0	47.0	261.1	165.3	0.03708	10–18
La	0.2	0.3	0.2	0.2	0.2	0.2	0.00013	6–8
Ce	0.6	0.7	0.5	0.4	0.5	0.5	0.00032	6–7
Pb	4.2	29.1	4.9	4.4	22.8	5.8	0.00060	4
Bi	0.1	0.4	0.1	0.1	0.4	0.1	0.00004	6–10

in the southern city centre (*Escola Musica* 41°58′69″N/2°49′31″E: adjacent to the busy main road feeding north into the centre), whereas the background site lies in urban parkland (*Parc Migdia* 41°58′10″N/2°49′28″E) 350 metres to the SE (150°). Data from both stations were obtained from 5 May to 30 June 2008, with 24-h $PM_{2.5}$ sampling being carried out by means of MCV CAV-A and DIGITEL DH80 high volume samplers ($30\,m^3\,h^{-1}$) equipped with quartz fibre filters (Munktell). Filters were treated and analyzed following the procedure described by Pey et al. [10]. This is based on the daily sampling of PM and subsequent analysis of major and trace elements by ICP-AES and ICP-MS (of acidic digestions of 1/2 of each filter), soluble anions and cations by ion chromatography, ammonium by colorimetry-FIA (water leached, 1/4 of each filter) and carbon by thermo-optical methods. Contents of Si and CO_3^{2-} were indirectly determined from the contents of Al, Ca and Mg, on the basis of prior experimental equations ($2Al_2O_3 = SiO_2$; $1.5Ca + 2.5Mg = CO_3^{2-}$). Blank field filters were used for every stock purchased for sampling and analyzed in the same batches of their respective filter samples. The corresponding blank concentrations were subtracted for each sample. For analysis control, reference material NIST 1633b was added to a fraction of a blank filter to check the accuracy of the analysis of the acidic digestions. The individual uncertainty of daily measurements due to analytical techniques was estimated following the method described by Amato et al. [11] and expressed as % (interquartile range) of species concentration (Table 1). An estimate of detection limit (DL) was performed for the *j*th analyte based on the following formula:

$$DL^j = \frac{\sqrt{\sigma_0^{2j} + \sigma_{BLK}^{2j}}}{V}$$

which combines estimates of the two uncertainties linked to the instrument σ_0 (ICP-MS, ICP-AES, HPLC, etc.) and the blank subtraction σ_{BLK} [12]; V is an average value of air volume sampled in 24 h. Additional measurements were made every 15 min for SO_2 (ultraviolet fluorescence), and atmospheric conditions (wind velocity and direction, precipitation, relative humidity and ambient temperature) were supplied by the local site in the meteorological network of the Generalitat of Catalonia.

3. Results

The *Sant Joan* fireworks fiesta is celebrated late in the evening of 23rd June, and is recorded on an hourly scale by our SO_2 data at *Parc Migdia* which show a sudden rise after 20:00 to a transient peak at 22:00 UTC time (from 2.0 to 6.8 $\mu g\,m^{-3}$), followed by rapid subsidence to background levels after midnight (Fig. 1 inset). In contrast, neither aerosol nitrate nor ammonium levels were significantly affected by fireworks combustion, as also noted in previous work during New Year celebrations in Germany [6]. Mass concentrations of $PM_{2.5}$ (averaged over 24 h) rose from 14 $\mu g\,m^{-3}$ on the 22nd June to 25 $\mu g\,m^{-3}$ on the 23rd June. The influence of fireworks

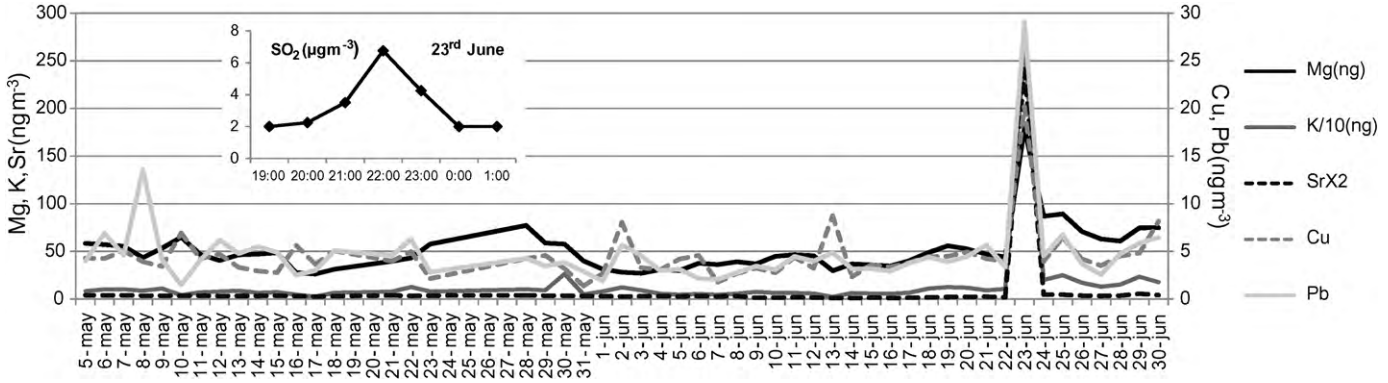

Fig. 1. Daily concentrations of Sr (x2), Mg, Cu, Pb and K (/10) at *Parc Migdia* urban background monitoring station, Girona. The prominent fireworks-related peak on 23rd June is followed by an aftermath of elevated levels compared to pre-fireworks background. Inset: hourly SO_2 levels registered on the 23rd of June.

on the PM content of filter samples for 23rd June in *Parc Migdia* is clear: they are unusually rich in metals, with K, Ba, Mg, Sr, Cu, Pb, Sn, Al, Bi, and Ga all rising suddenly to their highest value during the 8 week sampling campaign. The elemental increases relative to pre-fireworks background levels measured over the preceding weeks are, in decreasing order, Sr (x86), K (x26), Ba (x11), Co (x9), Pb (x7), Cu (x5), Zn (x4), Bi (x4), Mg (x4), Rb (x4), Sb (x3), P (x3), Ga (x2), Mn (x2), As (x2), Ti (x2) and SO_4^{2-} (x2).

Fig. 1 illustrates the scale of the fireworks metals peak at *Parc Migdia* on 23rd June, using five metals which best define the event, namely Sr, K, Mg, Cu and Pb. Interestingly, ambient concentrations of these and other metals during the week after the firework show do not fall back to pre-fireworks background levels but instead remain relatively elevated (Fig. 1). During this time the weather in Girona remained very hot and dry, with above average $PM_{2.5}$ concentrations (except after an early morning storm on 27th June). This observation of a post-fireworks concentration anomaly suggests that the transient but intense smoke event on the 23rd June created a reservoir of metalliferous particles which continued to contaminate the area days after the initial pollution episode. We envisage much of this reservoir as fine metalliferous PM attached to surface deposited particles later subject to daytime resuspension by wind, traffic and other activities. Further study is needed to ascertain for how long this "reservoir effect" can be detected within an urban area.

During the fireworks event light winds blew the main body of the dispersing smoke plume generally northwards over the city centre. The distinctive metalliferous fingerprint of fireworks emissions at the *Escola Musica* site was therefore again obvious, but with correspondingly reduced concentrations and a slightly different chemical mix. Once again pronounced increases were shown by Sr (x71), Ba (x6), Pb (x5), Bi (x4) although K levels dropped considerably (from x26 at *Parc Migdia* to x13 at *Escola Musica*), presumably due to less intense levels of black powder smoke close to ground level. Table 1 compares metal aerosol concentrations at the two monitoring sites averaging the 24-h values before, during and after the fireworks event.

The data from *Escola Musica* again show a post-fireworks aftermath of continued contamination, although as this site was less of a main focus of firework activity the effect is weaker (Table 1). Furthermore, there is a more obvious PM contribution from road vehicles at *Escola Musica*, as reflected by higher levels of background pre- and post-fireworks metals such as Ba, Zn, Cu, and Sb concentrations (Table 1), these elements being well established tracers not only for fireworks events but also for other anthropogenic emissions such as those from road traffic [13 and references therein]. The usefulness of these four metals in highlighting fireworks events is therefore somewhat compromised in sites with heavy traffic. In contrast, the element Sr is not only an excellent tracer for fireworks emissions, but also is unaffected by high traffic flows, with similar pre-fireworks background concentrations at both *Parc Migdia* and *Escola Musica* (Table 1).

In general, and with the obvious exception of 23rd June, background levels of trace metals at the two monitoring sites are controlled primarily by weather conditions and traffic density. Ambient PM concentration reach peaks during dry, mid-week periods and fall to prominent troughs during rainy spells, especially if these coincide with weekends. The first four rainy periods during this summer campaign period occurred at weekends, when daytime traffic flows were at their lowest, and each of these periods is

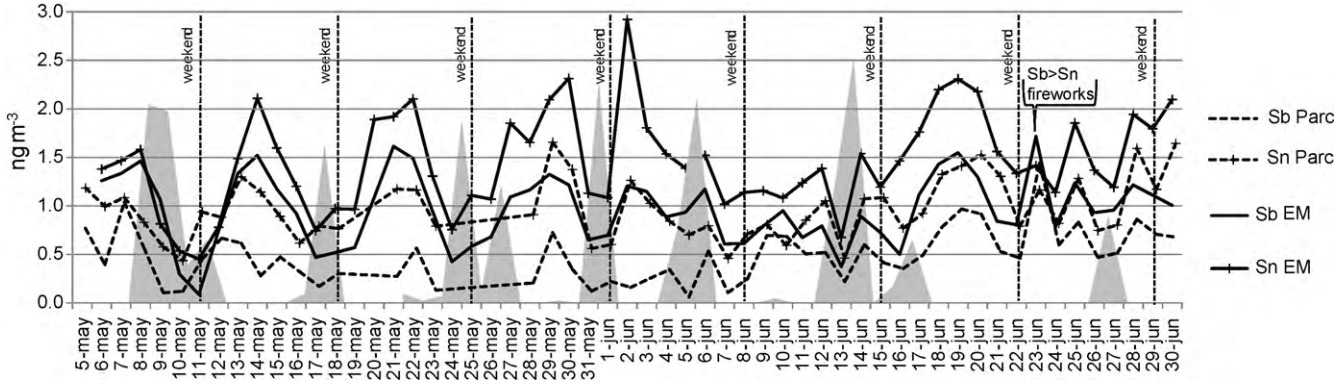

Fig. 2. Concentrations of the trace metals Sb and Sn in ambient air at *Parc Migdia* (Parc, urban background) and *Escola Musica* (EM, heavy traffic) monitoring sites in Girona. The repeated pattern of higher weekday and lower weekend levels is overprinted by cleansing rainfall (grey) events. During the *Sant Joan* fireworks event on 23 June Sb concentrations rise anomalously, exceeding those of Sn, making Sb a useful fireworks marker.

correspondingly marked by a prominent fall in PM concentrations. In contrast, the weekly PM maxima over this period consistently occurred between Tuesday and Thursday under dry conditions. Fig. 2 illustrates how trace metals record this fluctuation in PM concentrations, comparing levels of Sb and Sn, two trace metals with contrasting behaviours with respect to fireworks emissions. The obvious exception to a generally concordant pattern between Sb and Sn occurs over the *Sant Joan* fiesta, when Sb levels more than triple their pre-fireworks average whereas Sn levels, unaffected by fireworks, stay well below their average (Table 1 and Fig. 2). Fig. 2 therefore demonstrates that although contaminants such as Sb derive from mixed sources (in this case traffic and fireworks), comparing this metalloid with trace elements not present in fireworks (in this case Sn) can be a useful way to identify a fireworks event. Overviewing the chemistry of both sites leads us to conclude that the best firework elemental tracers in our study were Sr, K, SO_4^{2-}, Pb, and Bi, with Ba, Zn, Cu, and Sb also clearly showing firework peaks additional to those associated with traffic.

4. Discussion and conclusions

Most previous publications dealing with atmospheric PM emissions from fireworks have emphasised Sr, Ba, and K as especially typical tracers of firework emissions. With regard to other metals and metalloids there is less agreement, with different papers variously identifying some combination of Cu, Ti, Al, Ni, Cr, Zn, Cd, Mg, Co, Pb, Bi, and As [1–7,14–16]. It is clear that different fireworks vary enormously in the cocktail of metals they contain. Although K, as the black powder fuel and combined with S, is dominant, the main "special effects" trace additive can include a variety of other metals such as Al, Cu, Ti, or even Pb [17]. The case of Pb is of especial interest, given the high toxicity of this metal, as it is one of the few metals/metalloids for which legal atmospheric concentration limits exist (along with As, Hg, Ni, and Cd), although only for PM_{10} rather than $PM_{2.5}$. Despite this awareness, it is clear that in many countries any legal requirement for avoiding use of Pb in fireworks manufacture and combustion is being thwarted by imports from manufacturing countries less concerned with potential health implications. Some fireworks continue to contain Pb levels measurable in decigrams [17], and the effect of this on the chemistry of the resulting combustion plume is obvious. In both this current study in Girona, as well as in our study of Las Fallas smoke clouds in Valencia [5], Pb was a prominent component of the firework pollution plume, with average daily levels in Girona rising an order of magnitude higher following the display.

Despite the lack of legal controls on PM emissions emanating from firework combustion, the severity of the impact of such events on urban background atmospheric chemistry provides reasonable cause for concern [18]. There is already abundant published evidence that short-lived fluctuations in pollutants can induce changes in both lung and heart function [8,19–23]. Asthma symptoms, perhaps the most obvious risk factor, have been linked to 1-h PM_{10} and NO_2 concentrations rising from background values of 20–30 $\mu g\,m^{-3}$ to brief peaks at 40–70 $\mu g\,m^{-3}$ [24]. Similarly, 1-h exposure to elevated traffic levels has been associated with the onset of myocardial infarction [25], and hourly increases in $PM_{2.5}$ linked to myocardial ischaemia [26]. In the specific case of fireworks emissions it is the metalliferous component of atmospheric aerosols which is additionally implicated in negative health effects, not only with acute responses but also in their possible contribution to long term degenerative conditions such as Parkinson's and Alzheimer's diseases [e.g. [27,28]]. Presumably, those most immediately at risk from exposure to dense smoke clouds are people already debilitated by pre-existing illness, notably severe asthma or coronary heart disease, but the metalliferous and highly respirable nature of fireworks emissions makes them *per se* hazardous to the general population.

Finally, we emphasise that it is the additional burden of smoke emissions on already contaminated urban air which makes many fireworks events especially polluting. In the case of Girona, a fireworks event lasting an hour or two contributed to raising the daily average $PM_{2.5}$ mass from 13 $\mu g\,m^{-3}$ on 22nd June to 25 $\mu g\,m^{-3}$ on 23rd June. City centre concentrations of metals and metalloids such as Pb, Co, Ni, Zn, As, Al all increased markedly due to the fireworks display. All of these elements are listed in the Comprehensive Environmental Response, Compensation and Liabilities Act (CERCLA) Priority List of Hazardous Substances published by the US Agency for Toxic Substances and Disease Registry. The health effects of inhaling such a concentrated and complex chemical cocktail of different toxic substances in the form of micron sized particles remain unknown. Furthermore, our observation of a continued "reservoir effect" enhancement of ambient metal PM levels persisting for days after the fireworks event indicates that the effect on urban background PM is less transient than might be supposed.

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UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Southwest Region
501 West Ocean Boulevard, Suite 4200
Long Beach, California 90802- 4213

2010/02039:CCF

May 14, 2010

Donna Frye
Chair, Natural Resources & Culture Committee
San Diego City Council
202 C St. #10
San Diego, California 92101-3860

Dear Councilwoman Frye:

Thank you for contacting NOAA's National Marine Fisheries Service (NMFS), Southwest Regional Office, regarding the San Diego City Council's next steps concerning Children's Pool Beach (CPB), located in La Jolla, California. Following a conference call with my staff in the Protected Resources Division on April 28, 2010, your staff sent, via electronic mail, a copy of your draft report to be presented to the San Diego City Council identifying management options for CPB. Per your request, this letter provides our comments and recommendations regarding the proposed management options. In order to provide some context for these comments, we have added some background (Appendix 1) on the Marine Mammal Protection Act (MMPA), harbor seal biology and life history, and the historical and current use of CPB by harbor seals.

The presence of a harbor seal colony at CPB has been the focus of several lawsuits in the recent past. In 2009, the California State Legislature passed Senate Bill 428, which amended the conditions of the 1931 State trust granting the CPB area tidelands to the City of San Diego. Effective January 1, 2010, the trust was amended to allow for an additional use of the tidelands: a "marine mammal park for the enjoyment and educational benefit of children." While, there is no definition or recognition of the term "marine mammal park" under the MMPA and NMFS' implementing regulations, this amendment of the trust provides the City of San Diego with greater latitude in implementing management actions regarding the harbor seal colony at CPB.

COMMENTS AND RECOMMENDATIONS

My staff and I have reviewed the actions that were proposed by the San Diego City Council Natural Resources and Culture Committee, of which you are the Chair, and offer the following comments and recommendations.

1. **Direction to seek a Local Coastal Program amendment to prohibit the public from entering the beach during pupping season, 24 hours a day from December 15 through May 15.**

Harbor seals (*Phoca vitulina richardii*) at CPB are subject to many potential daily urban disturbances– traffic noise, car alarms, slamming doors, people shouting and laughing. Any of these disturbances may provoke a reaction from a harbor seal on the beach. This may include raising their head, looking around, or moving. The most disruptive of human interactions are those that lead to animals flushing into the water, causing animals to expend energy and prevent them from gaining the benefits of hauling out (e.g., rest or thermoregulation). When pups are on the beach, they can be injured or even killed by stampeding adult seals. Biologists have observed that the presence of people on the beach near the hauled out seals, or at the water's edge typically results in large numbers of seals flushing (Hanan 2004). By preventing the public from entering the beach, flushing can (in most cases) be prevented. Therefore, NMFS supports prohibiting the public from entering the beach.

For the harbor seals hauled out at CPB, the most critical time for protection from disturbance and harassment are during the last months of pregnancy and through pup weaning. The first full-term harbor seal pups are usually born at CPB in January. Based on these dates, implantation occurs no later than August. Therefore, adult females hauled out at CPB after August are likely pregnant. NMFS has received documented reports of abortions and premature parturition occurring in CPB harbor seals in November and December. Therefore, NMFS recommends treating December 1 as the beginning of the pupping season. This date is a conservative start date for pupping season and provides protection from human interference for late term pregnant females hauled out at CPB, and likely reduces the risk of abortion and premature parturition. After birth, pups nurse and are dependent on their mothers for approximately 4-6 weeks until weaning. The last pups of the season (typically born in April) may not wean until the end of May. Therefore, NMFS recommends treating May 30 as the end of the pupping season, as this date ensures that the majority of pups will be weaned.

NMFS has one comment regarding this restriction. The City Council might consider exempting certain categories of people from the general prohibition. For example SeaWorld personnel may need to access CPB if an entangled animal is observed on the beach. We recommend that the City Council consider a mechanism that will allow access in this, or other appropriate situations.

2. **Prohibit dogs on the Children's Pool Beach year round, 24 hours a day**

NMFS supports this action. Dogs may harass seals and cause them to flush into the water. There is also a risk of disease transmission between canines and pinnipeds. Therefore, prohibiting dogs from the beach is protective of the seals and dogs by reducing potential disturbance and preventing potential health issues.

3. **Seek private funding for a Park Ranger or Lifeguard full-time position with expert qualifications whose primary duty is to patrol the Children's Pool. Authorize the Park and Recreation Department or Lifeguard Services to create a Volunteer Docent Program led by this Park Ranger or Lifeguard**

NMFS supports this action. NMFS recognizes that at least some percentage of harassment of marine mammals is caused by well-meaning members of the public who do not understand the

impact their behavior may have on the animals or lack knowledge of applicable laws. La Jolla's CPB is a destination area for many tourists who may have no experience with wild marine mammals. Once informed, most people tend to maintain a greater distance and limit potentially disruptive behaviors. Therefore, the combination of enforcement and education should greatly help this situation.

While NMFS appreciates the efforts of the private organizations that have provided educational materials at CPB, we believe that the programs would be more organized and messages more consistent if they were maintained by a central group operating under the purview of the local government, such as the program envisioned by this proposal. NMFS would look forward to coordinating closely and assisting with such a program. Additionally, having a dedicated employee with enforcement authority would likely help prevent violation of city ordinances that are protective of seals or limit conflict, including purposeful violation of those city ordinances.

While NMFS has enforcement authority under the MMPA, limited staffing creates a challenge. NMFS has a toll-free hotline (1-800-853-1964) to report violations of marine laws (including the MMPA). In 2009, this number received a total of 154 calls regarding CPB. Although only three of the 154 calls resulted in a full investigation, all of the calls represent a significant investment for the local NMFS enforcement agent.

Under MMPA Section 109(a), no State may enforce a State law or regulation relating to the taking of the species of marine mammal without a transfer of management authority from the Secretary of Commerce. This has not occurred in California. However, States and local governments are free to implement and enforce ordinances, such as the closure of a beach, which may have a side benefit of preventing the harassment of a marine mammal.

4. **Seek an emergency amendment to the existing Coastal Development Permit to keep the guideline rope up year round**

NMFS supports this action with some reservations, as maintenance of the guideline rope does not ensure that harbor seals will not be harassed. For example, if a harbor seal hauls out on the "human" side of the rope, harassment of that seal may still be considered a violation of the MMPA, even if one is on the "correct" side of the rope. NMFS recommends that the public maintain a distance from any seal, regardless of where they are regarding the guideline rope. For most harbor seal haulout sites along the West Coast of the U.S., NMFS recommends a distance of 100 feet. However, with the relatively small area at CPB, NMFS has recommended that the public maintain a distance of at least 50 feet from any seal, while standing on CPB (as opposed to standing on the breakwater). As this is a viewing guideline and not codified in the regulations, NMFS has the flexibility to modify it to meet the individual circumstances of the geographic area and the natural history of the species. Because viewing distances are guidelines, they do not have the force of law, but harassment is a violation of the MMPA regardless of the distance from which it occurs.

Therefore, NMFS supports establishing the guideline rope year-round, as it would allow at least some measure of public awareness and protection to the harbor seals hauled out on the sand. However, we note that merely abiding by the guideline rope (standing on the "human" side) does

not guarantee that a person will not violate the MMPA. For example, humans approaching CPB from the water while harbor seals are on the beach may present more of a threat to the seals than humans on the beach, which may result in flushing en masse into the water.

CONCLUSION

Thank you for the opportunity to provide input on the proposed actions sent before the City Council of San Diego. We hope that our comments and recommendations will help inform your discussions. If you have any further questions, please do not hesitate to contact Sarah Wilkin at Sarah.Wilkin@noaa.gov or 562-980-3230 or Christina Fahy at Christina.Fahy@noaa.gov or 562-980-4023.

Sincerely,

for Rodney R. McInnis
Regional Administrator

cc: Copy to File: 151422SWR2005PR2267

Appendix 1.

BACKGROUND:

Marine Mammal Protection Act

Under the Marine Mammal Protection Act (MMPA), NMFS (exercising the authority of the Secretary of Commerce) is the resource trustee agency for cetaceans and pinnipeds (excluding walrus) in the United States and is responsible for implementing and enforcing the law. The stated goal of the MMPA is to ensure that marine mammal species and stocks do not "diminish beyond the point at which they cease to be a significant functioning element in the ecosystem of which they are a part" (16 U.S.C. §1361(2)). To implement this goal, the MMPA imposes a general moratorium on the "take" of a marine mammal in U.S. waters. It defines take to mean "to harass, hunt, capture, or kill or attempt to harass, hunt, capture or kill any marine mammal" (16 U.S.C. §1361(13)).

Pacific harbor seals – general biology/life history

Pacific harbor seals (*Phoca vitulina richardii*) are widely distributed throughout the North Pacific. Haulout sites are widely distributed throughout the California mainland and on offshore islands, including beaches, rocky shores, and intertidal sandbars. This subspecies inhabits near-shore coastal and estuarine areas from Baja California, Mexico to Alaska. Their migrations are limited to 300-500 kilometers, occasionally traveling these distances to find food or suitable breeding areas. The timing of harbor seal pupping occurs sequentially along the west coast of North America, with the earlier pupping seasons occurring in Baja California and southern California, and later seasons occurring in the Pacific Northwest and British Columbia, Canada. After birth, pups nurse and are dependent on their mothers for approximately 4-6 weeks until weaning. Harbor seals breed shortly after weaning their pups. Delayed implantation of the fertilized blastocyst occurs 1.5 to 3 months following mating. The gestation period is approximately 9 months.

For any individual or group of individuals in a breeding colony, there are two time periods in a given year when non-lethal disturbance or harassment would be the most harmful to harbor seals. The first is any interference with pregnant females that might result in the loss of young prior to birth (either through abortion of a fetus that cannot survive outside of the womb or the premature birth of a pup that lives a short time before dying). This type of reproductive failure can be harmful to the health of that female and, over time, may result in the collapse of the harbor seal colony. The second critical time period is immediately following birth, when mothers and their pups bond, so they can recognize each other if they become separated. Disruption of the bonding process usually leads to abandonment of the pup and eventual death without human intervention.

Harbor seals at Children's Pool Beach

There have been limited studies focusing on the harbor seals in La Jolla, but the animals likely colonized CPB because it provided suitable habitat. Genetic analyses have not been conducted to determine population structure or the origins of the founding animals for this rookery; however, one reasonable assumption is that they may have originated from some of the offshore islands (e.g., San Clemente Island) in southern California. In addition, there is no evidence that human intervention (via the release of rehabilitated seals) created this colony. Prior to colonization of CPB, some rehabilitated harbor seals were released from multiple La Jolla beaches located near known offshore haulout sites (rocks). However, these releases represent a small number of animals and there is no evidence that those released harbor seals were more likely to haul out on the mainland beaches versus offshore rocks following release. Currently, all harbor seals rehabilitated in San Diego County are released off Point Loma.

Currently, Pacific harbor seals use CPB to haul out year-round, and to give birth and nurse their pups. Harbor seals historically occupied the offshore rocks near La Jolla, but were not consistently observed on the mainland at CPB until the early 1990's. By 1995, harbor seals were using CPB daily (Yochem and Stewart 1998). This time period coincides with an observed increase in the harbor seal population off California. During this time, individual animals could be observed hauling out in areas containing suitable habitat (Hanan 1996), sometimes leading to the establishment of a haulout site or re-colonization of an historical haulout site. With a sloping, sandy beach that is north-facing and generally protected from tidal influence and high wave action, CPB provides suitable habitat for harbor seals.

The first observed pups confirmed born on CPB occurred in the late 1990's. NMFS conducts a statewide harbor seal census survey every few years and includes the animals at CPB. More frequent observations by volunteer groups and project monitoring reports indicate that the number of pups born annually appears to have stabilized at CPB, now averaging between 40-50 pups. Harbor seals, including those at CPB, display site fidelity, with female harbor seals often remaining close to the area they gave birth.

The term "rookery" is not defined in either the MMPA or through its implementing regulations. ~~The American Heritage Science Dictionary (2002) defines a rookery as: "A place where certain~~ birds or animals, such as crows, penguins, and seals, gather to breed." Harbor seals have been observed giving birth at CPB for approximately 10 years, and the timing and numbers of pups born are generally predictable from year to year. Therefore, NMFS considers CPB to be a harbor seal rookery and year-round haulout site.

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UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Office for Law Enforcement
Southwest Region
501 W. Ocean Blvd., Suite 4300
Long Beach, CA 90802

March 21, 2006

Mayor Jerry Sanders
City Administration Building
11th Floor
202 C Street
San Diego, CA 92101

Dear Mayor Sanders,

I am writing in regard to the marine mammals at La Jolla's Children's Pool and steps we can take to protect them and the people in the community. In the past few months, there have been numerous calls and other communications to NOAA's Office for Law Enforcement (OLE) regarding incidents of marine mammal harassment by the public at the Children's Pool Beach (CPB) in La Jolla, CA. As you know under the Marine Mammal Protection Act (MMPA), §16 U.S.C. 1372 (a)(2)(A), *it is unlawful for any person or vessel or other conveyance to take any marine mammal in waters or on lands under the jurisdiction of the United States.* Harassment is listed under the definition of 'take.'

> Take means to harass, hunt, capture, collect, or kill, or to attempt to... any marine mammal.
>
> Harassment (Level B) means any act or pursuit, torment, or annoyance which has the potential to disturb a marine mammal or marine mammal stock in the wild by causing disruption of behavioral patterns, including, but not limited to, migration, breathing, nursing, breeding, feeding, or sheltering but which does not have the potential to injure a marine mammal or marine mammal stock in the wild.

The CPB receives an estimated 80,000 visitors per month which increases the potential for seal/human interaction. OLE has placed two signs down on the beach which warn the public to keep a safe distance from the hauled out seals and sea lions. While the guidelines are useful, they have not prevented actions that could be considered harassment from occurring at the beach, particularly during pupping season. The OLE has received over 60 HOTLINE calls reporting alleged marine mammal harassment at the CPB since January 1, 2006. The agency responded to these complaints by increasing the number of patrols to the beach, especially on weekends but, resources do not afford us with the ability to maintain a constant presence.

Harbor seals haul out at CPB for breeding, nursing, molting, and resting. The pupping season at the CPB is from January through the end of April. Typically, the pup is born and weaned during the late spring. Nursing usually lasts about 3-6 weeks, averaging about 4 weeks until the pup is weaned. Unlike many other seal pups, harbor seals are able to swim at birth, but harbor seal mothers are very protective and the mother/pup bond is very important, particularly during the time immediately following birth.

California State Parks closed beaches in Arroyo Laguna and Piedras Blancas because of concerns of elephant seals being harassed at sites they are known to haul out to rest, give birth, care for their pups, and molt.

OLE is concerned that the public will continue to harass marine mammals and continue to be subject to citation under the MMPA at CPB. Therefore, we strongly recommend, as well, that the City close the CPB during the remainder of pupping season (through the end of April). The closure during this time will afford the City with time to decide, plan, and place into action a more permanent strategy for the CPB.

In the event you decide against a temporary closing of the beach, as conducted at Arroyo Laguna and Piedras Blancas, consider reinstating the CPB rope barrier that was once in place. Unfortunately, in the past the rope barrier did not deter the "determined" individual(s) from approaching the seals. The rope barrier will provide a clear message for those that have a sincere desire to respect the marine mammals present on the beach, and therefore will provide some level of heightened protection for the adult and newborn seals. The rope barrier will also aid in informing humans when they are more likely to be found in violation of the MMPA and potentially cited. This option has been supported by Susan Davis, Member of the U.S. Congress (House or Representatives).

OLE appreciates and looks forward to a continued opportunity to work with you in assisting you fulfill your goals as well as protect the animals and citizens of our community.

Sincerely,

Donald W. Masters
Special Agent in Charge

cc: Julie Teel, Office of the City Attorney
April Penera, City Manager's Office
Dale Jones, Director, Office for Law Enforcement
Rod McInnis, Regional Administrator, SW Region

NOAA FISHERIES SERVICE
Office of Law Enforcement
501 W. Ocean Blvd., Suite 4300
Long Beach, CA 90802

November 30, 2007

San Diego City Attorney's Office
Nina M. Fain, Deputy City Attorney
1200 Third Ave, Suite 1100
San Diego, CA 92101

Dear Ms. Fain,

I am writing in regard to the marine mammals at La Jolla's Children's Pool and steps we can take to protect them and the people in the community. In the past few months, there have been numerous calls and other communications to NOAA's Office of Law Enforcement (OLE) regarding incidents of marine mammal harassment by the public at the Children's Pool Beach (CPB) in La Jolla, CA. As you know, under the Marine Mammal Protection Act (MMPA), 16 U.S.C. 1372 (a)(2)(A), *it is unlawful for any person or vessel or other conveyance to take any marine mammal in waters or on lands under the jurisdiction of the United States.* Harassment is listed under the definition of 'take.' Take means to harass, hunt, capture, collect, or kill, or to attempt to… any marine mammal.

Harassment (Level B) means any act of pursuit, torment, or annoyance which has the potential to disturb a marine mammal or marine mammal stock in the wild by causing disruption of behavioral patterns, including, but not limited to, migration, breathing, nursing, breeding, feeding, or sheltering but which does not have the potential to injure a marine mammal or marine mammal stock in the wild.

Joe Cordaro, NOAA Wildlife Biologist, advises that harbor seals haul out at CPB for breeding, nursing, molting, and resting. The first full-term pups are usually born in early-mid January. Pups wean from their mothers in approximately 4-7 weeks. The last pups of the season may not wean until the end of May.

The CPB receives numerous visitors each month which increases the potential for seal/human interaction. OLE has placed signs on the landings above the CPB, which warn the public to keep a safe distance from the hauled out harbor seals. While the guidelines are useful, they have not prevented actions that could be considered harassment from occurring at the beach, particularly during pupping season. OLE continues to receive HOTLINE calls reporting alleged marine mammal harassment at the CPB.

OLE is concerned that the public will continue to harass marine mammals and continue to be subject to citation under the MMPA at CPB. Therefore, we strongly recommend, that the City close the CPB starting December 15 through May 30 or, at a minimum, consider reinstating the CPB rope barrier that was once in place. Unfortunately, in the past the rope barrier did not deter the "determined" individual(s) from approaching the seals. The rope barrier will provide a clear message for those that have a sincere desire to respect the marine mammals present on the beach, and therefore will provide some level of heightened protection for the adults and newborn seals. The rope barrier will also aid in informing people when they are more likely to be found in violation of the MMPA and potentially cited.

In previous years, OLE appreciates your practice of implementing the rope barrier. The rope barrier has been a needed step in the right direction, but closing the beach would make a safer environment for the nursing seals. OLE appreciates and looks forward to a continued opportunity to work with you in assisting you achieving your goals as well as protecting the animals and citizens of our community.

Sincerely,

Donald W. Masters
Special Agent in Charge
NOAA Fisheries/OLE

cc: April Penera, City Manager's Office
Dale Jones, Director, Office of Law Enforcement
Rod McInnis, Regional Administrator, SW Region
Russ Strach, Assistant Regional Administrator for Protected Resources, SW Region

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Perchlorate Behavior in a Municipal Lake Following Fireworks Displays

RICHARD T. WILKIN,*,† DENNIS D. FINE,‡ AND NICOLE G. BURNETT§

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Perchlorate salts of potassium and ammonium are the primary oxidants in pyrotechnic mixtures, yet insufficient information is available regarding the relationship between fireworks displays and the environmental occurrence of perchlorate. Here we document changes in perchlorate concentrations in surface water adjacent to a site of fireworks displays from 2004 to 2006. Preceding fireworks displays, perchlorate concentrations in surface water ranged from 0.005 to 0.081 μg/L, with a mean value of 0.043 μg/L. Within 14 h after the fireworks, perchlorate concentrations spiked to values ranging from 24 to 1028× the mean baseline value. A maximum perchlorate concentration of 44.2 μg/L was determined following the July 4th event in 2006. After the fireworks displays, perchlorate concentrations decreased toward the background level within 20 to 80 days, with the rate of attenuation correlating to surface water temperature. Adsorption tests indicate that sediments underlying the water column have limited (<100 nmol/g) capacity to remove perchlorate via chemical adsorption. Microcosms showed comparatively rapid intrinsic perchlorate degradation in the absence of nitrate consistent with the observed disappearance of perchlorate from the study site. This suggests that at sites with appropriate biogeochemical conditions, natural attenuation may be an important factor affecting the fate of perchlorate following fireworks displays.

Introduction

Detection of perchlorate in groundwater and surface water around the United States has fueled recent evaluations of the source, distribution, and biogeochemical processes governing perchlorate behavior in aquatic environments. Much of the current concern over this anion stems from the fact that perchlorate ingestion may pose an adverse human health risk because perchlorate interferes with the production of thyroid hormones required for normal metabolism and the development of mental function (*1*, *2*). Elevated perchlorate concentrations may also pose a risk to aquatic ecosystems. Fish from contaminated sites have been found to contain several thousands of parts per billion (ppb) of perchlorate in the head area and hundreds of ppb in the fillets (*3*). In addition, recent histological assessments show that fish from perchlorate-contaminated sites have increased thyroid follicular hyperplasia, hypertrophy, and colloid depletion at perchlorate concentrations as low as 100 μg/L and exposure times of 30 d (*4*, *5*).

The potential impact of perchlorate on human and ecosystem health is directly tied to its mobility and attenuation in the environment. Perchlorate salts are highly soluble in water and perchlorate ions weakly adhere to mineral and organic surfaces (*6*−*9*); thus, abiotic attenuation pathways of perchlorate are frequently considered to be unimportant. Attenuation of perchlorate in the environment, however, can be effectively mediated by microaerophilic or anaerobic microorganisms that transform ClO_4^- to Cl^- following the pathway $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow Cl^- + O_2$ (*10*−*13*). Perchlorate-reducing organisms can use a variety of organic carbon substrates as electron donors, such as glucose, acetate, vegetable oils, and natural organic carbon compounds present in soils and sediments (*6*, *8*, *14*, *15*). Biological transformation of perchlorate has been successfully utilized for drinking water treatment (*16*−*17*) and for in situ groundwater remediation (*18*−*21*).

Occurrences of perchlorate in groundwater and surface water stem from both anthropogenic and natural sources. Anthropogenic sources of perchlorate include ammonium perchlorate, a major ingredient of rocket fuel that powers the space shuttle and the U.S. nuclear missile arsenal. In addition, potassium perchlorate is a key ingredient in the production of fireworks, explosives, road flares, and other minor uses (*22*). Natural sources of perchlorate were generally thought to be restricted to fertilizers mined from Chilean caliche deposits (*23*). However, recent studies suggest a possible atmospheric origin for background levels of perchlorate, formed from chloride or hypochlorite during atmospheric lightning discharges or from reactions involving ozone, solar energy, and chloride (*24*−*26*). Other work proposes multiple possible sources of perchlorate to subsurface and surface environments such as mineralogical impurities, agricultural fertilizers, or in situ formation via electrochemical processes (*27*). Although fireworks are commonly referred to as a source of perchlorate to the environment (*28*−*29*), few data are available to evaluate impacts to groundwater or surface water resources (*30*). For example, Dasgupta et al. (*29*) note, in their recent examination of sources of perchlorate to the environment, that a knowledge gap exists regarding the relationship between fireworks displays and the environmental occurrence of perchlorate. This paper documents the time-dependent concentrations of perchlorate observed in a municipal lake following four fireworks displays from 2004 to 2006.

Materials and Methods

Sample Collection, Handling, and Analysis. Surface water samples were collected along the shoreline of a small lake (62 000 m^2) located in Ada, OK. The site is a park with no known source of perchlorate contamination. Sample collection times were centered on fireworks displays in July 2004, July 2005, November 2005, and July 2006. Figure 1 shows an aerial photograph of the lake, locations of sampling sites, and the location of the fireworks ignition site. Samples for perchlorate analyses were syringe-filtered (0.2 μm pore size) in the field into plastic bottles and kept refrigerated at 4 °C until analysis. Measurements in the field were made for pH,

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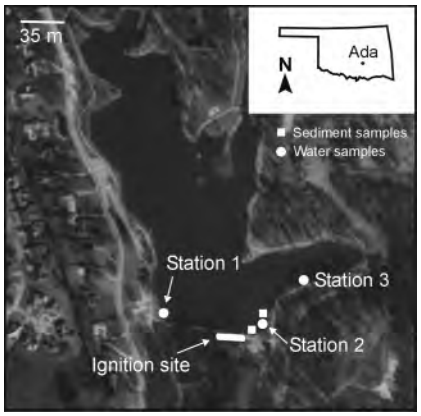

FIGURE 1. Study area, sampling locations, and fireworks ignition site.

specific conductance, and temperature. Samples for stable hydrogen and oxygen isotopic compositions were collected into 20-mL glass vials and sealed to prevent evaporation that can potentially alter $^{18}O/^{16}O$ and $^{2}H/^{1}H$ ratios. Oxygen- and hydrogen-isotopic ratios of H_2O were analyzed using a high-temperature conversion elemental analyzer linked to a continuous flow isotope ratio mass spectrometer (IRMS, Finnigan Delta plus XP). Filtered samples were also collected for element analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin-Elmer Optima 3300DV) and anion analysis by capillary electrophoresis (CE, Waters). Sediment samples were collected from the top 10 cm of the bottom sediments at locations near the surface water sampling sites. Sediments were stored at 4 °C in nitrogen-gas purged containers. Sample splits were used for solid-phase characterization, adsorption tests, and microcosm experiments.

Reagents and Standards. Water and acetonitrile (both LC/MS grade manufactured by Riedel-de Haen, Seelze, Germany), sodium perchlorate (minimum 99% purity), and 40% w/w methylamine in water were purchased from Sigma-Aldrich (Milwaukee, WI). Oxygen-18-enriched sodium perchlorate, $NaCl^{18}O_4$, was obtained from Isotec (Miamisburg, OH) and was diluted with reagent water to a concentration of 40 μg/L $Cl^{18}O_4$. The liquid chromatography (LC) mobile phase (200 mM methylamine) was prepared by adding 10 mL of 40% w/w methylamine to 490 mL of LC/MS water. Certified second source standards of perchlorate were purchased from Environmental Resource Associates (Arvada, CO).

Perchlorate Analysis. The determination of perchlorate in water was done using a liquid chromatography tandem mass spectrometry (LC/MS/MS) method based on EPA method 331.0 (*31*, *32*). An Agilent 1100 liquid chromatograph and a Finnigan TSQ Quantum Ultra triple-quadrupole mass spectrometer were used for the analysis. Sample volumes of 40 μL were injected, via an Agilent autosampler, onto a Dionex IonPac AS21 column (250 mm × 2 mm) (Dionex, Millford, MA). The flow rate of the mobile phase, 200 mM methylamine, was 350 μL/min. This allowed the perchlorate anion to elute from the column in ~8 min. All PEEK coated fused silica connecting tubing in the liquid chromatograph was replaced with PEEK tubing. Similarly, all Vespel graphite rotor seals in valves and the Vespel graphite injector seat in the LC injector were replaced with parts made of PEEK material. A postcolumn flow of 300 μL/min acetonitrile was added via a tee before the column flow entered the electrospray source. Optimization of the MS parameters was done using infusion of perchlorate into the mobile phase (further details are provided in the Supporting Information).

A value for the lowest concentration minimum reporting level (LCMRL) for this method, 0.011 μg/L, was calculated using the procedure described elsewhere (*33*). The method detection limit (MDL) was determined by analyzing seven samples prepared separately at the 0.010 μg/L level, calculating the standard deviation of the determined concentration, and multiplying the standard deviation by 3.15 (the 97% Student t value) (*32*). The MDL for the method was determined to be 0.003 μg/L (0.03 nmol/L). The quality control data for this study were collated from sample queues run between July 2004 and August 2006. Over this period, continuing calibration check standards of 0.025 and 0.100 μg/L had average recoveries of 109% (RSD = 9.2%, $n = 4$) and 103% (RSD = 5%, $n = 13$), respectively. Secondary source standards with certified concentrations at 0.151 and 1.51 μg/L had average recoveries of 99% (RSD 3.6%, $n = 6$) and 101% (RSD 2.6%, $n = 11$), respectively. During this study fifteen samples were spiked with perchlorate at concentrations between 0.100 and 10 μg/L. The average matrix spike recovery for these samples was 101% (RSD = 11%). The concentration of perchlorate in the samples that were spiked ranged from 0.017 to 11.9 μg/L.

Adsorption and Microcosm Experiments. Precautions were taken to minimize the alteration of sediment samples prior to use in batch adsorption and microcosm experiments. Fresh sediments (wet) were added to 50 mL bottles along with oxygen-saturated deionized water, and aliquots of a stock sodium perchlorate solution. Oxygen-saturated water was used in batch adsorption tests to inhibit potential microbial degradation of perchlorate. The bottles were sealed with screw caps and their contents were mixed on a mechanical shaker for 2 d. All samples were filtered through 0.2-μm syringe filters and analyzed for perchlorate by LC/MS/MS.

Microcosm experiments were conducted in 45 mL glass serum bottles. Duplicate experiments were established containing 1 g of wet sediment, plus solution containing 5 mg/L NO_3^--N, 1 mg/L ClO_4^-, or a mixture of 1 mg/L ClO_4^- and 5 mg/L NO_3^--N. All solutions were purged with nitrogen gas to remove dissolved oxygen. Sterile control experiments were set up with $HgCl_2$ and container controls were prepared by spiking sterile water in serum bottles with the stock nitrate and perchlorate solutions. At selected time intervals, samples were collected from the serum bottles and filtered through 0.2 μm syringe filters prior to sample storage and analysis. An analysis of holding times indicated that perchlorate concentrations were stable for time periods of at least 6 months in filtered solutions (see Supporting Information, Figure S1).

Results and Discussion

Method Improvement. One important modification to the reported LC/MS/MS method (*31*) that increased the overall method sensitivity of perchlorate determinations was to add acetonitrile postcolumn before the aqueous LC solvent entered the electrospray source. The addition of organic solvents to an aqueous mobile phase can help reduce the effects of surface tension, viscosity, and heat of vaporation (*34*). An increase in perchlorate response of 170% occurred immediately with as little as 50 μL/min addition of acetonitrile and continued with acetonitrile flows of up to 500 μL/min (Figure 2). For this analysis, a postcolumn flow of 350 μL/min of acetonitrile was used. This resulted in an 8-fold increase in the response of perchlorate and isotopically labeled perchlorate.

Perchlorate Background, Spiking, and Attenuation. Temporal trends in perchlorate concentrations show significant variations centered on the timing of fireworks displays (Figure 3). Perchlorate concentrations preceding fireworks displays, by up to 6 days in July 2005, November 2005, and

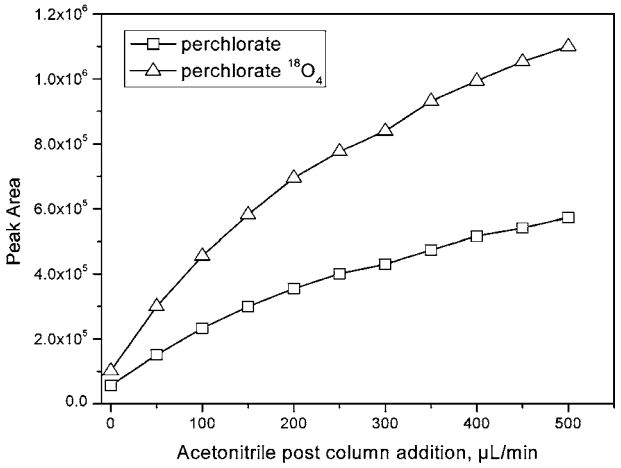

FIGURE 2. Increase in LC/MS/MS peak area response for replicate injections of 0.50 μg/L perchlorate and 1.0 μg/L labeled perchlorate as the flow rate of postcolumn acetonitrile increases.

July 2006, ranged from 0.005 to 0.081 μg/L (0.05 to 0.81 nmol/L), with a mean value of 0.043 μg/L (0.43 nmol/L; $n =$ 15). Prior to fireworks displays, the ClO_4^-/Cl^- mole ratio in Wintersmith Park surface water was 5.4×10^{-7}. This baseline ClO_4^-/Cl^- mole ratio is lower by a factor of 256× compared to the ratio estimated for modern bulk atmospheric deposition, approximately $1.4 \pm 0.1 \times 10^{-4}$ in New Mexico (*26*), suggesting perchlorate depletion in Wintersmith Lake surface water relative to chloride due to biological processes or chloride enrichment from other sources.

Sampling events within 14 h after the fireworks showed spikes in perchlorate values ranging from 24 to 1028× the mean baseline value. A maximum perchlorate concentration of 44.2 μg/L (444 nmol/L) was determined following the July 2006 display (Figure 3). These trends show significant increases in perchlorate levels that can be reasonably attributed to fireworks sources. Rainfall events do not obviously correlate with perchlorate concentrations which would indicate perchlorate inputs from surface runoff (Figure 3). Various factors potentially impact the absolute increase of perchlorate levels in surface water bodies adjacent to fireworks displays, such as the overall amount of ignited fireworks and efficiency of perchlorate oxidation which controls the mass of perchlorate introduced to the environment, wind direction and velocity which controls the dispersion and fallout of perchlorate-enriched particles, and sampling locations relative to the site of fireworks detonation. About 2−3× more fireworks were ignited during the July 2005 display as compared to the November 2005 display (city of Ada, personal communication), which is generally consistent with the observed perchlorate response in surface water (Figure 3).

In a previous study, Canadian surface waters in the Great Lakes Basin were analyzed for the presence of perchlorate (*35*). Sampling sites included Hamilton Harbor, Niagara River, Lake Huron, and Lake Erie. Surface water samples were analyzed by HPLC/MS/MS using isotopically labeled perchlorate. Perchlorate was detected at several sites at concentrations close to the reported method detection limit of 0.2 μg/L (2.0 nmol/L). Interestingly, perchlorate was detected in Hamilton Harbor, the location of Canada Day fireworks (July 2004). Perchlorate was detected 4 days after the event; a week later perchlorate was undetected at the same site (*35*).

In each of the fireworks events examined in this study, perchlorate concentrations attained a maximum level within 1 d following the display. Subsequently, concentrations decreased and reached the background level after 20−80 d

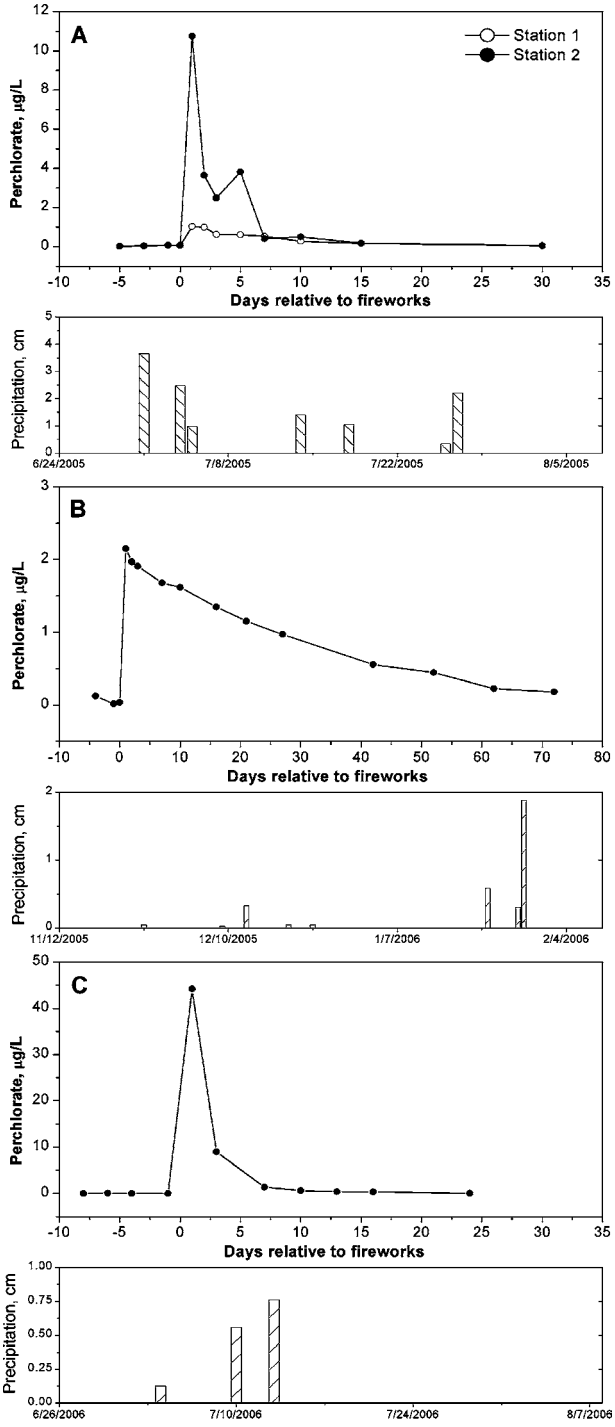

FIGURE 3. Perchlorate concentration trends and precipitation data centered on fireworks displays in (A) July 2005, (B) November 2005, and (C) July 2006. Samples taken from Station 3 before and after the 2004 July 4th display indicated perchlorate concentrations had changed from 0.08 (on July 2, 2004) to 6.42 μg/L (on July 5, 2004). Station 1 was sampled only in July 2005. Data for all sampling events are presented in the Supporting Information (Table S1).

(Figure 3). The reaction kinetics of perchlorate disappearance from the aqueous phase was modeled with a pseudo-first-order rate equation

$$dC/dt = -k_{obs}C$$

where C is the concentration of perchlorate in the aqueous phase (μg/L), k_{obs} is the observed first-order rate constant (d^{-1}), and t is time (d). Linear regression analysis of plots of

the natural logarithm of perchlorate concentration versus time gave straight-line results with R^2 values ranging from 0.81 to 0.99 (see Supporting Information Figure S2). Values of k_{obs} ranged between 0.03 and 0.28 d^{-1}. Rates of perchlorate removal observed in Wintersmith surface waters are similar to a microbial degradation rate, 0.14 d^{-1}, measured in sediment porewaters from a contaminated site (*15*). Perchlorate removal rates in Wintersmith Lake correlate with temperature. The fastest rate of perchlorate removal was observed in surface water with a mean temperature of 33.4 °C (July 2006, see Supporting Information Table S1); whereas, the slowest apparent rate occurred in surface water with a mean temperature of 12.4 °C (November 2005). The apparent activation energy (E_a) of the perchlorate removal process was estimated using the equation

$$E_a = -R\frac{d\ln k_{obs}}{d(1/T)}$$

where E_a is the apparent activation energy (kJ/mol), R is the gas constant (8.314×10^{-3} kJ/mol K), and T is temperature (K). Regression analysis yields an apparent activation energy of 60.5 ± 5.0 kJ/mol (see Supporting Information Figure S3), consistent with cellular and life-related reactions, mineral precipitation−dissolution reactions, but not with adsorption or diffusion processes (*36*).

Other Components in Pyrotechnics. White (*28*) lists over 53 organic and inorganic chemicals important in fireworks as fuels, oxidizers, binding agents, and for various coloration and sound effects. Perchlorate salts of potassium and ammonium are the most common oxidizers in modern fireworks displays, and presumably unreacted perchlorate salts are the compounds that lead to spikes in soluble perchlorate concentrations discussed above. Detonation of fireworks is expected to lead to the quantitative conversion of perchlorate to chloride following, for example, the decomposition reaction for potassium perchlorate:

$$KClO_4 \rightarrow KCl + 2O_{2(g)}$$

Thus, complete efficiency in perchlorate oxidation reactions during pyrotechnical displays should result in no remaining perchlorate (*37*). Not surprisingly, spikes in chloride concentrations were consistently observed after the fireworks displays, but were delayed relative to the timing of perchlorate spikes by 3−5 days (see Supporting Information Figure S4). Chloride concentrations were observed to increase by about 5−7 mg/L compared to pre-fireworks values or by about 25%. Only a small fraction (maximum of 0.3%) of this chloride could have been derived from degrading perchlorate that was present in the lake water; the main source of this chloride is apparently from the dissolution of combustion residues.

Other compounds containing strontium, barium, calcium, sodium, copper, antimony, aluminum, and magnesium are essential color-emitters used in pyrotechnical displays (*37*). Spikes in the concentrations of these other elements were not detected in Wintersmith Lake. The reasons for this are uncertain but may have to do with the more limited sensitivity of the analytical technique employed for these elements (ICP-OES) coupled in some cases with relatively high background concentrations (Ca, Na, K, Mg) and their lower mass abundance compared to perchlorate in the fireworks. Also the final chemical form, water solubility, and reactivity of metals associated with the remains of detonated fireworks have not been studied in detail.

Oxygen and Hydrogen Isotopes. Stable oxygen and hydrogen isotope ratios are valuable for hydrologic investigations, especially for water-balance evaluations in groundwater and surface water systems. Wintersmith Lake surface water had stable isotope ratios of oxygen and hydrogen that showed seasonal variations of about 1.5‰ and 8‰, respectively (Figure 4). Isotopic data show the effects of evaporation in that the meteoric water trend is not observed; rather data follow along a trajectory below the meteoric water trend having a slope of about 5 instead of 8. Ratios of $^{18}O/^{16}O$ and $^{2}H/^{1}H$ are more variable in the summer, because of greater precipitation and generally more intense evaporation compared to the late fall and winter. Considering the limited rainfall that occurred over the period that perchlorate concentrations were decreasing in Wintersmith Lake (Figure 3) and the overall evaporitic trend indicated by the isotopic data (Figure 4), dilution is not expected to be an important factor in lowering perchlorate levels in this system. Dilution of perchlorate concentrations via mixing of the lake water, however, is a possibility that was not assessed during this study.

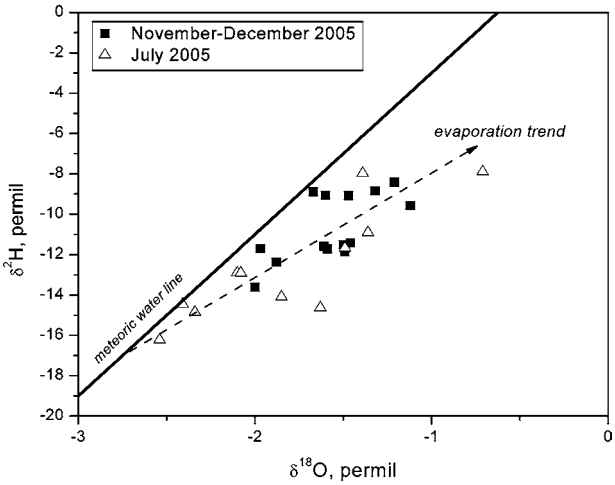

FIGURE 4. Plot of $\delta^{18}O$ versus $\delta^{2}H$ of samples collected from Wintersmith Lake.

TABLE 1. Selected Chemical Characteristics for Two Core Sections (0−10 cm Depth) from Wintersmith Lake in Ada, OK

core	% clay	% silt	% sand	sediment pH	TOC,[a] g kg^{-1}	TIC,[a] g kg^{-1}	TS,[b] g kg^{-1}
NBWR	15	12	73	8.46	35.2	4.1	0.16
NBWL	10	8	82	8.03	1.82	0.43	1.2

[a] Measured by carbon coulometry. Total carbon (TC) determined by combustion at 950 °C. Total inorganic carbon (TIC) determined by acid extraction using 2 N perchloric acid. Total organic carbon (TOC) is equal to TC − TIC. Total sulfur (TS) measured by sulfur coulometry via combustion at 1100 °C.

Adsorption. It is widely accepted that perchlorate does not appreciably sorb to solids and that its mobility and fate in the environment are largely influenced by hydrological and biological factors (*9*). Core samples retrieved from the bottom of Wintersmith Lake were composed predominately of sand-sized particles and the sediment pH for each core was between 8.0 and 8.5 (Table 1), similar to the mean pH of the overlying water column (8.51 ± 0.44; $n = 50$). Core section NBWR was ~20× more enriched in organic matter compared to core section NBWL (Table 1). Also the fraction of clay-sized particles is somewhat higher in the NBWR sample.

Constant-pH sorption tests were conducted with perchlorate loadings from about 10 to 450 μg perchlorate per g of sediment. Core section NBWL showed no potential to remove perchlorate from solution, as 96−102% of the spiked perchlorate was recovered in the aqueous phase (Figure 5). However, core section NBWR removed up to about 10 μg of perchlorate per g of sediment (100 nmol g^{-1}) (Figure 5). The measurable sorption capacity for this material may be related to a higher abundance of organic carbon and an overall finer

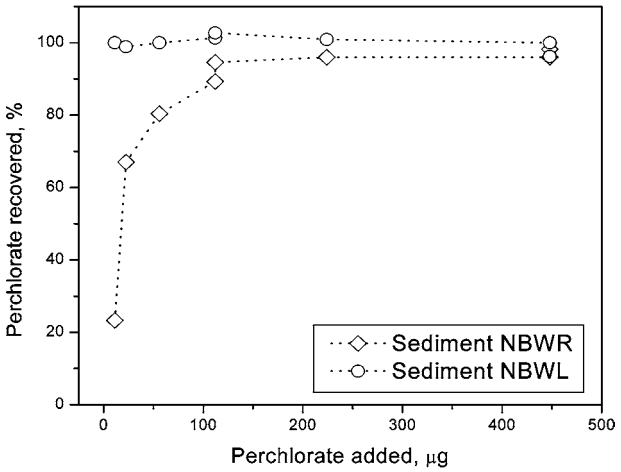

FIGURE 5. Results of sediment adsorption tests. Perchlorate recovered as a function of perchlorate loading to 1 g of sediment, pH 7.5 ± 0.3, and 2 day exposure time.

grain size (Table 1). As noted in previous studies, it can be difficult to discern between chemical adsorption and microbial degradation in batch experiments with perchlorate (*8*, *9*). The batch adsorption experiments were conducted over 48 h with initial aerobic conditions that should have prevented any microbial perchlorate degradation. The results of the adsorption tests suggest that sediments underlying Wintersmith Lake have only a minor capacity to remove perchlorate via sorption. However, this mechanism of removal cannot be completely discounted. More detailed assessments of the spatial distribution of organic carbon content and perchlorate adsorption capacity may allow for a better estimate of perchlorate adsorption and desorption.

Microcosms. Microcosm experiments show that Wintersmith Lake sediments contain microbial communities capable of reducing both nitrate and perchlorate, with nitrate reduction being favored (Figure 6). In nitrate-free microcosms, perchlorate was degraded from 1 mg/L after 18 d to at or below 0.05 μg/L; whereas, in microcosms with both perchlorate and nitrate present the start of perchlorate reduction lagged several days behind nitrate reduction, and up to 35 d was needed for perchlorate concentrations to decrease below 0.05 μg/L. Note that the mean value of nitrate in Wintersmith Lake was determined to be 40 μg/L. Interestingly, the lowest perchlorate concentrations obtained in the microcosms fall within range of the observed pre-fireworks background levels in Wintersmith Lake (Figure 6a), perhaps suggesting that microbial perchlorate reduction becomes unfavorable at very low concentrations (*38*). Simultaneous reduction of perchlorate and nitrate was observed. However, perchlorate reduction was clearly favored only after nitrate concentrations were reduced to below 200 μg N/L. Note that a transient period of ammonia production, perhaps due to dissimilatory nitrate reduction to ammonia, occurred prior to dentrification. Pseudo-first-order rate constants were determined by fitting perchlorate data in the initial nonlinear decay period. Rate constants ranged from 0.39 to 0.59 d^{-1} and are comparable to rates observed in previous microcosm studies on sediments from contaminated sites (*15*, *39*). Additional studies to examine the effects of initial concentrations of perchlorate and nitrate have not been conducted, but the results indicate that microbial perchlorate reduction can occur at initial perchlorate levels much greater than are observed resulting from several fireworks displays.

Implications. Spikes in perchlorate concentrations significantly above background levels were noted after four separate fireworks displays, and in one case concentrations in Wintersmith Lake reached 44 μg/L. Maximum concentrations observed in this study following fireworks displays exceed current action levels for drinking water (e.g., 6 μg/L State of California; 4 μg/L State of Texas, and 1 μg/L State of Massachusetts). It is unclear if aquatic organisms are affected at these concentration levels, although previous work indicates thyroid impacts in fish at perchlorate concentrations as low as 100 μg/L and exposure times of 30 d (*4*). Microcosm tests showed comparatively rapid intrinsic perchlorate degradation in the absence of nitrate consistent with the observed disappearance of perchlorate from Wintersmith Lake, indicating that natural attenuation may be an important factor affecting the fate of perchlorate in the environment following fireworks displays. The availability of organic carbon to provide energy for perchlorate reducing bacteria may be a key factor governing perchlorate attenuation rates in the environment. Results from this study highlight the need for additional studies of perchlorate behavior following fireworks displays in relation to surface water and groundwater quality, particularly in urban areas.

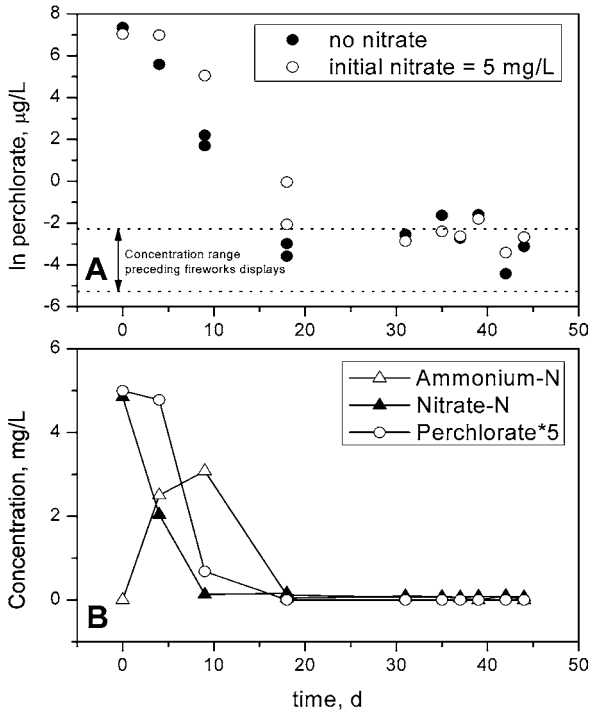

FIGURE 6. Results of microcosm studies showing degradation of perchlorate and nitrate as a function of time (sediment sample NBWR). (A) Perchlorate concentration change with and without nitrate with respect to time. (B) Microcosm concentration of ammonia-N, nitrate-N, and perchlorate with respect to time. Perchlorate concentrations were multiplied by 5 to make trends more apparent.

Acknowledgments

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Supporting Information Available

Additional information, data, and figures as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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World record rocket launch attempt- an assessment of pollution to controlled waters and toxicity[1]

27 July 2007

1. Introduction

1.1 The Environment Division's position concerning the record attempt

The Environment Division would, in all cases, strongly advise against any addition of chemical or waste products, or any large-scale disturbance, to the St Aubin's Bay area. However, the Division recognises the social context and benefits of the firework world record attempt, as well as, the traditional nature of displays to mark the finale of the Battle of Flowers.

Whilst the Environment Division does not support any potential risk to the environment, it has sought to provide a balanced perspective and to identify and quantify these risks and, more importantly, practical means by which they can be minimised.

The Division considers that the main potential risk to the environment will be through the physical setting and clearing-up of the firework area, rather than by the fireworks themselves. To this end, comprehensive guidelines to safeguard the bay area have been forwarded and discussed with Mr McDonald. Adherence to these will be closely monitored and assessed by the relevant Environment Officers.

Any significant harm to the aquatic environment or damage to the amenity value of the bay caused by the record attempt will be investigated as a pollution incident under the Water Pollution (Jersey) Law, 2000.

1.2 Background information

The paper assesses the likely risk of pollution to controlled waters and, where possible, the toxicity to the aquatic ecosystem resulting from the world-record attempt to launch the highest number of firework rockets at one time.

The record attempt will take place within the inter-tidal zone of St Aubin's Bay, and is scheduled during the Jersey Battle of Flowers Moonlight Parade, Friday 10 August 2007 at approximately 22.30hrs. The event is organised by Mr Terry McDonald.

A total of 111,000 rockets are planned to be fired simultaneously. These comprise, in equal numbers, of white, red and 'crackle' rockets. The estimated height that each rocket will reach is 40-60m. The rocket launch will last for approximately fifteen seconds.

The paper is based on information forwarded to date to the Environment Division by Mr McDonald. This includes Parts 1 and 2 of the Risk Assessment and a chemical breakdown of the rocket types to be used.

The record attempt has received some negative public comment regarding the potential impact on the marine ecosystem within St Aubin's Bay.

[1] Based on information to date, Part 1 & 2, Risk Assessment and chemical breakdown of rockets forwarded by Mr T. McDonald

2. Impact of the rocket launch on the marine ecosystem

2.1 Chemical composition of the rockets (pre-combustion) and concentration of chemicals

The composition, by weight, of the chemicals used in the record attempt was requested by, and has been forwarded to, the Environment Division. The weight per rocket has been raised to the total number of rockets (Table 1).

Table 1. Total weights (kg) and concentration (mg l^{-1}) in St Aubin's Bay of chemicals for the pre-ignited rockets used in the record attempt

Chemical	Formula	Weight (Kg)			Total Weight (Kg)	Concentration at high water[3] (mg l^{-1})	LD50 RAT (mg kg^{-1})
		Red*[2]	White	Crackle			
Potassium nitrate	KNO_3	207	207	317	732	0.0058	LD50 3,750 mg kg^{-1}
Potassium perchlorate	$KClO_3$	127	122	44	294	0.0023	n/a
Carbon	C	72	72	114	258	0.0020	-
Aluminium & magnesium alloy	Al + Mg	52	60	32	144	0.0011	LD50 >2000 mg kg^{-1}
Aluminium	Al	19	96	19	133	0.0011	n/a
Sulphur	S	17	17	33	67	0.0005	LD50 8 mg kg^{-1}
Strontium carbonate	$SrCO_3$	52	-	-	52	0.0004	5 mg/m^3 nuisance dust
Resinox (phenolic resin)	$C_{48}H_{42}O_7$	16	18	-	34	0.0003	n/a
Copper oxide	CuO	-	-	32	32	0.0003	LD50 278 mg kg^{-1}
Polyvinyl chloride	$(C_2H_3CL)n$	18	-	-	18	0.0001	LD50 fish 0.17 mg l^{-1} n/a
Lac	$C_{16}H_{24}O_5$	13	-	-	13	0.0001	n/a
Total		**592**	**592**	**592**	**1,776**	0.0140	

where: LD50 is the amount of a material, given orally all at once, which causes the death of 50% of a group of test rats.
n/a; data on LD50 not established.

The total weight of chemicals of the 111,000 pre-ignited rockets is approximately 1.8 tonne[4]. If this total quantity fell into St Aubin's Bay and was evenly diluted within the bay (through tidal and wave mixing) then the concentration at high water would be 0.014 mg per litre sea water (Table 1). Where data is available, values for constituent chemicals are lower than the LD50 values (where one litre of sea water is 1.03 Kg).

This figure represents a maximum concentration, given that the high-temperature combustion of the rockets will convert much of the initial weight into air-born gases.

For example, slightly more than one tonne (58% of the total weight) of the chemicals comprise of potassium nitrate and potassium perchlorate (constituents of gunpowder) which are used to propel the rocket. The majority of these two chemicals will be converted into a gaseous state during firing and will therefore not all directly enter St Aubin's Bay.

[2] where 37,000 rockets of each type will be fired
[3] includes St Aubin's Bay area taken inside the line between Noirmont and south end of Elizabeth Castle breakwater. High water taken on 10-08-07
[4] where total weight of the pre-ignited fireworks (inc. sticks, cardboard etc) is 5.7 tonne.

However, many of the resulting chemicals will, in the first instance, be deposited within a more limited 'fall-out' area defined by the angle and height of rocket firing and the wind strength and direction. Therefore, the initial concentration of chemical by-products within this more limited area will be greater.

This is particularly relevant for the insoluble metal oxides and sulphates produced during combustion. Being insoluble they will not easily be dispersed away from the fall-out area. The soluble products (chlorides, nitrates and perchlorates etc.) will, however, be more easily dispersed.

2.2 Uses, human health risks and toxicity of the chemicals in the pre-ignited rockets

Table 2 shows that most of the pre-combusted chemicals used in the record attempt are soluble and, in their raw state, of a low health risk (apart from copper oxide). However, of more importance are the chemicals, and their solubility and toxicity resulting from high temperature combustion.

Table 2 Uses, health risks and toxicity of chemicals used in the firework display (pre-combustion state)

Chemical	Uses	Human health risk	Toxicity
Potassium nitrate	Gunpowder (saltpetre)	Irritation to skin (itching), eyes and respiratory tract (coughing, shortness of breath).	Combustion over 400ºC causes decomposition, forming toxic nitrogen dioxide and oxygen
Potassium perchlorate	Gunpowder, has replaced unstable potassium chlorate	Irritation to skin, eyes and lungs.	Heating to decomposition releases toxic fumes such as potassium oxide. Highly soluble, easily dispersed (Schneider, 2001).
Carbon	Naturally occurring	-	Low, will form CO_2 on combustion.
Aluminium and magnesium alloy	Naturally occurring material	Medication to relieve heartburn, sore stomach, or acid indigestion. Irritant mucus membranes in large does.	Low
Aluminium	Light weight construction. Most abundant metal on earth.	No studies have found a correlation between aluminium oxide and neurological effect. Irritant to mucus membranes, contact dermatitis.	Highly insoluble as a solid. Flammable in powder form.
Sulphur	Manufacture of acids, bleaching. Naturally occurring chemical.	Sulphur required by the body. Sulphuric substances may affect behaviour and circulation.	Sulphur non toxic. By-product sulphuric substances are toxic.
Strontium carbonate	Manufacture of TV-tube glass, ceramic ferrites. Provides red colour in the rockets.	Irritation to skin, eyes, and respiratory tract if inhaled at 10 mg m^{-3}.	Slightly soluble in water, low health risk
Resinox (phenolic resin)	Reaction of phenols with simple aldehydes and used to make molded products (e.g. snooker balls, and as coatings and adhesives.	-	-
Copper oxide	Fungicides, seed dressings, boat anti-fouling paint.	Headache cough sweating nausea and fever may be caused by freshly formed fumes or dust of copper oxide.	Toxic to aquatic organisms.
Polyvinyl chloride	One of the most widely used plastics. Found in products such as packaging, cling film, bottles and materials such as window frames, cables, pipes, flooring, wallpaper and window blinds.	May cause cancer and birth defects	Low toxicity. Liberate toxic dioxins on ignition in fireworks. Molecular weight too high to be available to most organisms.
Lac	Varnishes, French polish (shellac)	Complex natural substance. Derived from tree resin.	Low

2.3 Human health risks and toxicity of the chemicals resulting from combustion of the rockets

The wide range of chemicals available, the intense heat of pyrotechnic flames, and the almost infinite number of ways in which they can be combined makes a detailed breakdown of combustion products difficult (von Oertzen, 2001).

For example, the combustion of the three elements of gunpowder (potassium nitrate, carbon and sulphur) results in the production of potassium carbonate, potassium sulphate, hydrogen sulphide and eight other chemical products. These chemicals are further enhanced by the use of perchlorate, the oxidisation of metals, metal salts, and binders that are used for colour or sound effects in the firework displays.

However, the record attempt consists only of white, red and crackle rockets. Apart from the constituents of gunpowder (potassium nitrate, potassium perchlorate, carbon and sulphur) and binders (resinox and lac), only strontium carbonate, aluminium and magnesium alloys and copper oxide are used for colours and sound.

This potential mix of chemicals is, therefore, likely to be less than a normal firework display that contains a multitude of colours and sound effects. For example, lead and barium and the blue coloured rockets (that give off high proportions of dioxins) are not being used in the record attempt.

It remains however that given the high temperature reaction that, potentially, a large array of chemical products will be formed during the record attempt. It is extremely difficult to quantify the type and quantities of the compounds that will be produced, on which an assessment to the risk of environmental pollution or toxicity can be made.

Literature further provides little information. Environmental papers generally report products of combustion as metal oxides, nitrates, chlorides, sulphates and carbonates etc. and do not give details of individual products (von Oertzen, 2001).

Given that specific information on potential pollutants of the record attempt is limited, a wider assessment of environmental impact has been made. Many of the chemicals that are deposited on the beach will be water soluble (perchlorates, hydrogen sulphate, chlorides etc.). The display is to take place approximately 3.5 hours before the beach is submerged by the rising tide and four days before the spring tides (10.82m springs). The tidal currents will help maximise the dilution of the soluble chemicals and mixing of the insoluble elements.

The insoluble chemicals include most of the metal oxides and sulphates produced from combustion of the rockets (including aluminium, magnesium and copper oxides). The fact that they are insoluble means that they are difficult to incorporate into the food chain (apart from direct ingestion by bottom feeding fish or wading birds). These will probably persist longer in the bay, although wave and tidal action will help to disperse these in the longer-term.

The concentration of these insoluble products is expected to be much lower than the soil guideline values (SGVs) that have been developed for the UK and the Dutch Intervention Guidance for groundwater. It is recognised that these values only cover a few of the chemicals produced from combusted fireworks. However, taking copper as an example, if all the copper fell in its raw state within the confines of the fall-out area then a concentration of 5.5 mg kg^{-1} sand would be expected.[5] This is below the Dutch Intervention Guideline of 75 mg kg^{-1} and the LD50 Rat of 470 mg kg^{-1}.

Given the lack of knowledge concerning the chemicals produced, an indication of the effects of firework displays on the environment can be gained from case examples. A 10-year study of an estimated 2,000 firework displays over water at Walt Disney's EPCOT centre in Florida (Debusk *et al*. 1992) found little effect on the aquatic ecosystem. The study concluded that minimal risk to the environment would be caused by infrequent firework displays.

3. Summary and Conclusion

1. The large number of firework fired during the record attempt and their resultant combustion products contain toxic and environmentally damaging chemicals.

2. Many of the products will be soluble and become highly diluted and removed from the open bay area. Insoluble chemicals will not easily be absorbed into the marine food chain and the limited analysis able to be undertaken indicates that the initial concentration will be below risk levels.

3. A case study shows that single one-off firework events are unlikely to result in large-scale damage to the environment. Particularly, for an ecosystem which is open and subject to regular wind and tide mixing.

4. It is considered that the greatest potential risk to the environment will be caused by the physical setting and clearing-up of the launch area (damage from vehicle and foot traffic, raking sand etc). The Environment Division has written a detailed paper to Mr McDonald that gives comprehensive guidelines on how the risks to the Bay's environment can be minimised.

5. The test firing, rocket launch and beach clean up will be closely monitored by the Environment Division with ongoing advice given.

6. Any significant harm to the aquatic environment or damage to the amenity value of the bay that is caused by the record attempt will be investigated as a pollution incident under the Water Pollution (Jersey) Law, 2000.

[5] Given area of fall-out 100x200m square, 15cm mixing of sand.

References

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Linda S. Adams
Secretary for Environmental Protection

California Regional Water Quality Control Board

San Diego Region

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April 5, 2010

Mr. Charles D. Wurster
President/CEO
San Diego Unified Port District
3165 Pacific Highway
San Diego, CA 92101
Dear Mr. Wurster:

SUBJECT: Regulation of Wastewater Discharges From Firework Events

This is in response to your letter dated March 3, 2010 requesting information on the California Regional Water Quality Control Board, San Diego Region's (San Diego Water Board) approach for future regulation of discharges from firework events.

NPDES Permits

Firework events in the San Diego Region are typically conducted over or adjacent to surface water bodies, including but not limited to, the San Diego River, San Diego Bay, Mission Bay, and the Pacific Ocean. Firework events result in the release of pollutants to these waters including aluminum, magnesium, strontium, barium, sodium, potassium, iron, copper, sulfate, nitrate, and perchlorate. Firework events also result in the release to surface waters of debris from exploded and unexploded shells such as paper, cardboard, wire and fuses. Based on these considerations, the San Diego Water Board considers pollutant releases from firework events over or adjacent to surface waters as point source discharges of pollutants subject to the National Pollutant Discharge Elimination System (NPDES) permit requirements of the federal Clean Water Act.

The San Diego Water Board plans to draft a general NPDES permit to regulate firework related wastes discharged into surface waters in the San Diego Region and bring it before the Board for adoption in the first half of 2011. The San Diego Water Board is currently evaluating data from monitoring conducted at SeaWorld San Diego for their firework events conducted over Mission Bay. This analysis is helpful in determining appropriate discharge and monitoring requirements for the proposed general NPDES permit.

Under the federal Clean Water Act and California's Porter Cologne Water Quality Control Act, discharges of pollutants to waters of the United States[1] are authorized by obtaining

[1] The USEPA has interpreted "waters of the United States" to include "intrastate lakes, rivers, streams (including intermittent streams) . . . the use, degradation, or destruction of which would affect or could affect interstate or foreign commerce," and "tributaries of [those] waters." [40 C.F.R. § 122.2(c) and (e) respectively]. San Diego River, San Diego Bay, Mission Bay, and the Pacific Ocean and most other surface water bodies in the San Diego Region are designated as waters of the United States.

and complying with the terms of an NPDES permit. Persons proposing to discharge pollutants must obtain an NPDES permit before they can lawfully discharge. Unauthorized discharges are normally subject to enforcement action by the San Diego Water Board.

Interim Approach

In the interim period, however, until adoption of a general NPDES permit for firework events, I will not recommend that the San Diego Water Board initiate any enforcement action for firework events over or adjacent to surface waters of the San Diego Region. This position is predicated upon implementation of Best Management Practices designed to minimize environmental impacts before, during and after firework events. Further, it is expected that any such firework displays comply with the requirements of other governmental organizations having jurisdiction over such events. My decision to not recommend any enforcement actions in this regard may change based upon new information on the water quality effects of firework events. In that scenario, I will meet and discuss any violations with the Port District or other responsible parties before beginning an enforcement action. Please note that my decision to not recommend any firework event enforcement actions during the interim period is not a restriction or shield against third party lawsuits under the citizen suit provisions of the Clean Water Act.

Applications for NPDES Permit Coverage

The San Diego Water Board is not requiring submission of an individual NPDES permit application or notice of intent for coverage under general NPDES permit requirements for firework event waste discharges to surface waters at this time. The Port District or any other person, however, pursuant to California Water Code Sections 13376 and 13260, may submit an NPDES permit application for firework event discharges at any time. In some cases, it might be beneficial for a single entity to submit an NPDES permit application covering multiple events for a particular water body, as long as that entity has the authority to oversee the operators to ensure full compliance with the permit conditions. For example, the Port of San Diego could submit an application covering events within San Diego Bay under its jurisdiction. The NPDES permit application should include, at a minimum, the following information:

1. A completed USEPA Application Form 1 – General Information (EPA Form 3510-1)
2. The number of annual shows;
3. The duration of each show;
4. Location from which fireworks are launched;
5. Number and size of shells dispensed per show;
6. Fireworks deposition zone;
7. Chemical composition of fireworks;
8. Best Management Practices designed to minimize environmental impacts before, during, and after fireworks events; and

9. A filing fee check or money order payable to the State Water Resources Control Board in the amount of $1,452.00

We look forward to working with the Port District and others on the development and implementation of the proposed general NPDES permit for firework events.

If you have any questions, please contact Brian Kelley at (858) 467-4254, email bkelly@waterboards.ca.gov or David Barker (858) 467-2989, email dbarker@waterboards.ca.gov.

Respectfully,

James G. Smith
Assistant Executive Officer

JS:dtb:bdk:mm

cc :

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CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD
SAN DIEGO REGION

TENTATIVE ADDENDUM NO. 1 TO
ORDER NO. R9-2005-0091, NPDES NO CA0107336
FOR THE DISCHARGE OF WASTE FROM
SEA WORLD AERIAL FIREWORKS DISPLAYS TO SAN DIEGO MISSION BAY
SAN DIEGO

The California Regional Water Quality Control Board, San Diego Region (hereinafter Regional Board) finds that:

1. On October 26, 2006, Brown and Caldwell submitted an incomplete report of waste discharge (RWD) on behalf of SeaWorld, San Diego for the discharge of waste to Mission Bay associated with their fireworks program. Additional information was requested on December 7, 2007 and received on January 19, 2007 to make the application complete.

2. The RWD indicates that nightly displays of fireworks occur during the summer months between April and September and other times during the year. Under the current Sea World Master Plan update, approved by the California Coastal Commission in 2001, Sea World may present up to 150 fireworks shows per year, with an anticipated average between 110 and 120 shows per year.

   The fireworks are launched from a barge located in the Pacific Passage Zone of Mission Bay, between Fiesta Island and the Sea World Shorelines. The average fireworks show lasts 5 to 6 minutes and dispenses approximately 250 shells; special events, such as the 4th of July and New Year's Eve, may dispense between 1,000 and 1,750 shells. Sea World subcontracts the logistics of fireworks, operations, transportation, setup, ignition and cleanup to Fireworks America, a licensed pyrotechnics company based in Lakeside, CA.

3. Typical fireworks constituents include aluminum, magnesium, strontium, barium, sodium, potassium, iron, copper, sulfate, nitrate and perchlorate. These constituents have a potential to adversely impact and/or contribute to degradation of water and sediment quality within Mission Bay.

   In addition, debris from unexploded shells as well as paper, cardboard, wires and fuses from exploded shells can also adversely impact the quality within Mission Bay. The area affected by these debris can vary depending on wind speed and direction, size of the shells, and other environmental and anthropogenic factors.

4. After each aerial fireworks display, crews conduct sweeps to gather floating debris from spent fireworks using handheld fishnets. In addition, the fireworks barge is swept immediately after each show to prevent solid waste and debris

from being swept into the water by the wind. Unexploded fireworks are disposed of by Fireworks America. Fireworks debris deposited on Fiesta Island is collected from the shorelines each morning following the aerial fireworks display. Solid waste typically consists of paper, paperboard or cardboard shells, and marginal amounts of wires and fuses.

Data for wet and dry debris retrieved by Sea World staff since 2002 was reviewed and it was determined that, on average, 11 pounds of fireworks related wet debris were collected each evening and 8 pounds of wet debris each morning.

5. Sea World conducted annual fireworks related monitoring of sediment and water quality parameters between 2001-2006. The final monitoring report prepared for Sea World, by Science Applications International Corporation, concluded that there were no significant spatial or temporal patterns in concentrations of critical metals in sea water or sediments in Mission Bay. It was also concluded that there is no indication of fireworks residue accumulation in the water or sediment of Mission Bay.

6. This action is exempt from the provisions of the California Environmental Quality Act (Public Resources Code, Section 21100 Et seq.) in accordance with California Water Code Section 13389.

7. This Regional Board has notified the Discharger and all known interested parties of the intent to amend Order No. R9-2005-0091.

8. This Regional Board in a public meeting has heard and considered all comments pertaining to the proposed discharge from the Sea World fireworks displays to Mission Bay.

IT IS HEREBY ORDERED, that Order No. R9-2005-0091 is amended as follows:

The following shall be added to Section III Discharge Prohibitions:

H. The discharge of waste from the aerial fireworks display shall not cause or contribute to the degradation of water or sediment quality in Mission Bay.

I. The discharge of waste from the aerial fireworks display shall be free of settleable material or substances that may form sediments, which will degrade benthic communities or other aquatic life.

J. Fireworks aerial displays shall be limited to the following dates: Easter through Labor Day and New Year's Eve of each year and shall not to exceed a maximum of 150 fireworks aerial displays per calendar year.

The following shall be added to Attachment A-Definitions:

Fireworks Deposition Zone: The aerial extent of fireworks particles and/or debris created by a single fireworks display within the tidal influence of Mission Bay waters.

The following shall be added to Section IX of the Monitoring and Reporting Program:

F. Fireworks Related Water Quality and Benthic Monitoring

1. Beginning in April 2008, the Discharger shall implement a fireworks monitoring program that will continue until September 2010.

2. To determine the level of impact to the receiving water and underlying sediment, the monitoring program shall document conditions of the vicinity of the receiving water discharge points, at reference stations, and at areas beyond the immediate vicinity of the discharge points where discharge impacts might reasonably be expected.

3. The following shall constitute the water quality monitoring locations:

Station Number	Location
RSW-001R	Area south of crown point shore and north of Vacation Isle shore **Reference Station**
RSW-001	Pacific Passage, 20 feet from the fireworks barge and in the direction of the fireworks deposition zone
RSW-002	Pacific Passage, center of the deposition zone as determined after each event
RSW-003	Pacific Passage, the outermost area of the fireworks deposition zone, at a point farthest away from the barge

4. Water quality analysis shall be conducted at all stations for the following constituents:

Table X. Water Quality Monitoring Requirements

Constituent	Units	Type of Sample	Frequency[1]
BIS (2-Ethylhexyl) Phthalate	mg/l	Grab	Semiannually
di-N Butylphthalate	mg/l	Grab	Semiannually
di-N Octylphthalate	mg/l	Grab	Semiannually
Diethylphthalate	mg/l	Grab	Semiannually

Dimethylphthalate	mg/l	Grab	Semiannually
Phenol	mg/l	Grab	Semiannually
Constituent	Units	Type of Sample	Frequency[1]
Naphthalene	mg/l	Grab	Semiannually
2,4-Dinitrotoluene	mg/l	Grab	Semiannually
2,6-DNT	mg/l	Grab	Semiannually
2,4,6-Trinitrotoluene	mg/l	Grab	Semiannually
Nitrobenzene	mg/l	Grab	Semiannually
Tetryl	mg/l	Grab	Semiannually
RDX	mg/l	Grab	Semiannually
Aluminum[2]	mg/l	Grab	Semiannually
Antimony[2]	mg/l	Grab	Semiannually
Arsenic[2]	mg/l	Grab	Semiannually
Barium[2]	mg/l	Grab	Semiannually
Beryllium[2]	mg/l	Grab	Semiannually
Cadmium[2]	mg/l	Grab	Semiannually
Chromium[2]	mg/l	Grab	Semiannually
Cobalt[2]	mg/l	Grab	Semiannually
Copper[2]	mg/l	Grab	Semiannually
Iron[2]	mg/l	Grab	Semiannually
Lead[2]	mg/l	Grab	Semiannually
Manganese[2]	mg/l	Grab	Semiannually
Mercury	mg/l	Grab	Semiannually
Molybdenum[2]	mg/l	Grab	Semiannually
Nickel[2]	mg/l	Grab	Semiannually
Potassium[2]	mg/l	Grab	Semiannually
Selenium[2]	mg/l	Grab	Semiannually
Silver[2]	mg/l	Grab	Semiannually
Strontium[2]	mg/l	Grab	Semiannually
Thallium[2]	mg/l	Grab	Semiannually
Tin[2]	mg/l	Grab	Semiannually
Titanium[2]	mg/l	Grab	Semiannually
Vanadium[2]	mg/l	Grab	Semiannually
Zinc[2]	mg/l	Grab	Semiannually
Perchlorate	mg/l	Grab	Semiannually
Total Nitrogen	mg/l	Grab	Semiannually
Phosphorus	mg/l	Grab	Semiannually
Sulfate	mg/l	Grab	Semiannually

[1] Samples shall be collected and analyzed in January and July of each year. Semiannually means at least once during the months of January and July.

[2] All metals shall be reported as total and dissolved. Hardness as $CaCO_3$ shall also be analyzed.

5. **Sediment Characteristics.** The Discharger shall prepare a monitoring plan that identifies the locations of sediment monitoring. A minimum of 3 locations representative of the area of greatest potential impact and within the fireworks deposition zone shall be selected. All monitoring locations shall be approved by the Regional Board.

Sediment samples for chemical analysis shall be collected from the top 2 centimeters of the grab. Samples shall be analyzed for the constituents listed in table below. Sediment chemistry ambient monitoring may be conducted using USEPA approved methods, or methods developed by NOAA's National Status and Trends for Marine Environmental Quality. For chemical analysis of sediment, samples shall be reported on a dry weight basis.

Constituent	Units	Type of Sample	Frequency[1]
BIS (2-Ethylhexyl) Phthalate	mg/kg	Core	Semiannually
di-N Butylphthalate	mg/kg	Core	Semiannually
di-N Octylphthalate	mg/kg	Core	Semiannually
Diethylphthalate	mg/kg	Core	Semiannually
Dimethylphthalate	mg/kg	Core	Semiannually
Phenol	mg/kg	Core	Semiannually
Naphthalene	mg/kg	Core	Semiannually
2,4-Dinitrotoluene	mg/kg	Core	Semiannually
2,6-DNT	mg/kg	Core	Semiannually
2,4,6-Trinitrotoluene	mg/kg	Core	Semiannually
Nitrobenzene	mg/kg	Core	Semiannually
Tetryl	mg/kg	Core	Semiannually
RDX	mg/kg	Core	Semiannually
Aluminum[2]	mg/kg	Core	Semiannually
Antimony[2]	mg/kg	Core	Semiannually
Arsenic[2]	mg/kg	Core	Semiannually
Barium[2]	mg/kg	Core	Semiannually
Beryllium[2]	mg/kg	Core	Semiannually
Cadmium[2]	mg/kg	Core	Semiannually
Chromium[2]	mg/kg	Core	Semiannually
Cobalt[2]	mg/kg	Core	Semiannually
Copper[2]	mg/kg	Core	Semiannually
Iron[2]	mg/kg	Core	Semiannually
Lead[2]	mg/kg	Core	Semiannually

Manganese[2]	mg/kg	Core	Semiannually
Mercury	mg/kg	Core	Semiannually
Molybdenum[2]	mg/kg	Core	Semiannually
Constituent	Units	Type of Sample	Frequency[1]
Nickel[2]	mg/kg	Core	Semiannually
Potassium[2]	mg/kg	Core	Semiannually
Selenium[2]	mg/kg	Core	Semiannually
Silver[2]	mg/kg	Core	Semiannually
Strontium[2]	mg/kg	Core	Semiannually
Thallium[2]	mg/kg	Core	Semiannually
Tin[2]	mg/kg	Core	Semiannually
Titanium[2]	mg/kg	Core	Semiannually
Vanadium[2]	mg/kg	Core	Semiannually
Zinc[2]	mg/kg	Core	Semiannually
Perchlorate	mg/kg	Core	Semiannually
Total Nitrogen	mg/kg	Core	Semiannually
Phosphorus	mg/kg	Core	Semiannually
Sulfate	mg/kg	Core	Semiannually

[1] Samples shall be collected and analyzed in January and July of each year. Semiannually means at least once during the months of January and July.

[2] All metals shall be reported as total and dissolved. Hardness as $CaCO_3$ shall also be analyzed.

6. **Infauna.** The Discharger shall prepare a monitoring plan that identifies the locations of benthic infauna monitoring. A minimum of 3 locations representative of the area of greatest potential impact and within the fireworks deposition zone shall be selected. All monitoring locations shall be approved by the Regional Board.

   For analysis of benthic infauna, two replicate samples of bottom sediment shall be collected and analyzed in January and July from a minimum of 3 locations. The benthic infaunal samples shall be collected using a 0.1-square meter modified Van Veen gran sampler. These grab samples shall be separated from those collected for sediment analyses. The samples shall be sieved using a 1.0 millimeter mesh screen. The benthic organisms retained on the sieve shall be fixed in 15 percent buffered formalin, and transferred to 70 percent alcohol within 2 to 7 days of storage. These organisms may be stained using Rose Bengal to facilitate sorting. Infaunal organisms, obtained during benthic monitoring shall be counted and identified to as low a taxon as possible.

   a. Number of species per 0.1-square meter

b. Total number of species per station
c. Total numerical abundance
d. Benthic Response Index (BRI)
e. Swartz's 75 percent dominance index
f. Shannon-Weiner's diversity index
g. Pielou eveness (J)

In addition to the community parameters, an annual evaluation shall be performed that includes more detailed statistical comparisons including community, temporal, and spatial analyses. Methods may include, but are not limited to, various multivariates, such as cluster analysis, ordination, and regression. Additionally analyses shall also be conducted, as appropriate, to elucidate temporal and spatial trends in the data.

An additional array of 10 randomly selected stations shall be sampled and analyzed annually for sediment chemistry and benthic fauna. The same procedures must be followed as outlined in F.5 and F.6, with the exception of the number of samples collected at each station. Only one sample is required from each of the 10 randomly selected stations. The stations shall be reselected each year by USEPA using USEPA probability-based Environmental Monitoring and Assessment Program. The area shall extend throughout the Pacific Passage.

The random benthic sampling requirement may be suspended as part of a resource exchange agreement to allow for participation in the Southern California BIGHT Regional Monitoring Surveys at the discretion of the Executive Officer. The benthic sampling may only be canceled for the year in which the BRIGHT Survey is conducted.

7. The following information shall also be recorded during each sampling event: wind direction and speed; weather (cloudy, rainy, etc); tidal conditions; any other noteworthy water condition.

8. An aerial 8 ½ x 11 map that clearly outlines the fireworks deposition zone shall be prepared for each sampling event.

This addendum becomes effective on the date of adoption by the Regional Board.

I, John H. Robertus, Executive Officer, do hereby certify the foregoing is a full, true and correct copy of an Addendum adopted by the California Regional Water Quality Control Board, San Diego Region, on December 12, 2007.

TENTATIVE

JOHN H. ROBERTUS
Executive Officer

Date

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The impact of fireworks on airborne particles

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The impact of fireworks on airborne particles

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Abstract

Fireworks are one of the most unusual sources of pollution in atmosphere; although transient, these pollution episodes are responsible for high concentrations of particles (especially metals and organic compounds) and gases. In this paper, results of a study on chemical-physical properties of airborne particles (elements, ions, organic and elemental carbon and particles size distributions) collected during a fireworks episode in Milan (Italy) are reported. Elements typically emitted during pyrotechnic displays increased in one hour as follows: Sr (120 times), Mg (22), Ba (12), K (11), and Cu (6). In our case study, Sr was recognised as the best fireworks tracer because its concentration was very high during the event and lower than, or comparable with, minimum detection limits during other time intervals, suggesting that it was mainly due to pyrotechnic displays. In addition, particles number concentrations increased significantly during the episode (up to 6.7 times in one hour for the $0.5<d<1$ μm size bin). Contributions (e.g. Cu, elemental carbon and nitrogen oxides) to air pollution due to the large traffic volume registered during the same night were also singled out.

The original application of Positive Matrix Factorization and Multiple Linear Regression allowed, as far as we know, here for the first time, the quantification of the fireworks contribution to atmospheric particulate matter and the resolution of their chemical profile. The contribution of fireworks to the local environment in terms of PM10 mass, elements and chemical components was assessed with 4-hour time resolution. PM10 mass apportioned by fireworks was up to 33.6 μg m^{-3} (about 50% of the total PM10 mass). Major contributors were elemental and organic carbon (2.8 and 8.1 μg m^{-3}, respectively) as well as metals like Mg, K, Sr, Ba, and Cu (0.4, 0.7, 0.07, 0.1, and 0.1 μg m^{-3}, respectively).

Keywords: fireworks, chemical composition, number size distribution, PMF

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1. Introduction

In recent years concern for air pollution effects both on short-term and on long term has increased (Pope and Dockery, 2006; and therein literature). Therefore, many studies are currently carried out to characterise anthropogenic emissions especially in urban areas where large populations live.

One of the most unusual sources of pollution in atmosphere is the displacement of fireworks to celebrate festivities worldwide as well as specific events. The burning of fireworks is a huge source of gaseous pollutants such as ozone, sulphur dioxide, and nitrogen oxides (Attri et al., 2001; Ravindra et al., 2003) as well as of suspended particles. The aerosol particles emitted by fireworks are generally composed of metals (e.g. potassium, magnesium, strontium, barium, and copper), elemental carbon and secondary compounds like nitrate and organic substances (Kulshrestha et al., 2004; Drewnick et al., 2006; Moreno et al., 2007; Wang et al., 2007). The issue of exposure to elevated particle concentrations during celebrations with fireworks has implications in many countries of the world where pyrotechnic exhibitions often last for several hours/days (e.g. during Diwali Festival in India, Las Fallas in Spain, Lantern Festival in Beijing and New Year's celebration world-wide). The complex nature of particles emitted during fireworks may cause adverse health effects as reported in Ravindra et al. (2001). Nevertheless, some authors (Perry, 1999; Dutcher et al., 1999) concluded that fireworks unlikely pose a significant public health hazard, as they are relatively rare, detonate at altitudes well above the ground and generally burn outdoors, where the emitted pollutants can be dispersed in a large volume of air.

An additional effect of fireworks is the visibility reduction due to the generation of a dense cloud of smoke that drifts downwind and slowly disperses. The impact of fireworks on visibility and human health is particularly evident when the pyrotechnic exhibition is performed during stable meteorological conditions (Clark, 1997).

In this paper, we report on the chemical-physical characteristics of ambient aerosol measured during fireworks burnt in Milan (Italy) to celebrate the win of the football World Cup; due to the short duration of the fireworks exhibition, we considered it as a case study. The main goal of this paper is the assessment of the fireworks emissions environmental impact through the aerosol characterisation in terms of number (10 min resolution), mass and chemical composition (4-hour time resolution) as well as 1-hour resolution elemental data. In addition to particulate matter, trace gases concentrations, meteorological parameters, and atmospheric stability conditions were taken into account. Owing to the occurrence of this episode during a longer monitoring campaign, the apportionment of the fireworks source was possible applying Positive Matrix Factorization (PMF) and Multiple Linear Regression (MLR) to the whole dataset; as far as we know, this is the first attempt to identify and quantify the fireworks source contribution using a receptor model.

2. Experimental

The effect of pyrotechnic displays on air quality was studied in Milan (Italy) in July 2006, during the night between 9th and 10th, when the Italian team was celebrated for the win of the 2006 FIFA World Cup.

2.1 Site and sampling

Major pyrotechnic displays were located in the Cathedral's square downtown Milan; additional celebrations with many minor fireworks displays and a huge amount of crackers and sparkles were burnt everywhere in the town, starting soon after the end of the football match (at about 10:45 p.m.). Due to the peculiarity of the episode, the duration of the celebrations is not easy to assess (a reasonable estimate might be approximately 1 - 2 hours). The samplings were carried out at the University campus on the roof of the Institute of Physics, at about 10 m a.g.l.. The monitoring station was about 3 km far from the city centre so that the measurement related to the advected and diffused smoke cloud (as generally done in literature studies on this topic).

PM10 was sampled starting at 12 a.m., local time, from July 9th to 11th, every 4 hours. Samplings were carried out in parallel on PTFE filters (diameter: 47 mm, pore size: 2 μm) and quartz fibre filters (diameter: 47 mm, pre-fired at 700°C for 1 hour) using CEN-equivalent samplers operating at a flow rate of 2.3 m^3 h^{-1}.

Fine (d_{ae}<2.5 μm) and coarse (2.5<d_{ae}<10 μm) PM fractions were also collected with hourly resolution, using a streaker sampler. The streaker sampler separates particles in two different stages using a pre-impactor (which removes particles with d_{ae}>10μm) and an impactor. The latter is made of a Kapton foil on which coarse particles are collected. The fine fraction is then sampled on a Nuclepore filter (0.4 μm pore diameter). The Kapton foil and Nuclepore filter are paired in a cartridge rotating at constant angular speed (1.8° h^{-1}); this produces a circular continuous deposition on both stages. It should be noted that mass concentration in streaker samples is not available. Further details on the sampler, its cut-off diameters, and its control unit can be found elsewhere (Prati et al. 1998); it should be noticed that mass concentration in streaker samples is not available.

2.2 Laboratory analyses

Before and after the samplings the filters were exposed for 48 hours on open but dust-protected sieve-trays in an air-conditioned weighing room (T = 20 ∂ 1 °C and R.H. = 50 ∂ 5 %). The gravimetric determination of the mass was carried out using an analytical microbalance (precision 1 σg), which was installed and operated in the weighing room. Calibration procedures checked the microbalance performance.

PTFE filters were analysed for elemental composition by Energy Dispersive X-Ray Fluorescence technique (details can be found in Marcazzan et al., 2004), obtaining concentration values for Mg, Al, Si, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Sr, Ba, Pb. Other elements (i.e. V, As, Se, Zr, and Mo) were in principle detectable, but they often resulted below the minimum detection limit (MDL), which was in the range 2 – 20 ng m^{-3} for most elements. Experimental overall uncertainties were in the range 10-15 %.

One half of the quartz fibre filters was analysed for water-soluble major components (SO_4^{2-}, NO_3^-, and NH_4^+) by ion chromatography (IC). A special care was used in IC analyses of particulate matter collected on quartz fibre filters due to

high blank levels (minimum detection limits: 167, 359 e 46 ng m^{-3} for SO_4^{2-}, NO_3^-, and NH_4^+, respectively); information about extraction procedures and blanks correction can be found in Fermo et al. (2006). The overall uncertainty for ionic concentrations was estimated in 10 %.

One punch (area: 1.5 cm^2) cut from the quartz fibre filter was analysed by TOT (Thermal-Optical Transmittance) method (Birch and Cary, 1996) to quantify elemental and organic carbon. The technique detection limit was 0.2 σgC m^{-3} and the precision was 5%.

Nuclepore and Kapton substrates from the streaker sampler were analysed by Particle Induced X-ray Emission analysis (PIXE) at the LABEC-INFN accelerator facility in Florence, Italy, whose set up is described in Calzolai et al. (2006). The concentration of 19 elements (Na, Mg, Al, Si, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Sr, Ba, Pb) was obtained. As for ED-XRF analysis, other elements were in principle detectable, but they often were below the minimum detection limit (lower than 10 ng m^{-3} for V, As, Se, Zr, Rb, Mo). The accuracy of hourly elemental concentrations was in the range 2% - 20%.

2.3 Additional measurements

An Optical Particle Counter (Grimm, mod.1.107) measured number size distributions in the 0.25-32 σm range (31 size bins).

To evaluate atmospheric dispersion conditions, ^{222}Rn short-lived decay products measurements were performed using the experimental methodology reported in Marcazzan et al. (2003). Mixing layer heights (MLH) with hourly resolution were obtained by means of a box model suitably set up by the group of the Institute of Physics using ^{222}Rn concentration measurements as input data (Pacifico, 2005). MLH evaluations by our box-model were in good agreement with thermal inversions heights from radio-soundings data by the nearby Milan-Linate airport as well as with other modelling studies based on thermodynamic variables (Casadei et al., 2006).

Meteorological parameters (wind speed and direction, relative humidity, pressure, temperature, solar radiation and precipitation) were also measured at the Institute of Physics monitoring station.

Trace gases data recorded at monitoring stations of the Regional Environmental Protection Agency were also available (Figure 1): NO_2 and NO at the 1-J station (near the University campus and the motor-way) and NO_2, NO and CO at the 2-V station (city centre) and 3-L (on the ring-round). Moreover, hourly traffic volumes in the city centre were recorded at the station 4-S (city centre).

2.4 Receptor model

The fireworks episode occurred during a longer field campaign, which was performed during two weeks in summer and two weeks in winter 2006, with the same characteristics as those described in paragraphs 2.1, 2.2, and 2.3. The complete

PM10 data set (180 samples) was analysed by PMF to identify and apportion (by MLR) major aerosol sources. PMF resolved seven sources (re-suspended soil, construction works, industry, traffic, secondary sulphates, secondary nitrates and fireworks). In this paper, only results on the fireworks source will be described and discussed (another paper in preparation deals with the other six sources).

PMF is an advanced factor analysis technique computing a weighted, non-negative constrained least squares fit. It imposes non-negativity constraints to the factors and uses realistic error estimates from data standard deviations, as described in Paatero (1997). Data values and errors, missing values and below detection limit data were calculated according to Polissar et al. (1998) and used in this work as inputs for the PMF.

In PMF studies, a weak variable (according to signal to noise ratio criterion, as in Paatero and Hopke, 2003) can sometimes be inserted in the fit with the normal variables if it represents a tracer of a specific source (Qin et al., 2006). This approach was here adopted for Sr, considered the best tracer of the fireworks source in our case study (see paragraph 3.3). It was not really a weak variable but it had a much lower signal to noise ratio respect to other variables. In this work, instead of reducing the weights of Sr, we doubled them to highlight the role of this fireworks tracer in the fit. At the same time, it was necessary to down-weight some variables by increasing their uncertainties by a factor from 2 to 4 to obtain a better distribution of their scaled residuals (Kim et al., 2003). The coefficients of adjustment for the weights were determined with trial and error method until the model resolved the fireworks source, together with the same six sources found in a previous analysis where Sr was not used as input for PMF.

Rotational ambiguity is always a problem in factor analysis (Paatero et al., 2002); in this work, after a systematic study of the rotational range of the solution, FPEAK=0 was chosen. MLR was performed to regress the total mass against the factor scores; the regression coefficients were then used to transform the factor profiles given in arbitrary units in parts per million ones and to quantitatively apportion the mass contributions among the resolved sources.

3. Results and discussion

3.1 Mass concentration and meteorological conditions

During the case study period, meteorological conditions were quite stable. The wind speed was about 1 m s^{-1} as average value between 10:30 p.m. and 12 a.m. on the fireworks night and the prevalent wind direction was changing from south-westerly to westerly direction.

During the fireworks night a ^{222}Rn strong accumulation was registered (Figure 2); the variation of Radon concentration between the minimum (8.6 Bq m^{-3}) on July 9th afternoon and the maximum (26.4 Bq m^{-3}) in the following day was a good indicator of the nocturnal mixing layer depth, which was lower than 100 m.

In Figure 2, PM10 mass and ^{222}Rn concentration on 9th-10th July 2006 are shown. On 10th July, PM10 concentration increased up to 63.9 µg m^{-3} in the time interval between 12 a.m. and 4 a.m., when the pyrotechnical displays contribution

was maximum at our monitoring station, as singled out by the chemical markers analysis (see paragraph 3.3). However, fireworks were not likely the only cause of PM10 growth during that night; indeed, the concomitant strong accumulation of ^{222}Rn concentration suggests that this increase was likely due both to sources emissions and to the strong atmospheric stability.

As far as we know, currently in the literature there is no quantification of the fireworks contribution to the PM mass, as this estimation is difficult and not straightforward. With the aim of apportioning the fireworks source, in this paper the receptor model approach has been possible owing to the availability of a large number of chemically characterised PM samples with 4-hours temporal resolution. According to the PMF source apportionment the fireworks contribution began to be remarkable in the 8 p.m. – 12 a.m. time interval, accounting for 13.1 μg $m^{-3}$of the PM10 mass (27 %), reached its maximum at 33.6 μg m^{-3} (53 %) in the 4 hours after midnight and decreased to 4.2 μg m^{-3} (8 %) from 4 a.m. to 8 a.m.

3.2 Gaseous pollutants

CO concentration and traffic volumes increased soon after the end of the match (10:45 p.m.) at the monitoring stations 2-V and 4-S near the Cathedral's Square, as shown in Figure 3a. A similar pattern in CO concentration, i.e. maximum value between 11 p.m. and 12 a.m. with a 3-fold increase in one hour, was also recorded at the station 3-L, located next to the city ring-road (see Figure 1) and about 1.5 km far from major pyrotechnic displays. In Figure 3b NO_2 temporal patterns recorded at the stations 2-V, 3-L and 1-J are reported. At the 2-V and 3-L stations the concentration increase was recorded simultaneously with the CO increase while at the station 1-J (near University Campus) a delay in the maximum concentration occurred.

Ravindra et al. (2003) observed NO_2 increases during the pyrotechnic displays. On the contrary, in our case the experimental results indicate that the increase in trace gases concentration was mainly due to the high number of vehicles circulating soon after the end of the match to celebrate the national team more than to fireworks emissions. Indeed, it is important to observe that the location of the 3-L monitoring station compared to the city centre and the prevalent wind direction (see paragraph 3.1) suggest that fireworks unlikely affect air quality in that area. Moreover, the NO_2 temporal trend observed at the station near major pyrotechnic displays (2-V) and at the 3-L station are comparable, indicating that no significant NO_2 emissions can be ascribed to fireworks in our case study. The NO_2 peak occurring at 2 a.m. in the 1-J station was explained by traffic flows, likely due to people going back home, as also confirmed by Cu temporal pattern (another traffic tracer) represented in Figure 4.

3.3 Chemical composition

On 9th July, starting from 11 p.m., the hourly concentrations of some elements in the fine fraction strongly increased. Similar results were also found PM10 elemental data with 4-hour resolution (in Table 1 mass and chemical components

concentrations are given for the episode); nevertheless, for sake of brevity, the data with the highest time resolution are represented in Figure 4. At our monitoring station, the highest values were registered on 10th July between 1 a.m. and 2 a.m.; this is consistent with the location of major fireworks considering wind speed and direction. To quantify the elements concentration increase during the episode, the maximum concentration was compared to the value of the day before (9th July, not affected by fireworks), averaged between 12 a.m. and 10 p.m. In case of below MDL hourly data, one-half of the MDL value was used. Remarkable increases in Sr (120 times), Mg (22 times), K (12 times), Ba (11 times), and Cu (6 times) concentration were observed. No increases were detected in the coarse fraction elemental concentrations and Sr, Mg, K, Ba, and Cu concentrations were below or comparable to MDL (not shown), indicating that ambient aerosol after the fireworks event was preferably confined in the fine fraction.

Sr, Ba, and Cu compounds are used to give red, green, and blue fireworks, respectively (Kulshrestha et al., 2004; Wang et al., 2007; Moreno et al., 2007). Different Ba compounds can give the green colour, but the increase in chlorine concentration measured during the fireworks night and the nitrate concentration comparable or lower than other nights one, suggested that $Ba(ClO_3)_2$ was more likely used (Perry, 1999). K is one of the major components of fireworks (Liu et al., 1997; Dutcher et al., 1999; Perry, 1999; Kulshrestha et al., 2004; Drewnick et al., 2006): 74% of black powder consists of KNO_3, which provides the main oxidizer to the burning. Also potassium perchlorate or, less commonly, chlorate can be used in the black powder. Mg gives origin to bright electric white fireworks and it is used as metallic fuel (Moreno et al., 2007; Wang et al. 2007).

In this work, Sr was recognised as the best fireworks tracer because its concentration was very high during the event and lower than, or comparable with, MDL during other time intervals, suggesting that it was mainly due to pyrotechnic displays (see also PMF results in Table 2). On the contrary, Cu and Ba can also have a contribution coming from traffic (Vecchi et al., 2007 and therein literature) and K and Mg are widespread elements emitted by many sources (e.g. biomass burning for K and soil dust for Mg).

From 4-hours resolution PM10 data, the concentration ratios between levels registered in the 12 a.m. - 4 a.m. time interval and the average values of the day before (during the period free from the event, i.e. between 12 a.m. and 8 p.m.) were calculated; results for elements, organic and elemental carbon, and ions are reported in Figure 5. As expected, the most significant increases were observed for Sr, Mg, Ba, K, and Cu (elements ratios were smaller than those reported for 1-hour resolution elemental data because the longer sampling time included periods with lower concentrations). Indeed, these elements can be all considered fireworks tracers.

The nitrate concentration ratio was comparable to the one measured during other summer nights at the same sampling site (as an example, see the comparison with 6th July night, in Figure 5) because of the lower night-time temperature, which limited losses due to volatilisation. In agreement with results by Drewnick et al. (2006), in our case study no nitrate increase due to fireworks was observed.

The EC ratios (3.1) found in this work are in good agreement with black carbon increases reported by Babu and Moorthy (2001) and by Wang et al. (2007).

Opposite to what found by Wang et al (2007), no anomalous growth in secondary components was observed the day after the pyrotechnical displacement: the increases in sulphate and ammonium were similar to the ones measured during other summer afternoons at the same sampling site. However, it should be taken into account that secondary compounds formation may change in relation to local meteorological condition, pollutants mixture and duration and strength of the episode.

In Figure 6, the fireworks chemical profile obtained by PMF is also reported as an original contribution to the characterisation of fireworks emissions. Major components are carbon compounds (both EC and OC) and metals. The fireworks source profile confirms Sr as the best tracer in our case study as, contrarily to other fireworks indicators, it was found only in this chemical profile while, for example, Ba was also detected in the traffic profile, and K was found in a number of sources (not shown here). In Table 2 the PMF apportionment for major PM10 components detected during pyrotechnic displays is reported. As already reported for PM10 mass concentrations, also elements, ions, and carbon components peaked in the 12 a.m. - 4 a.m. time interval. Total carbon (TC = EC+OC) due to fireworks accounted for 11 μg m^{-3} of the PM10 mass (i.e. about 50-55 % of the measured total carbon). Major elemental contributions apportioned by PMF and due to the pyrotechnic displays were Mg (0.4 μg m^{-3}), K (0.7 μg m^{-3}), Cu (0.07 μg m^{-3}), Sr (0.1 μg m^{-3}), and Ba (0.1 μg m^{-3}) corresponding to 81%, 77%, 68%, 100% and 91% of their measured concentration, respectively. These results are in very good agreement with experimental observations discussed so far.

3.4 Number size distribution

During the fireworks night, starting from 11 p.m., the number concentration in all size ranges increased. The growths were different for each size bin, but the maximum concentration was always found on 12:10 a.m.. The ratios between the number of particles measured on 12:10 a.m. and 11 p.m. were as follows: 6.7 for particles in the range 0.5<d<1 μm, 2.8 for particles in the range 2.5<d<10 μm, 2.6 for particles in the range 1 <d<2.5 μm and 1.7 for particles with d<0.5 μm.

The delay (about 1.5 hours) in the occurrence of the maximum concentration compared to end of the match may be explained considering the distance of our sampling site from the city centre (where the major fireworks exhibition was performed and the largest traffic volume observed) together with the low wind speed, the wind direction and the atmospheric stability conditions. From 12:10 a.m. to 1:40 a.m. the particles number concentration in all size ranges decreased as follows: -20% for particles with d<0.5 μm, -70% for particles in the range 0.5<d<1 μm, -50% for particles in the 1<d<2.5 μm range and -35% for particles in 2.5<d<10 μm range. Between 1:40 a.m. and 3 a.m. another increase was observed in all size bins, and particularly in particles with diameters smaller than 0.4 μm. It is interesting to note that a growth in Cu hourly concentration (fine fraction) and in NO_2 concentration (at 1-J monitoring station near the University

campus) was also measured between 2 a.m. and 3 a.m. (see Figures 3b and 4). As these increases were contemporary, the growth in particle number concentration might be ascribed to traffic (contributing to Cu, NO_2, and fine particles), because of people going back home after celebrations. Moreover, these results suggested that traffic emissions were mainly in the finest fractions. After 3 a.m., particles number concentrations definitively decreased until the next morning, when the number of particles increased again because of typical working day's activities.

Particles number temporal pattern in each size range was compared to Sr concentration (taken here as fireworks tracer). The correlation coefficients between Sr (fine fraction) and number concentration were calculated using hourly-resolved data between 10 p.m. on 9th July and 10 a.m. on 10th July. The highest correlation coefficients (R>0.95) were registered in the 0.45 – 1 µm, and particularly in the 0.70 - 0.80 µm, size bin (R=0.98). The high correlation between Sr and the 0.7-0.8 µm size range is consistent with what found by Perry (1999), who reported 0.7 µm as mass mean diameter of potassium (in that work considered the indicator for fireworks) observed after fireworks emissions transport.

In Figure 7, Sr temporal pattern (1-hour resolution) and particles number concentrations (10-minutes resolution) in the 0.25-0.3 µm, 0.70 - 0.80 µm, and 8.5-10 µm size intervals are shown, as examples. A very good agreement between Sr and particle number in the 0.70 - 0.80 µm size range in the increase phase and in the first part of the decrease phase was evident, while differences can be noticed after 2 a.m. However, it must be considered that, in this case study, fireworks display was the only source of Sr while airborne particles in general can be originated by different sources. In Figure 7 can also be noted that, even if particles in the 0.25-0.3 µm and 8.5-10 µm size ranges increased during the fireworks period, a poorer correlation (R=0.72 and R=-0.13, respectively) was found with Sr concentration.

Taking into account the good correlation between Sr and particles in 0.4-1µm size range during the increase phase, and evaluating the time necessary to Sr to reach values similar to those presented before fireworks, a rough estimate of the time necessary to particles in this size-range to diffuse (with low wind speed conditions) can be evaluated in about 12 hours.

4. Conclusions

The fireworks exhibition was used to study the chemical composition and the size distribution of airborne particles observed during such events. The influence of additional emissions due to the traffic registered just after the football match was also discussed.

Atmospheric aerosols originated by fireworks had a typical signature as singled out by the few works on this topic (see references given in the text). Results obtained by hourly elemental analysis showed that in the fine fraction many metals (i.e. Sr, Mg, K, Ba and Cu) increased significantly during the celebrations (e.g. Sr up to 120 times in one hour) while no differences were observed in the coarse fraction concentrations. It is worth noting that, although fireworks cause short-lived air pollution events, fine particles are responsible for adverse health effects, and the bioreactivity of fine metal aerosols is of particular concern (Moreno et al, 2007; and therein cited literature).

The availability of a large number of chemically characterised samples allowed the PM10 and major chemical components apportionment during the pyrotechnic displays. Although our fireworks event had short duration, the PM10 concentration ascribed by PMF to the fireworks source was not negligible (up to 33.6 μg m^{-3}). In addition, fireworks accounted for a large part of the metal concentrations (e.g. up to 70-100% of the measured values for Mg, K, Cu, Sr, and Ba). Obviously, the impact of this source type can vary considerably in relation to fireworks duration and type, being more serious when stable atmospheric conditions occur (Clark, 1997). The assessment of the fireworks source chemical profile and of the contribution of fireworks to local environment gives an original contribution towards understanding the aerosol characteristics and burden during fireworks displays.

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Figure captions

Figure 1: map of the monitoring stations.

Figure 2: PM10 mass (in σg m^{-3}) and ^{222}Rn concentration (in Bq m^{-3}) on 9^{th}-10^{th} July 2006 in Milan.

Figure 3: a) CO (in mg m^{-3}) and traffic volume (number of vehicles); b) NO_2 (in σg m^{-3}) concentrations at three different monitoring stations on 9^{th}-10^{th} July 2006 in Milan.

Figure 4: fireworks elemental markers, fine fraction data with hourly resolution (in ng m^{-3})

Figure 5: Ratios between the concentration of different chemical components registered in the time interval 12 a.m. - 4 a.m. (fireworks displays) on 9^{th}-10^{th} July night and the average value measured for the same species during the day before (grey). Similar ratios (white) calculated for the night between 6^{th} and 7^{th} July (free from fireworks) are given for comparison.

Figure 6: fireworks source profile (in mg mg^{-1}) as resolved by PMF

Figure 7: Sr hourly temporal pattern (in ng m^{-3}) together with particles number concentration (particles m^{-3}) in the 0.25-0.30 σm, 0.70-0.80 σm and 8.5-10 σm size intervals

Table captions

Table 1: 4-hour resolution chemical components and elemental concentrations (in ng m^{-3}) during the fireworks episode

Table 2: Contribution to PM10 mass and major chemical components concentration (in ng m^{-3} and as percentage of their measured concentration) due to the fireworks source obtained by PMF. By convention, concentration values lower than experimental minimum detection limits have been labelled as <MDL.

Table 1

Date	**9/7**	**9/7**	**10/7**	**10/7**	**10/7**	**10/7**
Time interval	**4 p.m. - 8 p.m.**	**8 p.m. - 12 a.m.**	**12 a.m. - 4 a.m.**	**4 a.m. - 8 a.m.**	**8 a.m. - 12 p.m.**	**12 p.m. - 4 p.m.**
PM10 mass	46 400	48 200	63 900	51 400	71 100	55 500
SO_4^{2-}	4 232	3 130	3 622	2 687	2 830	5 147
NO_3^-	<360	1 115	4 499	2 326	2 326	3 683
NH_4^+	1 169	1 102	1 575	868	1 644	2 548
OC	7 870	9 806	13 491	11 672	12 071	10 490
EC	1 293	1 959	5 372	4 070	4 694	1 748
Mg	<100	183	598	246	127	182
Al	355	519	680	451	720	609
Si	802	1 023	1 368	967	1 790	1 344
S	1 303	803	1 176	1 276	1 024	1 759
Cl	<70	<70	233	98	115	121
K	158	369	991	369	364	267
Ca	308	369	645	723	1 475	744
Ti	28	32	46	37	53	34
V	<6	<6	<6	<6	<6	<6
Cr	<4	<4	11	10	<4	<4
Mn	7	16	30	35	30	20
Fe	468	847	1 731	1 581	1 374	586
Ni	4	2	5	7	6	5
Cu	20	43	105	56	65	25
Zn	82	85	190	270	276	90
Br	5	6	9	12	6	4
Pb	9	16	57	25	41	14
Sr	<3	55	139	18	11	<3
Ba	<20	41	156	22	31	24

Table 2

		9/7 4 p.m. - 8 p.m.	9/7 8 p.m. - 12 a.m.	10/7 12 a.m. - 4 a.m.	10/7 4 a.m. - 8 a.m.	10/7 8 a.m. - 12 p.m.	10/7 12 p.m. - 4 p.m.
EC	*ng m^{-3}*	<MDL	1 101	2 827	357	211	<MDL
	(%)		(56)	(54)	(9)	(6)	
OC	*ng m^{-3}*	<MDL	3 144	8 075	1 019	601	<MDL
	(%)		(39)	(52)	(9)	(5)	
Mg	*ng m^{-3}*	<MDL	168	433	<MDL	<MDL	<MDL
	(%)		(62)	(81)			
Al	*ng m^{-3}*	<MDL	102	262	<MDL	<MDL	<MDL
	(%)		(20)	(38)			
Si	*ng m^{-3}*	<MDL	151	387	49	29	<MDL
	(%)		(15)	(29)	(5)	(2)	
K	*ng m^{-3}*	<MDL	269	692	87	52	<MDL
	(%)		(64)	(77)	(27)	(13)	
Ca	*ng m^{-3}*	<MDL	111	284	36	21	<MDL
	(%)		(30)	(44)	(5)	(1)	
Mn	*ng m^{-3}*	<MDL	5.2	13	<MDL	<MDL	<MDL
	(%)		(33)	(44)			
Fe	*ng m^{-3}*	10.3	400	1 028	130	77	10
	(%)	(2)	(48)	(57)	(10)	(5)	(1)
Cu	*ng m^{-3}*	<MDL	27	70	9	5	<MDL
	(%)		(66)	(68)	(14)	(9)	
Zn	*ng m^{-3}*	<MDL	38	98	12	7	<MDL
	(%)		(45)	(52)	(5)	(3)	
Sr	*ng m^{-3}*	<MDL	54	140	18	10	<MDL
	(%)		(100)	(100)	(99)	(98)	
Ba	*ng m^{-3}*	<MDL	51	130	16	<MDL	<MDL
	(%)		(90)	(91)	(47)		
PM10 mass	*ng m^{-3}*	<MDL	13 087	33 610	4 240	<MDL	<MDL
	(%)		(27)	(53)	(8)		

Figure 1

Figure 2

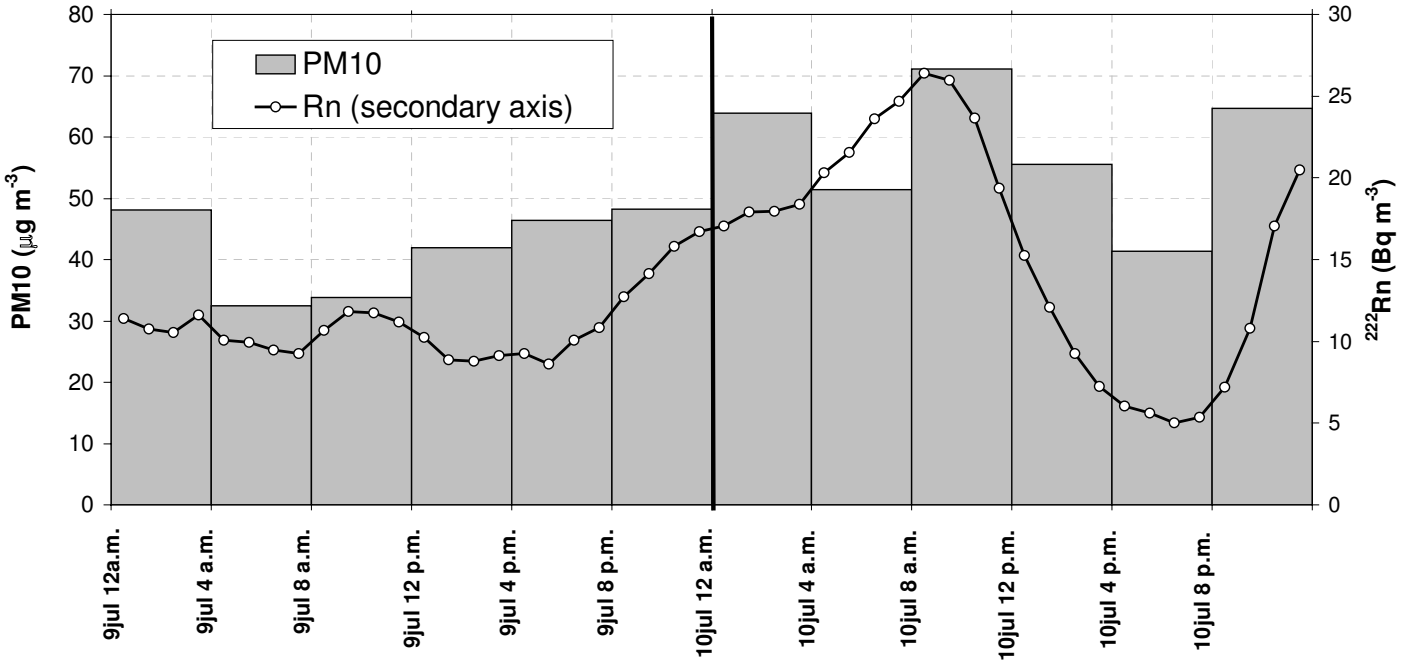
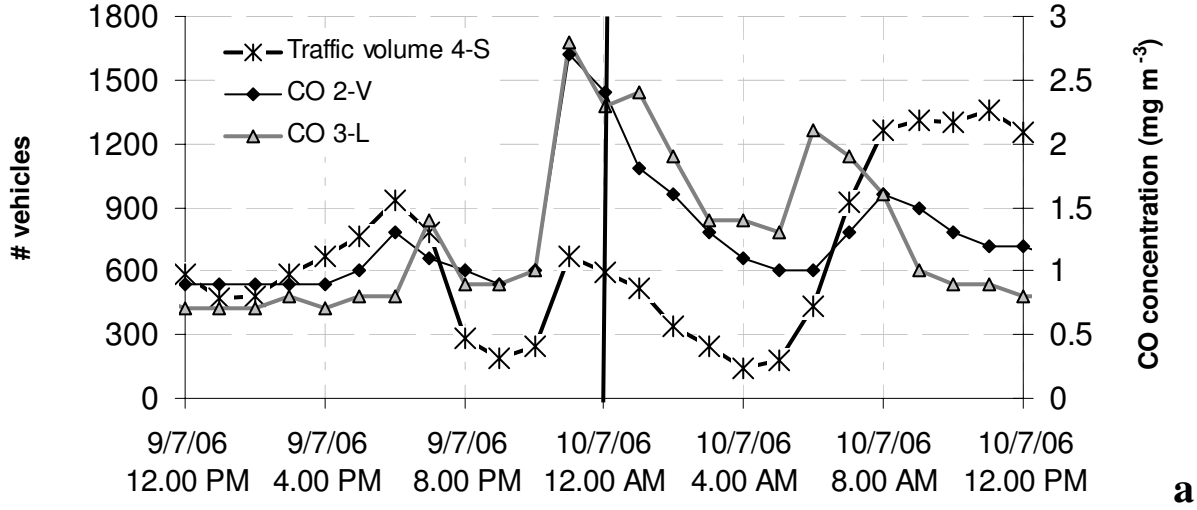


Figure 3



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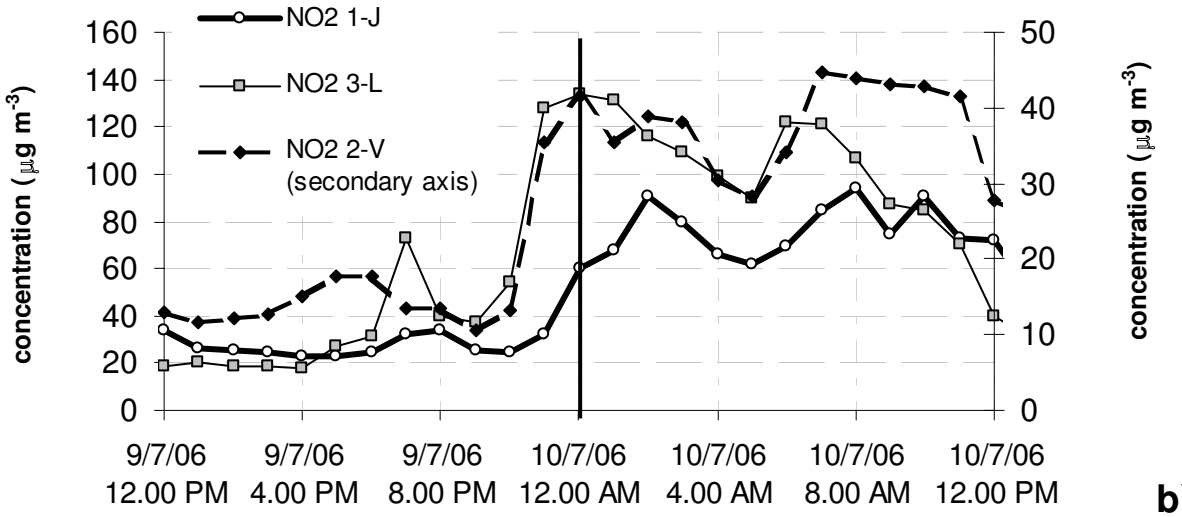

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Figure 4

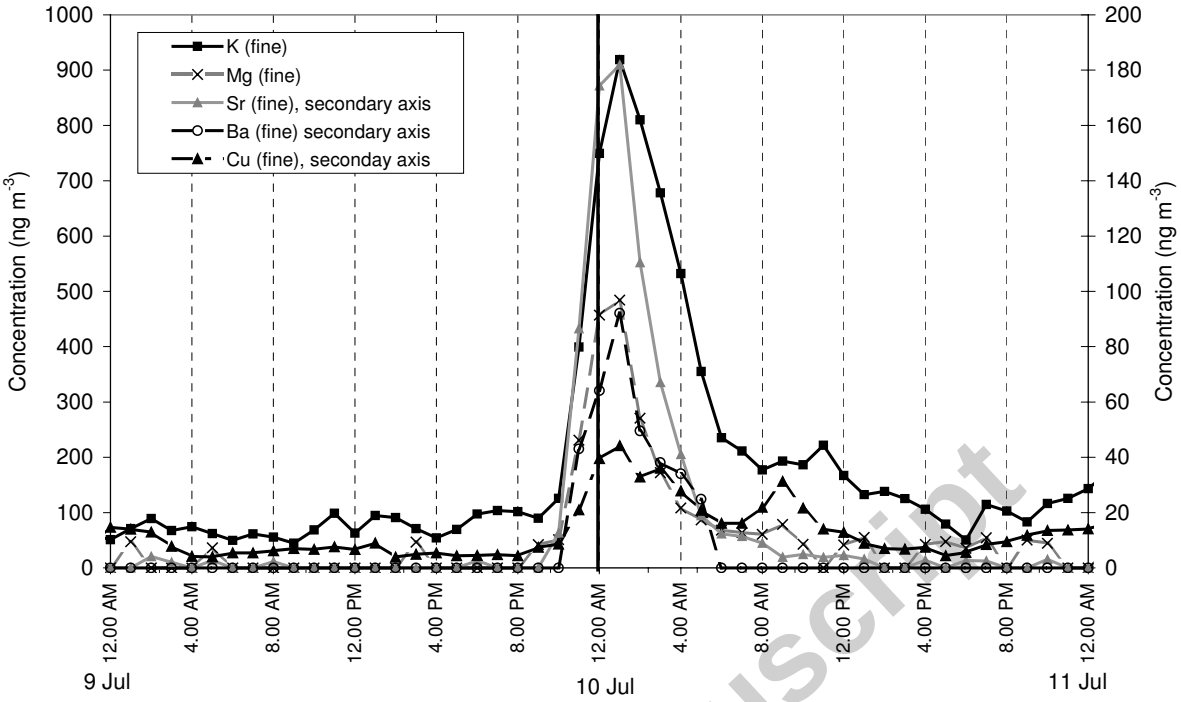


Figure 5

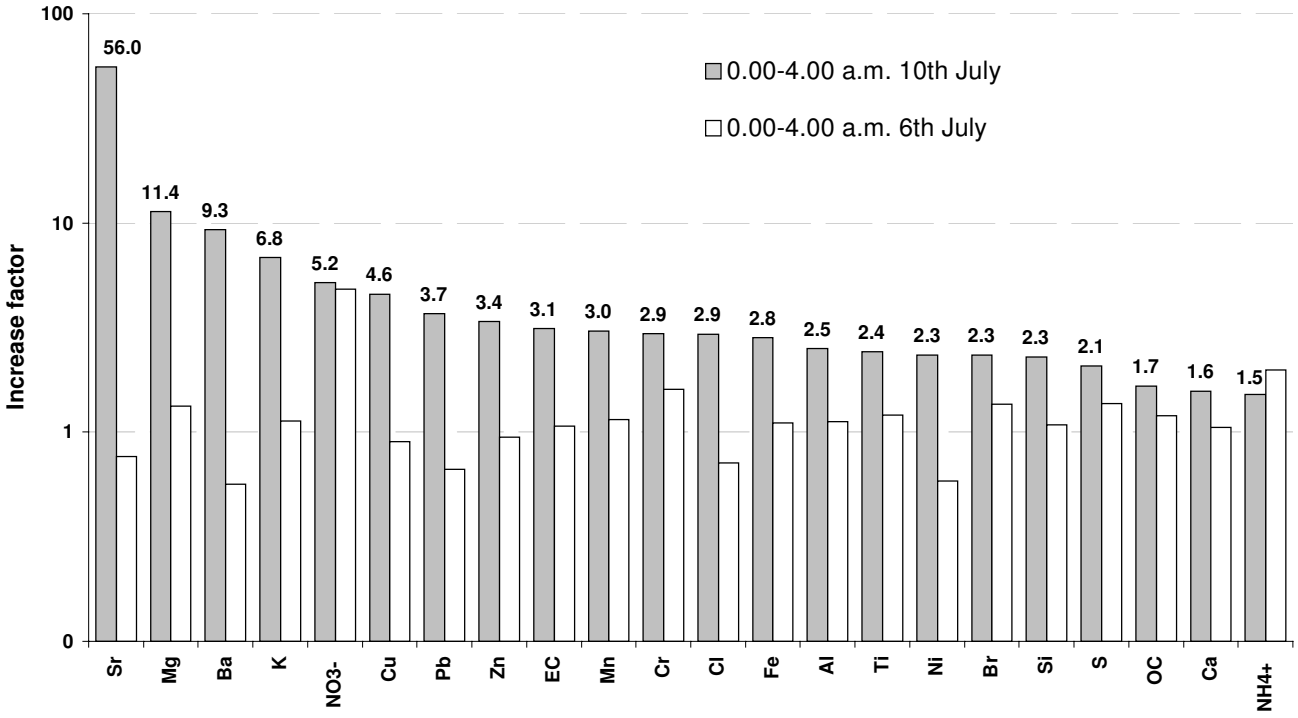


Figure 6

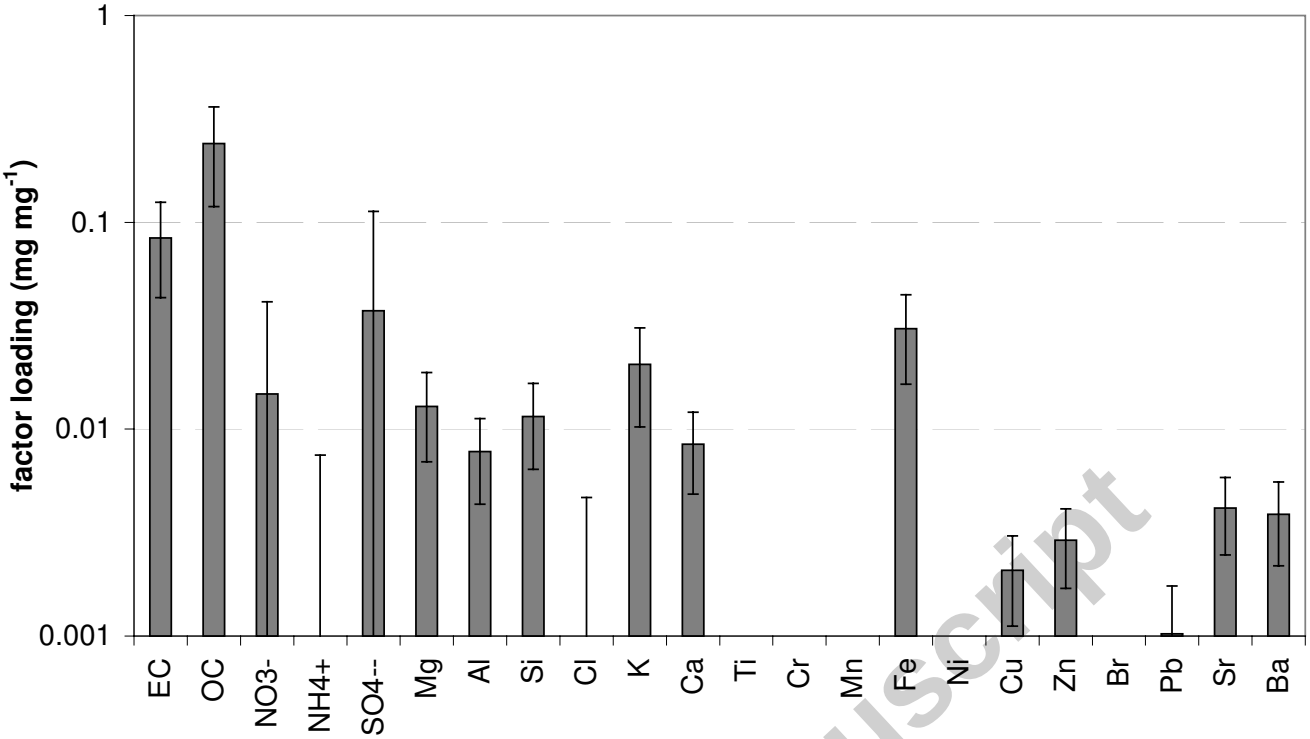
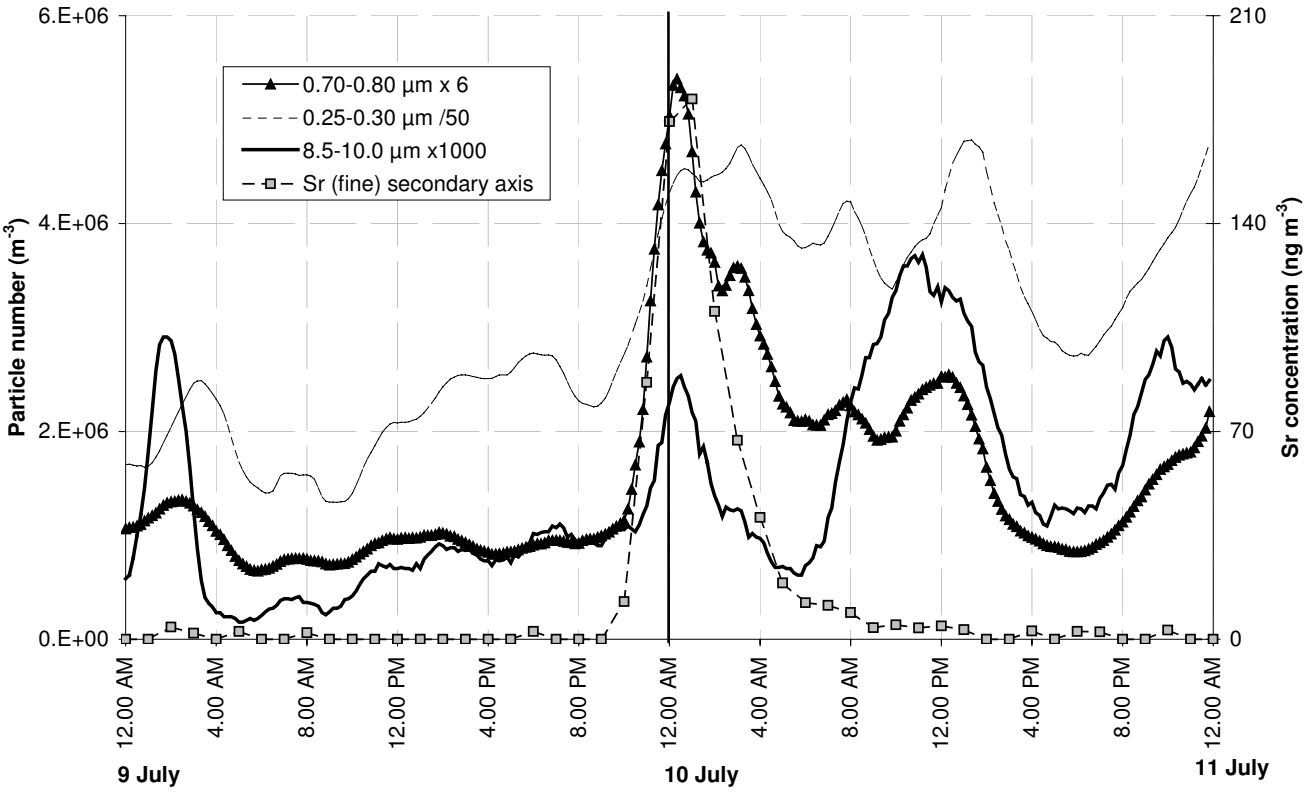


Figure 7



State of California
Regional Water Quality Control Board
San Diego Region

EXECUTIVE OFFICER SUMMARY REPORT
(December 12, 2007)

ITEM: 10

SUBJECT: NPDES Permit Revision: SeaWorld San Diego. The Regional Board will consider amending SeaWorld's existing NPDES permit to establish waste discharge requirements for discharges of waste from SeaWorld's aerial fireworks displays to Mission Bay, San Diego. (Tentative Addendum No. 1 to Order No. 2005-0091, NPDES No. CA0107336) (Michelle Mata)

PURPOSE: Tentative Addendum No. 1 to Order No R9-2005-0091 NPDES No. CA0107336 would, if adopted, amend Order No. R9-2005-0091 for SeaWorld San Diego to establish waste discharge and monitoring requirements for their aerial fireworks displays over Mission Bay.

PUBLIC NOTICE: A Public Notice of this agenda item was published in the San Diego Union Tribune on November 8, 2007, for the Board Meeting scheduled for December 12, 2007. Copies of the tentative Addendum No. 1 were mailed out on November 2, 2007 to SeaWorld and to all known interested parties and agencies. The tentative Addendum was made available for public review via the Regional Board web page on November 5, 2007.

DISCUSSION: On October 23, 2007, SeaWorld San Diego submitted a Report of Waste Discharge (RWD), prepared by Brown and Caldwell, for an Amendment to Order No. R9-2005-0091, NPDES Permit No. CA0107336 for the discharge of wastes from SeaWorld’s aerial fireworks displays over Mission Bay.

Fireworks displays have been a part of SeaWorld entertainment since 1968. From 1968 to 1985, fireworks were used for special events. In 1985, the frequency of fireworks displays increased to nightly from mid-June through Labor Day, and since 1997, the schedule has expanded to include three additional weekends starting Memorial Day weekend. Fireworks displays are also conducted for special events, private parties and

celebrations. The SeaWorld Master Plan Update, which was approved by the Coastal Commission in 2001, allows up to 150 shows per year. Currently the park averages between 110 and 120 shows per year.

The average fireworks show lasts 5 to 6 minutes and dispenses approximately 250 shells; special events, such as the 4th of July and New Year's Eve, may dispense between 1,000 and 1,750 shells. Fireworks are launched from a barge moored in the Pacific passage Zone of Mission Bay, between Fiesta Island and the SeaWorld shorelines. SeaWorld subcontracts the logistics of fireworks, operations, transportation, setup, ignition and cleanup to Fireworks America, a licensed pyrotechnics company based in Lakeside, CA.

There have been concerns over the possible environmental effects of fireworks displays on sediment and water quality. Constituents of concern include aluminum, magnesium, strontium, barium, sodium, potassium, iron, copper, sulfate, nitrate and perchlorate. These fireworks constituents have a potential to adversely impact and/or contribute to degradation of water and sediment quality within Mission Bay. In addition, debris from unexploded shells as well as paper, cardboard, wires and fuses from exploded shells can also adversely impact the quality within Mission Bay. The area affected by these debris can vary depending on wind speed and direction, size of the shells, height of the explosion, and other environmental and anthropogenic factors.

SeaWorld conducted annual fireworks related monitoring of sediment and water quality parameters between 2001-2006 as part of a Coastal Commission permit requirement. The final monitoring report prepared for SeaWorld, by Science Applications International Corporation, concluded that there were no significant spatial or temporal patterns in concentrations of critical metals in sea water or sediments in Mission Bay. It was also concluded that there is no indication of fireworks residue accumulation in the water or sediment of Mission Bay.

If adopted, Addendum No. 1 would establish waste discharge requirements (WDRs) for discharges of waste from SeaWorld's aerial fireworks displays to Mission Bay, San Diego. The WDRs include monitoring of water quality,

sediment and benthic infauna for fireworks related constituents.

Comments have been received from SeaWorld San Diego. A written Responses to Comments document and an Errata sheet will be included in the Supplemental Agenda Package.

KEY ISSUE:

1. Although the tentative Addendum includes a monitoring and reporting program designed to assess the potential adverse effects of fireworks related constituents on water quality, sediment and benthic infauna, the monitoring requirements may need to be revised after review of the data submitted to ensure that the program is adequate.

2. It is uncertain whether the current BMP's are sufficient in reducing impacts of fireworks related debris on water quality, sediment and benthic infauna. The BMP's will be reviewed periodically to evaluate their effectiveness and to determine if additional measures or changes to the current measures are needed.

LEGAL CONCERNS: None.

SUPPORTING DOCUMENTS:

1. Map
2. Transmittal letter for Tentative Addendum No. 1 to Order No. R9-2005-0091, NPDES No. CA0107336.
3. Tentative Addendum No. 1 No. R9-2005-0091, NPDES No. CA 0107336.
4. Order No. R9-2005-0091, NPDES No. CA0107336
5. Comment letter from SeaWorld San Diego dated November 28, 2007.

SIGNIFICANT CHANGES: The tentative Addendum would establish requirements for the SeaWorld aerial fireworks which were previously not regulated by the Regional Board.

COMPLIANCE RECORD: N/A – The discharge of fireworks wastes from SeaWorld has not previously been regulated by the Regional Board and, therefore, no compliance record has been established.

RECOMMENDATION(S): Adoption of Tentative Addendum No. 1 to Order No. 2005-0091, NPDES No. CA 0107336 is recommended.

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December 9, 2010

VIA ELECTRONIC MAIL ONLY gibson@waterboards.ca.gov
David W. Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court – Suite 100
San Diego, CA 92123

In reply refer to:
Reg. Measure ID 375971: MMATA
Place 656901

Re: Tentative Order No. R9-2010-0124, General Permit No. CAG99902
General Waste Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson:

This law firm represents the National Fireworks Association ("NFA"). As and for a response to the November 17, 2010 Notice Of Public Workshop (the "Notice") by the California Regional Water Quality Control Board, San Diego Region ("San Diego Water Board"), requesting topics of discussion, comments and suggestions relating to Tentative Order No. R9-2010-0124, General Permit No.CAG99902 (the "Tentative Order"), the NFA submits the following information:

COMMENTS AND TOPICS OF DISCUSSIONS

By reason of the fact that the San Diego Water Board has not issued a complete, final document for review and consideration by the public, essentially preventing the NFA from furnishing a full and proper response, at this time it offers various topics of discussions in the form of questions.[1]

[1] The NFA expressly reserves all rights to challenge the actions of the San Diego Water Board in proceeding in this manner as a violation of various provisions of the Federal and State Administrative Procedures Act, as well as to demand legal and equitable relief, including injunctive relief and attorneys' fees.

THE CREADORE LAW FIRM P.C.

QUESTIONS:

1. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks displays cause an adverse impact to the quality of either receiving waters or surface water and, if so, please identify?

2. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks displays can exceed prevailing actionable levels of reported pollutants to either receiving waters or surface waters and, if so, please identify?

3. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks displays cause acute or chronic toxicity in receiving waters or surface water and, if so, please identify?

4. Is the San Diego Water Board relying upon any scientific or engineering research or test results that substantiate the need for testing sediment at depths of 50' and, if so, please identify?

5. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that the display of fireworks "have the reasonable potential to cause or contribute to an exceedence of a water quality standard, including numeric and narrative objectives within a standard." And, if so, please identify?

6. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks products in sediments "in quantities that alone, or in combination, are toxic to benthic communities and, if so, please identify?

7. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks displays create "pollutants....in sediments at levels that will bio-accumulate in aquatic life to levels proven to be harmful to human health" and, if so, please identify?

8. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that public displays of fireworks *discharge pollutant wastes* to surface waters and, if so, please identify?

9. What information has the San Diego Water Board received from SeaWorld that establishes that fireworks have a demonstrable adverse impact upon the quality of either receiving waters or surface water?

10. Given that "Under the terms of the Tentative Order any person who discharges or proposes to discharge pollutant wastes from a public display of fireworks to surface waters in the San Diego region *may submit a Notice of Intent...*" (emphasis added), under what circumstances will a person be exempt or excused from having to file a Notice of Intent?

11. Can a sponsor seek a waiver of enrollment and, under what circumstances shall a waiver be provided, and upon what terms?

12. Upon what circumstances will the San Diego Water Board demand "the joint submission of an NOI from both the sponsor and the person operating the fireworks event."?

13. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks displays produce or generate wastewater and, if so, please identify?

14. Is the San Diego Water Board relying upon any studies that have determined that fireworks displays involve a process of production or manufacturing, and, if so, please identify?

15. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks are demonstrably equivalent to munitions or ammunition, and if so, please identify?

16. Is the San Diego Water Board relying upon any scientific or engineering research or test results that argue or conclude that fireworks display products constitute "pollutant wastes" as asserted in the Tentative Order, and if so, please identify?

17. What dispute process is available to Sponsors in the event that the Notice of Enrollment includes "additional or increased monitoring due to specific circumstances of the discharge,"?

18. Under what circumstances will the San Diego Water Board impose "additional or increased monitoring" requirements, and how and when will it do so?

19. Which receiving waters or surface waters are known to the San Diego Water Board to have documented and reported adverse impacts attributed specifically to particulate matter and miscellaneous debris associated with fireworks displays?

20. What background data did the San Diego Water Board rely upon in developing the requirements in the Order?

21. What studies to establish (water quality-based effluent limitations (WQBELs) have been conducted by the SDWB?

22. Why are sediment quality objectives being pursued in connection with an order relating to surface water and receiving waters?

23. What are the established effluent limits germane to fireworks displays intended to be enforced by the San Diego Water Board?

24. What are the established receiving water limits?

25. What are the "other requirements" referenced in the Order, (source, Tentative Order, page 12)?

26. Is the San Diego Water Board relying upon any scientific or engineering research or test results in deciding to impose Best Management Practices ("BMP") that are more stringent than current custom and practice and, if so, please identity?

27. How many sweeps of a fireworks display event satisfies the BMP as proposed in the Tentative Order? [BMP 'f']

28. What are "dangerous fireworks", and how are they materially different from display fireworks? [BMP 'e'].

29. How many fireworks display events does one permit cover?

30. Are all-volunteer organizations subject to the Tentative Order?

31. Can co-sponsors jointly apply and, if so, how?

32. Under the Tentative Order, are the terms "discharger" and "permittee" synonymous?

33. Can a 'Discharger' be determined to be liable under the terms of the Tentative Order where it is not an enrollee and, if so, under what circumstances?

34. Under what circumstances will the San Diego Water Board require a person to also apply for an individual NPDES permit?

35. What is the intended definition of "discharger" pursuant to the Tentative Order?

36. What is the intended definition of "receiving waters" pursuant to the Tentative Order?

37. What is the intended definition of "surface water" pursuant to the Tentative Order?

38. Is the San Diego Water Board relying upon any scientific or engineering report or study that either suggests or concludes that fireworks mortars are designed to function as conveyances of pollutants and, if so, please identify?

39. Is the San Diego Water Board relying upon any scientific or engineering report or study that either suggests or concludes that a mortar containing a finished, non-ignited fireworks shell is considered a non-point source?

40. Under what circumstances would the San Diego Water Board determine that the discharge from a fireworks display will not affect, or have the potential to affect, the quality of the waters of the state, prompting the refund of all or part of the annual fee?

41. Under what circumstances would the San Diego Water Board determine to extend a waiver to fireworks displays in accordance with § 13269 of the Porter-Cologne Water Quality Control Act (Ca. Water Code, Division 7)?

42. Under what circumstances would the San Diego Water Board determine to waive the monitoring requirements described in § 13269 of the Porter-Cologne Water Quality Control Act (Ca. Water Code, Division 7)?

43. Under what circumstances would the San Diego Water Board determine that the discharge from fireworks displays will not affect, or have the potential to affect, the quality of waters of the state, prompting a exemption in accordance with § 13269 of the Porter-Cologne Water Quality Control Act (Ca. Water Code, Division 7)?

THE CREADORE LAW FIRM P.C.

COMMENTS:

Comment 1

The requirements of the Tentative Order in its present form are not proportional to the activity sought to be regulated. The NFA strongly believes that the additional expense to each Discharger, in terms of the annual fee and to comply with the requirements prescribed in the Tentative Order, will approximate or exceed the expense of the fireworks display, effectively eliminating fireworks displays that rely upon volunteer donations, and further burdening the budgets of municipalities that provide fireworks displays for its citizens.

Comment 2

The BMP relating to unexploded fireworks is confusing, as well as contradictory: on the one hand the BMP requires the operator to collect and return to the manufacturer/wholesaler all unexploded fireworks, including duds and misfires while, on the other hand, the regulation mandates managing the collected material as hazardous waste [compare BMP 'g' and 'e']. Display fireworks duds and misfires which are collected are not waste, but viable product which can be reworked and reused.

Comment 3

The expense associated with segregating material for disposal as hazardous materials is prohibitively expensive and outweighs any documented benefit to be derived.

The NFA remains ready to assist the San Diego Water Board in achieving responsible regulation, and you may direct any questions regarding this communication to my attention.

Respectfully submitted,

THE CREADORE LAW FIRM, P.C.
Attorneys for the National Fireworks Association

Donald E. Creadore

Item No. 6
Supporting Document No. 5

Fireworks & Stage FX America
"The Difference is Quality"

PO Box 488, Lakeside, CA 92040
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Email: info@fireworksamerica.com

December 9, 2010

Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123-4353

Dear Honorable Members of the Board:

I am commenting on the proposed Tentative Order R9-2010-0124 regarding the public display of fireworks.

Fireworks & Stage FX America, Inc. has been producing fireworks displays for 16 years in the San Diego Area. We are concerned that the Tentative Order raises many complicated and vague requirements for our sponsors and threatens to end fireworks displays, thereby jeopardizing the future of our business. We are aware of the allegations made regarding the effects of fireworks. We have yet to see any reports that justify the allegations. Talk is cheap and this issue seems to be breeding a lot of cheap talk.

Now the Water Board is proposing to enact an order that includes permit fees and water monitoring. This is not necessary, this is not needed. There is no proof to justify the order. This order will put people out of work. This order will put at least one business out of business and more people on the unemployment line. This order will affect many other businesses adversely. Quite simply, this tentative order should be dropped. The consequences far outweigh any good you may think comes from this order.

We are all concerned about our environment and treasure our water and want to maintain good water quality. We have seen recent reports in the Union Tribune that our water quality is the best it has been in many years. San Diego has gotten a good report card from the very groups that are condemning fireworks. We are producing more fireworks than we have in the past, and our water quality is getting better! What is the reasoning behind the tentative order?

There is no politically correct way to state the obvious. DROP THE TENTATIVE ORDER.

Respectfully,

Joseph R Bartolotta
President

Item No. 6
Supporting Document No. 5

Michelle Mata - Concerns about Fireworks Permitting

From: James Unger <junger@hornblower.com>
To: <mmata@waterboards.ca.gov>
Date: 12/9/2010 2:01 PM
Subject: Concerns about Fireworks Permitting

From: James Unger <junger@hornblower.com>
To: <mmata@waterboards.ca.gov>
Date: 12/9/2010 2:01 PM
Subject: Concerns about Fireworks Permitting

Regional Water Quality Control Board
9174 Sky Park Ct # 100
San Diego, CA 92123-4353
Dear Hon. Members of the Board:
Thank you for the opportunity to comment on the proposed Tentative Order R9-2010-0124 regarding the public display of fireworks. Hornblower Cruises and Events has been putting on fireworks for over 20 years, for corporate events, as well as observed the July 4th and New Years Eve civic fireworks events. We are concerned that the proposed Tentative Order raises many complicated and vague requirements and threatens to end important traditions which thousands of citizens count on each year. We are also not aware of any evidence that our show has caused or is otherwise a threat to the environment.
First, we believe that the extensive permitting, monitoring and BMPs proposed in the Tentative Order could add tens of thousands of dollars and make the economics of shows prohibitive. Several people have estimated that the water and sediment monitoring alone, could cost between $30,000 and $100,000 per event, depending on the amount of testing required to prove a negative. We do not believe that we would be capable of raising the additional funds necessary to implement these regulations.
Second, we do not understand why once or twice yearly fireworks shows are being singled out for regulation. These shows have been going on for decades, and no one has ever suggested that these patriotic and civic displays present a water quality problem. In fact, your order provides no evidence that once or twice a year fireworks shows, cause any problems. Massive fireworks shows are done each year over bodies of water throughout the United States, but do not require the monitoring and permitting suggested in the proposed Tentative Order. We do not believe that there is a problem, and ask that you exempt our short and occasional shows from these regulations. We understand no other fireworks event in the country other than SeaWorld San Diego has a fireworks permit under the Clean Water Act, and our show is much smaller than SeaWorld's summer-long show. We also understand inland fireworks over inland water bodies are exempted. The scientific evidence, even at SeaWorld, shows no significant environmental impacts.
Lastly, fireworks are part of the social fabric of our community. It is hard to imagine that a single American has not watched fireworks at some point on the Fourth of July or New Years Eve. They are part of a public celebration of our collective liberty that brings the entire community together. We are at a loss to understand why the Regional Board would want to regulate out of existence something that is so important to so many. We hope the Regional

Board will reconsider the merit of this proposed regulation, or find that infrequent , professionally administered fireworks events have minimal impact and do not require any onerous regulation.
Sincerely,

Jim
--
Jim Unger
Vice President, San Diego
Hornblower Cruises & Events
[redacted]

December 9, 2010

Attn: Michelle Mata
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Via Electronic Mail
mmata@waterboards.ca.gov

RE: SUPPORT Tentative Order No. R9-2010-0124, NPDES Permit No. CAG999002 *General Waste Discharge Requirements For Discharges to Water Associated With Public Displays of Fireworks*

Dear Ms. Mata and Regional Board members:

Please accept these comments in strong support of the proposed water quality protections to be provided upon adoption of Tentative Order No. R9-2010-0124 by the Regional Board.

As Board staff is well aware, when fireworks explode over or adjacent to surface waters, chemicals contained in the fireworks are discharged directly into the water bodies themselves—not to mention can blow over the audience. The chemical-laden paper casings of spent fireworks, as well as unexploded "dud" fireworks also fall from the sky into the surface waters, and are often not recoverable. Both the federal and state Clean Water Acts require that permits be obtained before such discharges occur, and we appreciate the Board finally addressing this mandate.

Fireworks generally contain perchlorate salts, a luminum, magnesium, titanium, barium copper, chloride, and potassium nitrates. Other hazardous chemical compounds often associated with fireworks include, but are not limited to: arsenic salts, strontium salts, lithium salts, calcium salts, sodium, barium, cadmium, copper, aluminum, titanium, lead, mercury and magnesium. These chemicals are widely regulated by the Board, and no other circumstance exists where such intentional point source discharges are tolerated unpermitted. Failure to regulate these obvious discharges not only threatens water quality, it also calls into question the integrity of the Board's entire permitting scheme.

Pollution is pollution. Toxic chemicals are toxic chemicals. Discharge of them, unpermitted, into our waterways is not appropriate. We are confident that in the greatness of our nation we are not so limited that we cannot learn to celebrate without polluting our precious waters. It is critical that the permitting, reporting, and monitoring provisions of the draft permit be maintained until the full impacts of these discharges are understood.

Sincerely,

Laura Hunter

Laura Hunter
Associate Director of Programs

2727 Hoover Avenue, Suite 202 ◆ National City, CA 91950
619.474.0220 ◆ 619.474.1210 fax ◆ www.environmentalhealth.org

JERRY SANDERS
MAYOR

December 9, 2010

Executive Officer Gibson and Honorable Board Members
San Diego Regional Water Quality Control Board
9175 Sky Park Court, Suite 100
San Diego, CA 92123- 4340
Attn: Michelle Mata

Re: City of San Diego Comments regarding Tentative Order R9-2010-0124

Dear Mr. Gibson and Honorable Board Members:

Thank you for the opportunity to comment on the Tentative Order issued by the Regional Water Quality Control Board (RWQCB) considering the possible adoption of an order establishing General Waste Discharge Requirements and a NPDES Permit scheme for the discharges associated with the public display of fireworks. I write to express my grave concern that the proposed tentative order results in a series of damaging consequences that are not fully contemplated, nor vetted within the document. These consequences not only threaten the viability of future fireworks displays in San Diego, but also set a national precedent, devoid of adequate factual or scientific basis. The following repercussions must be seriously considered and fully vetted prior to moving forward with any new legislation.

The proposed tentative order will produce a de facto ban on most if not all fireworks displays in the San Diego region, including July 4th fireworks shows.

For a large number of Americans, few things are more sacrosanct than the right to watch July 4th fireworks. Many of these shows, such as the one in La Jolla and Ocean Beach, are beloved traditions that stretch back decades. Many of the organizations that sponsor fireworks displays operate on a shoe-string budget, oftentimes funded through individual community members and small local business donations. In many cases, local businesses go so far as to place coffee cans on their counters in attempt to raise the funds needed to produce the show. The proposed regulatory requirements would result in exorbitant and prohibitive expenses that these organizations simply will be unable to bear.

Fireworks have never been categorized as a point source discharge in the history of the Clean Water Act.

The Clean Water Act has been in existence for almost forty years. Since inception, fireworks shows have been enjoyed throughout the nation, yet no other state or local agency has applied the Act as proposed by the RWQCB. Regulation in the field creates a new and misguided interpretation of long-standing federal legislation.

No scientific evidence exists to suggest that fireworks, especially shows held only once a year, generate environmental impacts that would require such extreme measures.

The City of San Diego is committed to ensuring the quality of our water. However, insufficient data exists that indicates infrequent firework displays result in negative impacts to water quality. In fact, all available scientific evidence shows just the opposite – these fireworks shows have no measurable impacts whatsoever. Monitoring reports from regular shows, such as those sponsored by SeaWorld, demonstrate minimal impacts on water and sediment quality after 25-years of nightly fireworks shows. These findings are further detailed in our attached comments.

Based on the severity of the consequences listed above, I appreciate your serious consideration of our attached comments. I fully support the efforts of our community organizations that struggle to ensure these celebrations can be honored throughout the year and offer such a valuable benefit to the public. Regulations of the proposed magnitude should not be applied to fireworks displays that are only held on an occasional basis. As such, I urge your reconsideration and look forward to working toward a fair and equitable resolution.

Sincerely,

JERRY SANDERS
Mayor

Attachment: City of San Diego Comments to Tentative Order No. R9-2010-0124

City of San Diego Comments for the December 16, 2010 Workshop Regarding Tentative Order No. R9-2010-0124; NPDES Permit No. CAG999002; General Waste Discharge Requirements for Discharges Associated with the Public Display of Fireworks to Surface Waters within the San Diego Region

The City of San Diego respectfully requests that the Regional Water Quality Control Board (RWQCB) consider the following issues concerning the proposed Tentative Order:

1) **Proposed regulations will result in the cancellation of most, if not all fireworks displays in San Diego.**

   Cost associated with monitoring and compliance create an undue financial hardship for community organizations that sponsor fireworks shows, as these organizations rely on community and business donations to fund the events. Preliminary estimates indicate monitoring costs in excess of $30,000 for basic compliance. This amount exceeds the total budget for most shows and will result in a de facto moratorium on fireworks shows throughout the region.

2) The permitting and monitoring requirements associated with the proposed tentative order are disproportionate to water quality impacts.

   The hypothetical benefits achieved through the proposed permit requirements do not justify the costs. Local water quality consultants have estimated that the water quality and sediment monitoring required by the terms of the Tentative Order will cost between $30,000 and $100,000. This enormous financial burden is not supported by any scientific data or information that demonstrates the need for the report. Therefore, the Tentative Order violates California Water Code section 13267(b)(1), as no reasonable relationship can be shown between the costs of the Tentative Order's water quality and sediment monitoring requirements and any significant impact occasional firework displays may have on water quality.

3) Fireworks are not a "point source" discharge under the Clean Water Act.

   The CWA defines a point source as "any discernible, confined and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged." The City of San Diego does not agree that the legal authority of CWA applies. The Tentative Order would constitute the first and only interpretation in the county that public fireworks displays are a "point source" discharge under CWA.

4) Current monitoring data does not support the need for the level of monitoring proposed in the Tentative Order.

Significant water quality, sediment quality, and sediment toxicity data is available from Sea World, a current permit holder, who has displayed over 3,500 firework shows since 1985. Review of their reports indicates negligible environmental concerns. This demonstrated minimal impact on water and sediment quality after 25-years of fireworks displays calls into question if occasional displays will have any appreciable impact on water and sediment quality.

Furthermore, the Tentative Order states, "Sea World's public fireworks events represent the highest level of water and sediment effects because (1) Mission Bay is unique due to the restricted circulation of waters within the bay and the shallow depth of the bay in the vicinity of the fireworks events, and (2) the high frequency of repeat fireworks events throughout the year at the same location results in maximum pollutant loading." If the conditions associated with Sea World's fireworks shows constitute the most fertile environmental conditions for potential water impairment, then fireworks detonated on an infrequent basis over various depths and circulation patterns should not be held to the same standards.

5) If the order becomes effective as proposed, small-scale fireworks displays should be authorized under a de minimis exemption.

If the proposed Tentative Order is adopted as written, provisions should be included to treat occasional fireworks displays as a de minimis exemption under any general NPDES permit. State law defines a de minimis source as "types of wastes that have low pollutant concentrations and are not likely to cause or have a reasonable potential to cause or contribute to an adverse impact on the beneficial uses of receiving waters yet technically must be regulated under an NPDES permit." Occasional public fireworks displays undoubtedly fit within this de minimis definition, however; it remains questionable that whether NPDES permitting requirements apply in general. As evidenced in available monitoring reports, little to no water quality impacts are associated with these displays.

6) The definition of "significant distance" is unclear as it relates to the prohibition of fireworks in Areas of Special Biological Significance (ASBS).

This term should be clarified and defined.

The City of San Diego appreciates your consideration of the comments listed above. It is our hope that the Tentative Order can be significantly modified to ensure the continued operations of community fireworks displays on the 4th of July as well as during special events throughout the year. If you have any questions or require additional information, please contact Ruth Kolb at (858) 541-4328.

Item No. 6
Supporting Document No. 5

From: Joan Salat <ranchodlm@sbcglobal.net>
To: <mmata@waterboards.ca.gov>
Date: 12/9/2010 8:13 AM
Subject: Fireworks Contaminant Concerns

To Whom It May Concern,

Valley Center had a fireworks display which left about 10 lbs of exploded debree within 150 feet of Moosa Creek. The creek had water in it with 2 different types of tadpoles and toads. The runoff from the explosion site will end-up in Moosa Creek with rains. I had samples taken by a professional Lab recommended by the Valley Center Water District for soil and water before and after the fireworks display. Although the water samples were inconclusive or contaminated, the soil samples showed nearly 3 times the level of Perchlorate on the children's baseball field 2 days after the display than before. All of this evidence has been turned over to the Fire Dept. in V.C. including photos of the burnt debree, the tadpoles and toads in Moosa Creek. I personally collected about 10 lbs. of the debree since the fireworks committee failed to do so. I am concerned about the children playing on the baseball field and the runoff into Moosa Creek. I am also deeply concerned that since our small town had a 10 minute display leaving behind 10 lbs. of toxic debree, what would a larger display deposit into lakes and oceans with maybe 20 to 30 lbs. of toxic debree or more? My photos include debree filled with wires, burnt fuses and laquered layers of paper. What might this do to aquatic life? Later today I will forward photos of the debree and other evidence on file with Fire Marshall George Lucia. I have the debree evidence stored and hope to have it professionally analyzed for metals, and toxic chemicals. If you wish to have more information about this evidence, please feel free to contact me.

Sincerely,

Joan Salat,LCSW,BCD
13275 Betsworth Rd.
Valley Center, Ca. 92082
(760)751-9277

Michelle Mata - 27 of hundreds of photos taken after the Valley Center Fireworks Event 2010

From: Joan Salat <ranchodlm@sbcglobal.net>
To: <mmata@waterboards.ca.gov>
Date: 12/9/2010 4:59 PM
Subject: 27 of hundreds of photos taken after the Valley Center Fireworks Event 2010

From: Joan Salat <ranchodlm@sbcglobal.net>
To: <mmata@waterboards.ca.gov>
Date: 12/9/2010 4:59 PM
Subject: 27 of hundreds of photos taken after the Valley Center Fireworks Event 2010

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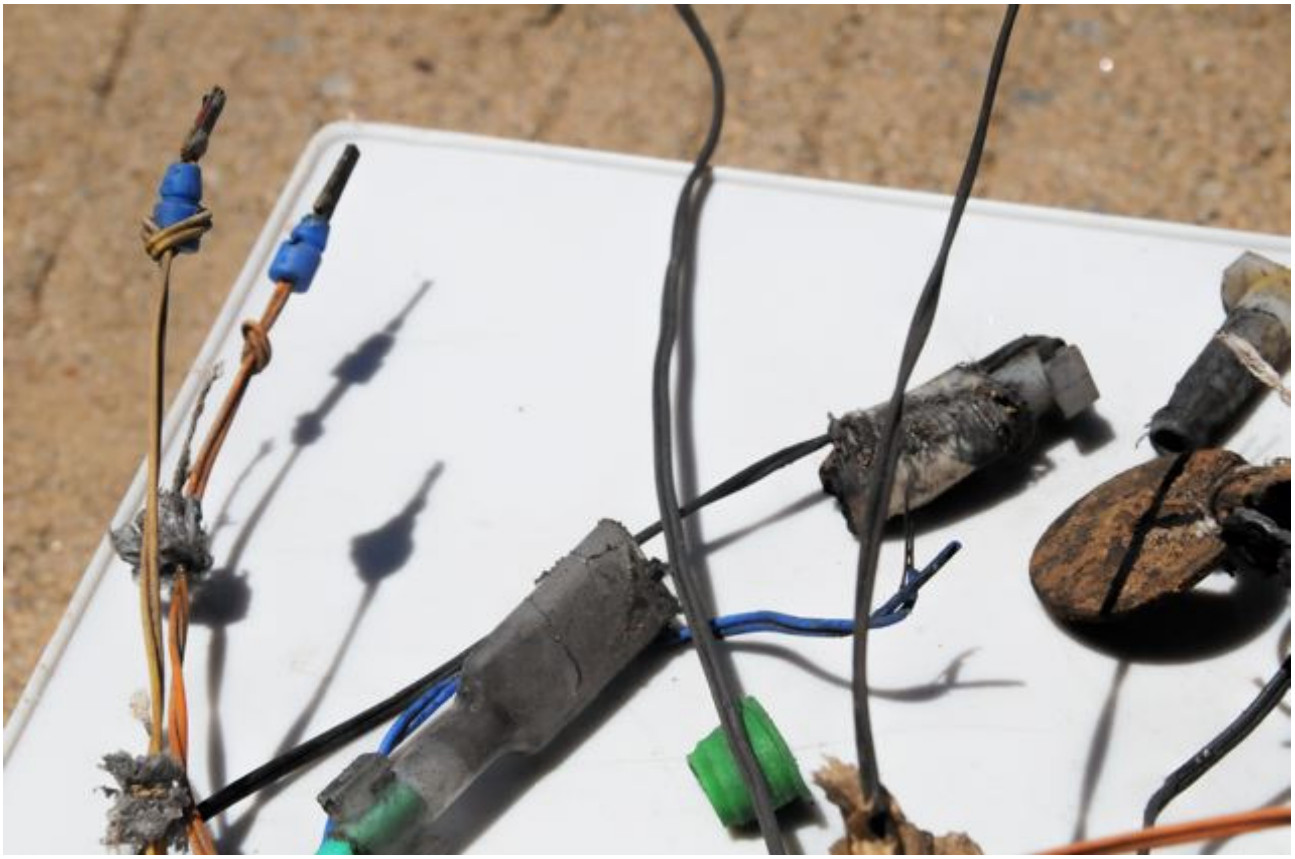

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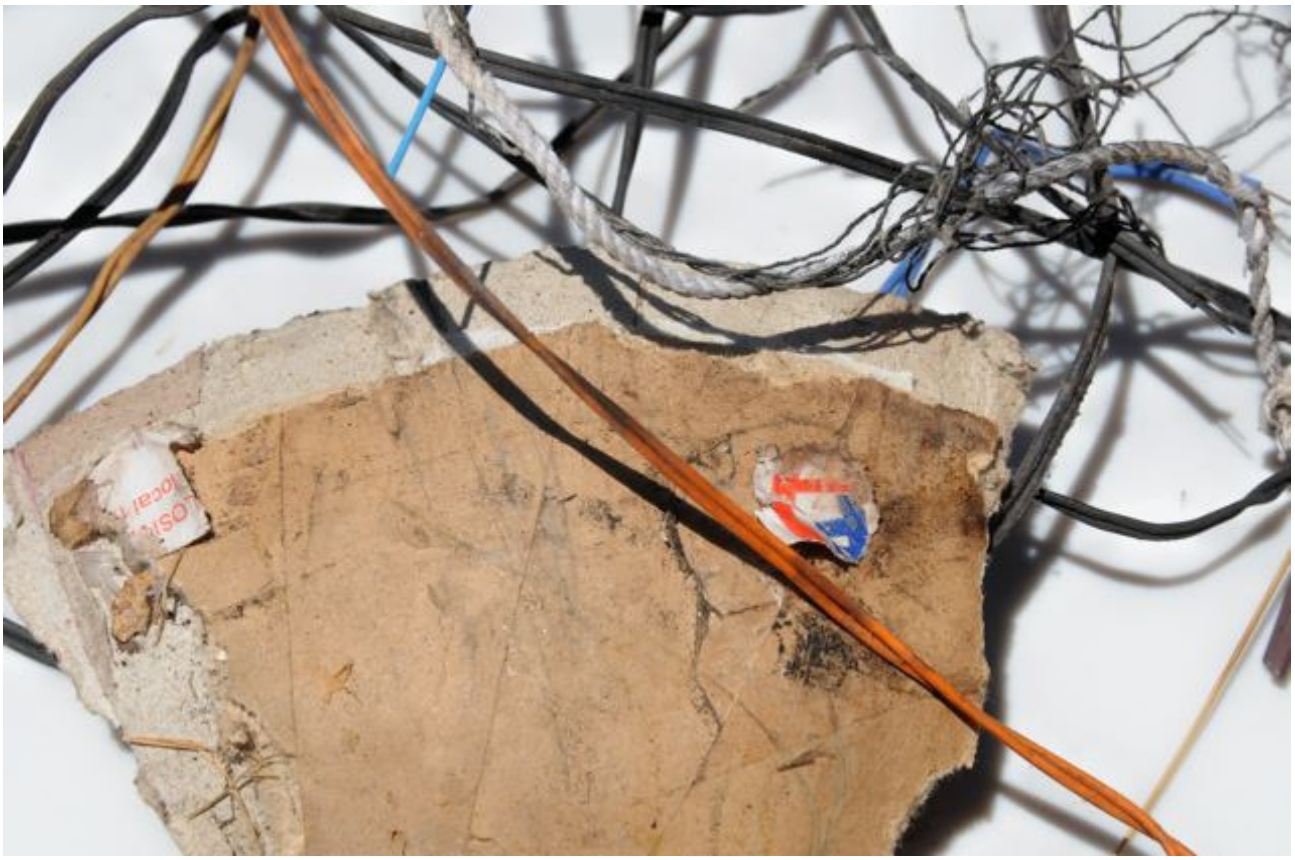

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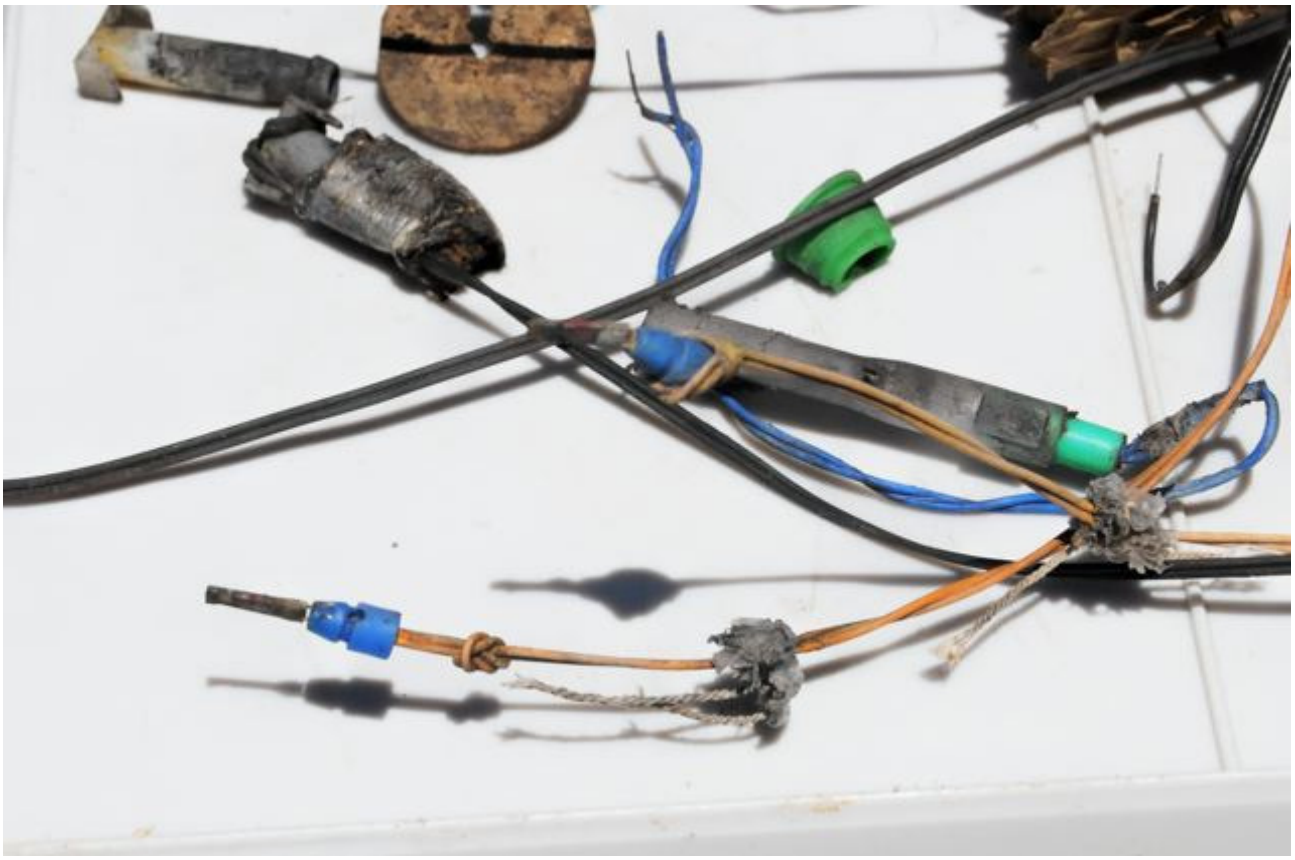

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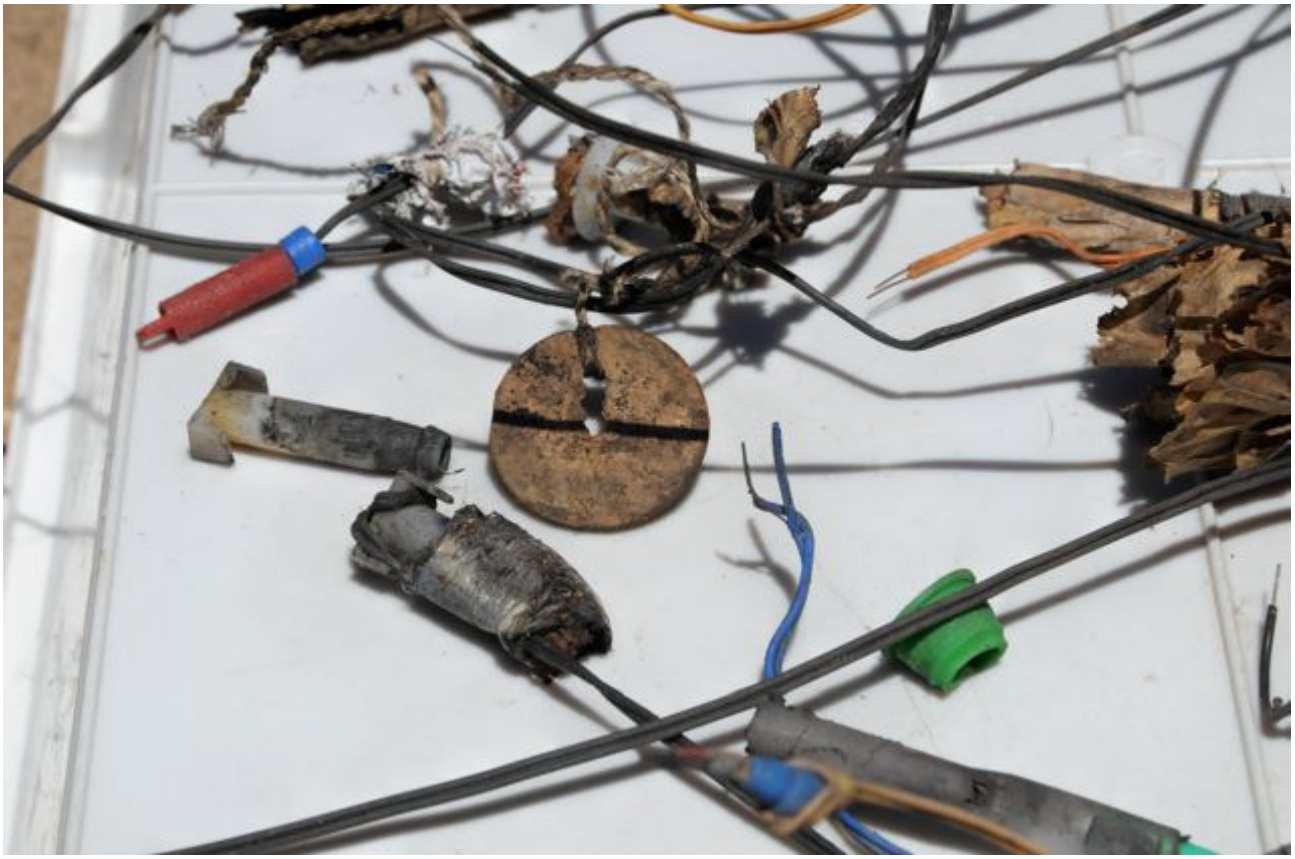

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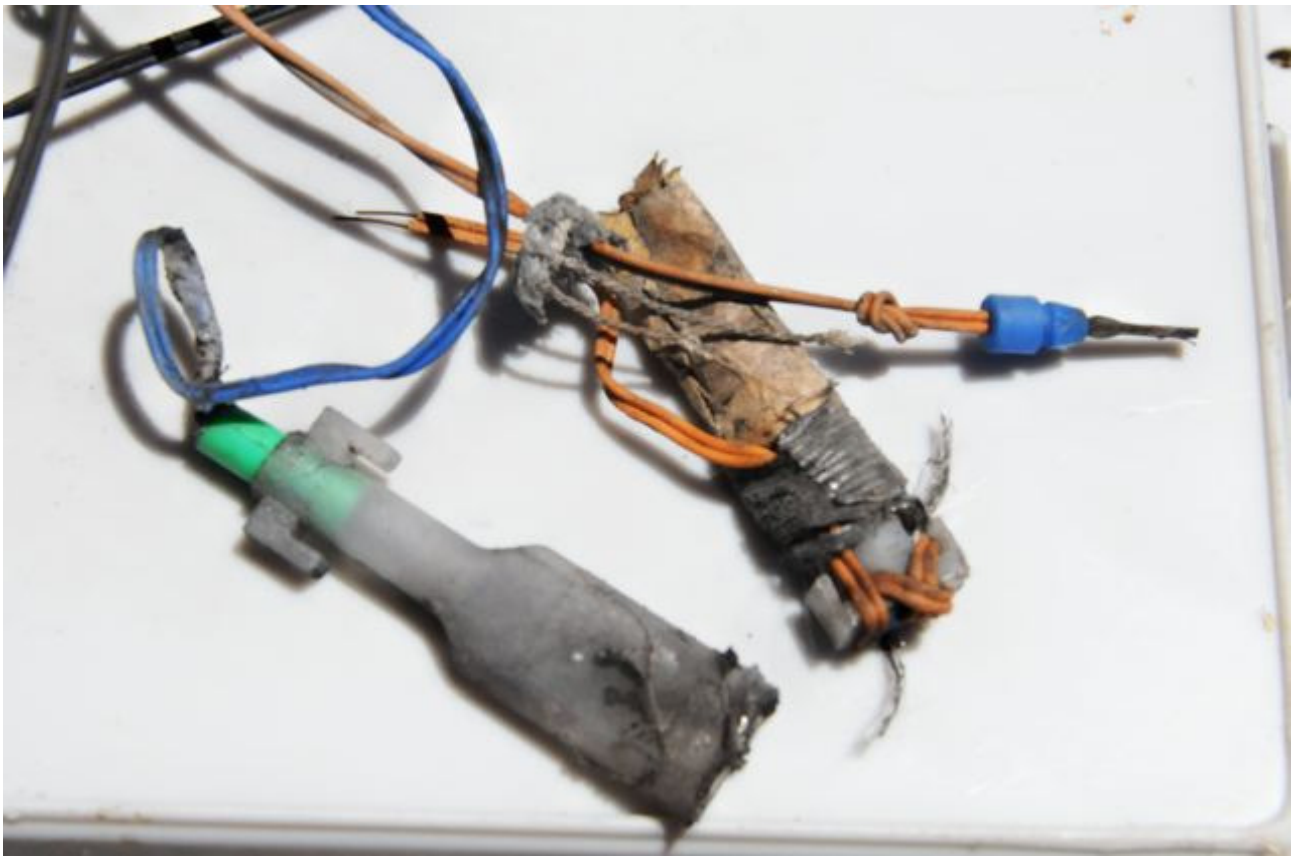

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LATHAM & WATKINS LLP

December 9, 2010

VIA E-MAIL & FEDERAL EXPRESS

Executive Officer Gibson and Honorable Board Members
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123-4340

Re: Revised Tentative Order No. R9-2010-0124, NPDES No. CAG999002; General Waste Discharge Requirements for Discharges Associated With The Public Display Of Fireworks To Surface Waters in the San Diego Region

Dear Mr. Gibson and Honorable Board Members:

Thank you for the opportunity to submit comments in advance of the December 16, 2010 workshop on Tentative Order No. R9-2010-0124 regarding General Waste Discharge Requirements for the Public Display of Fireworks in the San Diego Region ("Tentative Order"), released by the San Diego Regional Water Quality Control Board ("Regional Board") on September 23, 2010. We submit these comments on behalf of the La Jolla Community Fireworks Foundation ("LJCFF"), a non-profit corporation organized for the purpose of promoting patriotism and community spirit by preserving La Jolla's Fourth of July tradition with a public fireworks display.[1]

We are very concerned that the Regional Board staff has proposed a new, unnecessary and nation-wide precedent-setting regulatory regime for future public fireworks displays, without any significant public input and, more importantly, without any scientific basis. Quite simply,

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[1] As noted in our prior correspondence of November 19, 2010, these comments cannot begin to address fully all of the significant legal, technical, economic, and practical considerations that may arise from the current Tentative Order because several key components of the Tentative Order have not been made available to the public, including, by the Regional Board's own admission, potential modifications "including clarifying language, adding definitions and modifying receiving water monitoring requirements." However, in the interest of ensuring a productive and meaningful public workshop on December 16, 2010, we submit these comments with regard to the Tentative Order as it currently stands. We look forward to providing further comments on the Revised Tentative Order, when that document becomes available, and participating in a further public workshop at that time.

the Tentative Order is a regulation seeking a problem. There have been no showing of problems or water quality issues presented to the Regional Board that justify the issuance of this Tentative Order and the onerous regulatory requirements set forth therein. The burdensome regulations, testing and reporting requirements will almost certainly prevent most coastal communities in the San Diego region from participating in a patriotic fireworks tradition that dates back over 200 years. Importantly, no regulatory body in the nation has found it necessary or appropriate to regulate any one of the countless fireworks displays that have occurred during the almost forty years that the Clean Water Act has been in existence. And any attempt to justify the terms of the Tentative Order based on the current fireworks displays put on by SeaWorld is preposterous when one considers that the SeaWorld events occur for over 100 consecutive days from a barge in an enclosed, shallow bay, whereas, by way of example, Fourth of July fireworks are a once-a-year event that last a matter of mere minutes.

We would therefore request that the Regional Board withdraw this Tentative Order, and, as has been done for inland fireworks displays, issue a General National Pollution Discharge Elimination System ("NPDES") permit exemption for public fireworks displays that occur from the same coastal location between four to ten times a year.[2] In the alternative, the Regional Board should revise the Tentative Order to implement a de minimis exception for those public fireworks displays which occur from the same coastal location less than ten times a year and/or detonate no more than a reasonable annual threshold of pyrotechnical material, a threshold that can be reached through consultation with water quality consultants.

I. THE TENTATIVE ORDER WOULD REGULATE FOURTH OF JULY FIREWORKS OUT OF EXISTENCE

A. Implementation of the Tentative Order Would Result in Cancellation of Most Coastal Community Fireworks Displays

First and foremost, the Regional Board must understand that the Tentative Order as it now stands would result in the cancellation of most, if not all, San Diego area community fireworks displays as a result of the high cost of compliance with the Tentative Order's demanding regulations, testing and reporting requirements. Financed by small individual community contributions, these long-standing patriotic celebrations would be permanently shut down if communities are forced to produce enough capital to comply with the unnecessary and duplicative provisions of the Tentative Order.

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[2] The first order of business before the Regional Board purports to regulate all fireworks is to understand the source of fireworks displays that take place within its jurisdiction. The Tentative Order makes clear that no effort has been undertaken to understand the nature and extent of the regional fireworks displays that it is now attempting to regulate. For example, the Tentative Order contains no information on how the frequency or specific location of public fireworks displays affect receiving waters. Before issuing a blanket General Permit that purports to apply to all public fireworks displays, the Regional Board must make a greater effort to study the very activity that it now seeks to regulate.

As an example, the La Jolla Cove fireworks display has been an annual community celebration for over 25 years. This year's 2010 display lasted 23 minutes, at a total cost of approximately $30,000. Yet the Tentative Order proposes water quality and sediment monitoring that local water quality consultants have estimated will cost between $30,000 and $100,000, thereby doubling or quadrupling the cost of the event and making any single event cost prohibitive. The City of Santee, in a prior comment letter on the Tentative Order, estimated that the basic cost of compliance with the Tentative Order is likely $20,000. Even this lower cost estimate would still almost double the cost of most local celebrations, making such events impossible to finance and produce.

Far from some large corporate event, the $30,000 budget for the La Jolla celebration is gathered through contributions from local individuals and businesses that range on average from $50 to $200. There are no deep-pocketed corporations putting on the event. Instead, the event is sponsored by the local population as a matter of civic and national pride. In fact, this year more than others, the loss of a major business donor due to the economic climate almost cancelled the event. Had hundreds of average La Jolla citizens not stepped in to make small contributions, the tradition would have ended. Yet, notwithstanding the recent economic decline, the Tentative Order now expects these same citizens to produce almost double or quadruple the amount of contributions to prove a negative – that no significant sediment or water quality impacts occur from occasional fireworks displays; this simply won't happen.

It is our understanding that fireworks displays in Ocean Beach, Carlsbad, Oceanside and other small San Diego communities are produced on similar budgets and similarly financed through community contributions; the substantial additional expense required under the Tentative Order for permit compliance paperwork, water monitoring and sediment testing will certainly crush the budgets of these shows. The end result of the Tentative Order will thus be a massive cancellation throughout the San Diego region of most, if not all, small community fireworks celebrations, with no resulting improvement in water quality.[3]

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[3] For example, the Best Management Practices ("BMPs") required under the Tentative Order are no more protective than clean-up procedures already taken by event organizers, but certain aspects of these BMPs would render the cost of these events entirely prohibitive. By way of example, the Tentative Order requires unexploded fireworks and debris to be collected, which is already done following displays and has been for decades, but the Tentative Order would require event organizers to treat the debris as hazardous waste. Tentative Order, at VI.C.3.g.

First, the Regional Board does not have the statutory authority to define what is or is not hazardous waste under California's Hazardous Waste Control Law (CAL. HEALTH & SAFETY CODE § 25100 *et seq.*); this impermissible *ultra vires* act is void and unenforceable. Second, even if the Regional Board could act here, the Tentative Order demonstrates no scientific basis to deem the minor amounts of debris from fireworks displays, which are essentially cardboard and crate paper, as hazardous waste. Third, the treatment of this material as hazardous waste requires a hazardous waste removal and

II. THE REGIONAL BOARD HAS NO JURISDICTION TO REGULATE PUBLIC DISPLAYS OF FIREWORKS AS THEY ARE NOT A "POINT SOURCE" UNDER FEDERAL CLEAN WATER ACT

A. Fireworks Are Not A "Point Source" Under the Clean Water Act

Even if San Diego communities could conceivably raise enough capital annually to finance the permit fee, water monitoring and sediment testing requirements of the Tentative Order, the Regional Board has no legal jurisdiction to require these community organizers to comply with the terms of the Tentative Order. As explained below, occasional public fireworks displays detonated above or near water cannot be considered a "point source" under the federal Clean Water Act (33 U.S.C. § 1251 *et seq.*) ("CWA"), and thus the Regional Board has no legal basis for regulating these displays.

The CWA empowers states to administer the NPDES permit program, under which entities such as the Regional Board are authorized to issue and administer NPDES permits. 33 U.S.C. § 1342(b). However, the CWA requires such permits only when pollutants are discharged from a "point source." 33 U.S.C. § 1362(12). As explained below, the legislative history of the CWA, EPA regulations, and federal case law all confirm that individual fireworks displays are not "point sources" under the CWA and thus cannot be regulated by the Regional Board under the NPDES program.

The CWA defines a point source as "any discernible, confined and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged." 33 U.S.C. § 1362(14). The legislative history of the Act suggests that Congress meant to cover discharges that were at least "frequent," or that resulted in some "measurable" waste entering the water. *Northwest Envtl. Def. Ctr. v. Brown*, 617 F.3d 1176, 1183 (9th Cir. 2010). Here, the evidence shows that occasional celebratory and civic public fireworks displays are neither "frequent" nor result any "measurable" amount of waste entering the water; thus, it makes sense that no regulatory body in the nation, including the U.S. Environmental Protection Agency ("EPA") which has primary jurisdiction for nationwide enforcement of the CWA, has ever attempted to regulate such displays as a "point source" under the CWA.

Given the Regional Board's sudden and unexplained desire to enter into this previously unregulated area, one wonders what exactly is the Board's definition of "point source." Is it a barge-launched display such as the SeaWorld shows? Is it a coastal land-based display where the fireworks are projected over water? Or is it a coastal land-based display where the fireworks

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disposal program, as well as transportation and disposal of the material at a hazardous waste facility. The additional and unnecessary expenses of creating a hazardous waste plan and paying special transportation and disposal fees for this debris would likely run into the tens of thousands of dollars, adding to an ever-growing list of costs which will preclude the smaller community fireworks events from occurring.

explode over land? Notably, the Regional Board already provides a permit exemption for inland fireworks; yet at some ill-defined location near the coast, this now-exempt activity apparently becomes a regulated "point source" under the Regional Board's odd interpretation of the CWA. The Tentative Order does not explain how far inland a theoretical "point source" must be launched from before it becomes a non-regulated fireworks display. Additional considerations that should play into the Regional Board's interpretation of "point source" include the amount of pyrotechnics, the specific mechanics of how this activity is performed, how close the activity may be come to bodies of water or other watersheds, etc. But none of these issues is addressed in the Tentative Order.

The federal regulations interpreting the definition of "point source" focus on various industrial categories such as dairy products processing; grain mills; the textile industry; cement manufacturing; feed lots; fertilizer manufacturing; nonferrous metals manufacturing; steam electric power generating; leather tanning; asbestos manufacturing; and coal mining. 40 C.F.R. § 405 *et seq.* While the regulations include explosives manufacturing as a specified category of regulated point sources, fireworks displays are not referred to anywhere in the regulations. Given the breadth of regulations existing with regards to other potential "point sources," a logical conclusion from this conspicuous regulatory absence is that Congress does not consider occasional public fireworks displays detonated above water to constitute a "point source" discharge under the CWA.

Federal courts have held that activities and "sources" such as people, grazing cows, and even a building from which trash and runoff ran into a river are not "point sources." *United States v. Plaza Health Labs., Inc.*, 3 F.3d 643 (2d Cir. 1993); *Oregon Natural Desert Ass'n v. Dombeck*, 172 F.3d 1092 (9th Cir. 1998); *Hudson Riverkeeper Fund v. Harbor at Hastings Assocs.*, 917 F. Supp. 251 (S.D.N.Y. 1996). The Second Circuit has stated that the definition of "point source" and the examples given by Congress "evoke images of *physical structures and instrumentalities* that systematically act as a means of conveying pollutants from an industrial source to navigable waterways." *Plaza Health Labs., Inc.*, 3 F.3d at 646 (emphasis added). The individual fireworks displays at issue here do not systematically convey pollutants because they take place only once or twice per year, nor are they an industrial source of pollutants. Similarly, occasional fireworks displays are not comparable to the year-after-year deliberate bombing of water-based targets and disposal of million of pounds of military munitions into the ocean surrounding Vieques Island. *Weinberger v. Romero-Barcelo*, 456 U.S. 305, 307 (1982).

By enacting the CWA, Congress intended to target "industrial and municipal production of pollutants," not infrequent activities such as fireworks displays. *Plaza Health Labs., Inc.*, 3 F.3d at 650. The Tentative Order would constitute the first and only interpretation in the country that public fireworks displays are a "point source" discharge under the CWA. It simply cannot be reasonably argued that occasional coastal fireworks displays fall within the definition of "point source" discharge under federal law.

B. Regulation of Fireworks as a "Point Source" Would Lead To Absurd Conclusions

Any attempt by the Regional Board to label occasional public fireworks display as a "point source" under the CWA would inevitably lead to a slippery slope of endless regulation

with illogical results. For example, if once- or twice-yearly fireworks displays constitute a "point source," then the Regional Board by necessity must also require a NPDES permit for any plane flying over the ocean whose engines discharge particulates, or any person entering the ocean with non-waterproof sunscreen, or even a person caught littering in a body of water. All of these sources produce far more cumulative "pollutants" and occur infinitely more frequently than a 23-minute Fourth of July fireworks display; yet the Regional Board has correctly not seen fit to regulate any of these discharges as a point source under the CWA. No doubt the Regional Board recognizes that it does not have the legal authority to do so under the CWA, and such regulation would result in an endless permitting fiasco. The Regional Board should now apply those same principles here and provide a general NPDES permitting exemption for occasional public fireworks displays.

The characterization of Fourth of July fireworks displays as a "point source" is a fantastic and inappropriate expansion of the term as it is used in the CWA; it is tantamount to the San Diego Regional Board legislating a new definition of the term. Fireworks displays have been a national tradition dating back to the founding of the United States and have never been held by the EPA or any court to be subject to NPDES permitting. Indeed, the Regional Board staff cites no legal authority or regulatory guidance from U.S. EPA or any other state agency to support its contention that this activity suddenly needs regulation. In fact, neither the U.S. EPA, Congress, the US, California State Water Resources Control Board, nor any other Regional Board in the State of California or similar body in another State has defined a Fourth of July Fireworks display as a "point source." The precedent proposed by Regional Board staff is especially breathtaking given the massive coastal Fourth of July fireworks events that occur over water in Boston, New York, Philadelphia, Baltimore, and Washington D.C., among others. If the CWA requires the regulation of occasional public fireworks displays, why has the U.S. EPA and other state and local agencies with NPDES permitting authority chosen to ignore the aforementioned displays that occur in their very own backyards? The reason is obvious; no such regulation is required.

In this case, the Regional Board would overstep its authority and embark on a complete re-write of the CWA. Occasional public fireworks events occurring between four and ten times per year (or which comprise no more than a certain threshold of pyrotechnics) should be treated as exempt from any NPDES permitting requirements.

III. EVEN IF THE REGIONAL BOARD HAS JURISDICTION TO REGULATE PUBLIC DISPLAYS OF FIREWORKS, SUCH DISPLAYS QUALIFY AS A DE MINIMIS EXCEPTION TO NPDES PERMITTING

Even assuming that the Regional Board has the legal authority to regulate infrequent public fireworks displays under the CWA, which is not the case, the Tentative Order should be amended to treat fireworks displays that occur on the order of four to ten times a year as a de minimis exception under any general NPDES permit.

State law defines a de minimis source as "types of wastes that have low pollutant concentrations and are not likely to cause or have a reasonable potential to cause or contribute to an adverse impact on the beneficial uses of receiving waters yet technically must be regulated

under an NPDES permit." 23 Cal. Code of Regs. § 2200, n. 15. Occasional public fireworks displays, such as the La Jolla community display, undoubtedly fit squarely within this de minimis definition (even assuming the NPDES permitting program applies at all), as the evidence in the record demonstrates that there are little to no water quality impacts associated with these displays, as explained more fully below.

The Tentative Order appears to be incorrectly and inappropriately based on the unique facts of SeaWorld's daily fireworks displays. But even if the SeaWorld fireworks shows could be analogized here, water monitoring data obtained from those same Mission Bay barge-launched displays confirm that even after such a high level of frequency in a shallow water body with limited circulation, public fireworks displays result in almost undetectable levels of various constituents above background levels and thus constitute a de minimis source.

A. Daily SeaWorld Fireworks Displays Cannot Form the Basis of a NPDES Permit for a Single Event Fireworks Display

Lacking any substantive scientific evidence to support its regulatory grab, the Regional Board appears to have inappropriately based the requirements and procedures of the Tentative Order on the wholly irrelevant ongoing fireworks displays that occur at SeaWorld. As noted above, the SeaWorld fireworks shows occur for over 100 consecutive days per year, and are shot from a barge in a shallow, enclosed bay. In fact, the Tentative Order itself directly acknowledges the unique circumstances of the SeaWorld events stating, "SeaWorld's public fireworks events represent the highest level of water and sediment effects because (1) Mission Bay is unique due to the restricted circulation of waters within the bay [and] the shallow depth of the bay in the vicinity of the fireworks events, and 2) the high frequency of repeat fireworks events throughout the year at the same location results in maximum pollutant loading."

In stark contrast to the SeaWorld shows, the Fourth of July fireworks displays in La Jolla and communities around San Diego occur on a single day, last 15-25 minutes, and are often shot in proximity to the open ocean, a body of water that has a significantly different tidal structure which quickly dissipates any combusted residue that might enter the water. Applying the SeaWorld monitoring data to occasional Fourth of July fireworks displays results in an inappropriate "apples to oranges" regulatory comparison and illustrates why the Tentative Order cannot stand as currently drafted.

Even assuming the SeaWorld water chemistry data could be applied to significantly less frequent fireworks displays in proximity to entirely different bodies of water, such data demonstrates that these displays should be treated as a de minimis source. The Tentative Order itself acknowledges that after over 100 consecutive days of fireworks and three years of testing, "[w]ith the exception of Zinc, bis (2-ethylhexyl) phthalate, and perchlorate, water chemistry sampling to date, showed little evidence of pollutants within the receiving water column at levels above applicable water quality criteria or detected reference site levels. Comparison of the average concentrations of all the metals in water samples to California Toxics Rule (CTR) saltwater criteria shows that concentrations fall below both continuous exposure and maximum exposure concentrations." Tentative Order, at 7.

Given that most regional coastal fireworks displays occur only once or twice per year and would therefore demonstrate even lower levels of contaminants, if any, the SeaWorld data supports a finding that these fireworks displays are a de minimis source, which are not likely to cause or have a reasonable potential to cause or contribute to an adverse impact on the beneficial uses of receiving waters.

B. 2010 Big Bay Boom Monitoring Demonstrates No Water Quality Impact

In an obviously more comparable situation, yet still far bigger than the typical community fireworks show, recent monitoring data from the July 2010 Big Bay Boom also confirms that annual fireworks displays result in little to no environmental impact to surrounding bodies of water. The Big Bay Boom is a once-a-year Fourth of July fireworks display that occurs around the north bay area of San Diego Bay. A recent monitoring report for the Big Bay Boom notes that, "We have reviewed the data and found that the vast majority of metals analysis results indicated that total concentrations either declined between the pre-fireworks and post-fireworks sampling events, or increased less than 10 percent (an arbitrary value)." Yet the Tentative Order makes no mention of this data and instead only relies on the inapplicable results of SeaWorld's water quality and sediment chemistry monitoring.

With the preponderance of the evidence showing that there are no discernable water quality impacts related to once- or twice-yearly fireworks events, the Regional Board should revise the Tentative Order to implement a de minimis exception for those fireworks displays that occur on the order of four to ten times a year or otherwise meet a measurable size threshold.

IV. THE TENTATIVE ORDER'S MONITORING REQUIREMENTS ARE DISPROPORTIONATE TO WATER QUALITY IMPACTS AND THEREFORE VIOLATE MANDATORY COST-BENEFIT ANALYSES

California Water Code section 13267(b)(1) provides:

> The burden, including costs, of [monitoring program] reports shall bear a reasonable relationship to the need for the report and the benefits to be obtained from the reports.

As stated above, local water quality consultants have estimated that the water quality and sediment monitoring required by the terms of the Tentative Order will cost between $30,000 and $100,000. Yet the Regional Board has failed to support this enormous financial burden with any scientific data or information that demonstrates the need for the report. In fact, as shown above, all available information in the record supports a finding that there are no discernable water quality impacts related to occasional public fireworks displays. Therefore, the Tentative Order is in direct violation of California Water Code section 13267(b)(1), as no reasonable relationship can be shown between the outrageous costs of the Tentative Order's water quality and sediment monitoring requirements and any impact on water quality. Indeed, the monitoring costs equal or ***exceed*** the costs of the entire fireworks displays.

Notably, the U.S. Supreme Court recently confirmed that it is proper to compare costs and benefits when applying the CWA where the benefits are significantly disproportionate to the

costs, especially when the benefits are de minimis, as here. *Entergy Corp. v. Riverkeeper, Inc.*, 129 S. Ct. 1498, 1510 (2009).

V. THE TENTATIVE ORDER MAY REQUIRE INDIVIDUAL COMPLIANCE WITH THE CALIFORNIA ENVIRONMENTAL QUALITY ACT FOR EACH PUBLIC FIREWORKS DISPLAY

Adding to the list of significant permitting costs and unintended consequences, each organization hoping to produce a future public fireworks display may be required to comply with the California Environmental Quality Act (Cal. Pub. Res. Code § 21000, *et seq.*) ("CEQA") if the Tentative Order is approved as it currently stands. While the Tentative Order makes clear that the Regional Board's approval of the General Permit would be exempt under CEQA pursuant to California Water Code section 13389, it is unclear under the terms of the Tentative Order whether the Regional Board's act of reviewing and issuing each individual fireworks permit contemplated under the General Permit would be deemed a "discretionary act" subject to CEQA. Cal. Pub. Res. Code § 21080(a). If so, each time an entity seeks a fireworks permit from the Regional Board, that entity or even the Regional Board itself may be required to prepare CEQA environmental documents such as an initial study, environmental impact report and/or negative declaration. The costs of preparing such environmental analyses can reach as much as several hundred thousand dollars, dwarfing the current budgets of community fireworks presentations and making the prospect of future fireworks display even more unrealistic.

This CEQA scenario is not far-fetched; it is the exact position taken by the Coastal Environmental Rights Foundation ("CERF") in the recent state court litigation filed in 2010 against the La Jolla fireworks display. There, CERF asserted in court pleadings that the City of San Diego's permitting decision for the 2010 La Jolla community Fourth of July fireworks display was a discretionary project subject to CEQA because "it is a project requiring the exercise of judgment or deliberation by the City prior to Project approval." If the Regional Board agrees with this radical interpretation of CEQA, does the Regional Board plan to act as the lead or responsible agency under CEQA for all fireworks shows in San Diego County, including, but not limited to, the Fourth of July, New Years Eve, the Summer Pops concert series, Humphreys Concerts By the Bay, and the San Diego Bay Parade of Lights? Such an unprecedented increase in regulatory responsibility would no doubt require significant time, effort and resources on the part of the Regional Board staff, in addition to those additional tasks already contemplated in the Tentative Order.[4]

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[4] We further note that the Tentative Order appears to encroach on several other regulatory jurisdictions, thereby creating additional and unnecessary regulatory responsibilities for the Regional Board and its staff. As currently drafted, the Tentative Order requires documentary proof of United States Coast Guard Marine Event Permits, State Fire Marshal's licenses and city permitting "[n]o later than 30 days in advance of any planned public display of fireworks." Tentative Order at E-5. This begs the question of why the Regional Board would set itself up as the regulatory and permitting clearinghouse for these events. By doing so, the Regional Board is assuming an oversight role of state fire

The public is entitled to understand the extent to which CEQA would apply to regional fireworks displays, and how the Regional Board plans to manage this potentially substantial increase in regulatory workload, including how such CEQA compliance would impact the Regional Board's ability to timely issue annual fireworks permits. Under the terms of the Tentative Order as it now stands, these important issues have been entirely unaddressed.

VI. THE TENTATIVE ORDER WILL RESULT IN HARMFUL UNINTENDED CONSEQUENCES

In addition to the cancellation of most long-standing fireworks celebrations, the Tentative Order will lead to several harmful indirect effects on the surrounding San Diego region, as described below.

A. The Tentative Order Will Lead To Increased Public Safety Risk

Preventing professionally conducted public coastal fireworks displays would likely inevitably result in an increase in untrained individuals seeking to set off their own private fireworks displays, which implicates important public safety concerns. The Chief of the City of San Diego Fire Department ("SDFD"), Javier Maniar, spoke to this very issue in a recent declaration made in connection with the litigation filed against the La Jolla fireworks display. Chief Maniar stated:

> The SDFD favors public Fourth of July fireworks displays that are produced by professional pyrotechnic companies and permitted by the City, such as the La Jolla fireworks; professionally managed fireworks are more beneficial to the community than private fireworks because they reduce the incidents of injuries and accidental fires. I know from my experience and training that private fireworks set off by minors and untrained individuals injure thousands of people in the United States each year.

Moreover, current statistics confirm that private fireworks result in significant numbers of injuries and substantial property damage each year. According to the U.S. Consumer Product Safety Commission, roughly 7,000 people were treated in hospital emergency rooms for injuries associated with fireworks in 2008.[5] The National Fire Protection Association estimates that fireworks in 2008 nationally caused an estimated 22,500 reported fires, including 1,400 structure

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officials, the U.S. Coast Guard and municipal land use planning. This authority grab is outside of the area of expertise of the Regional Board and goes well beyond the defined role of the Board and purpose of general NPDES permits.

In addition, the Regional Board chronology for annual submissions in support of the General Permit is not consistent with the schedule of how these local and federal agencies will actually respond to fireworks permitting requests, which may be only a week or two before the event.

5 This information can be found at http://www.cpsc.gov/cpscpub/pubs/012.html.

fires, 500 vehicle fires and 20,600 outside and other fires.[6] These fires resulted in one civilian death, 40 civilian injuries and $42 million in direct property damage. In addition, private fireworks can lead to an increased risk of wildfires, a serious issue well known to all residents of the San Diego region. Importantly, Chief Maniar noted that "[t]he risk of wildfires from fireworks is significantly increased with unauthorized, unsupervised private fireworks displays." The City of Temecula thus sums it up perfectly: "The safest way to enjoy fireworks is to attend a public display conducted by trained professionals."[7]

B. The Tentative Order Will Cause Economic Harm to Businesses that Rely on These Events

Furthermore, the cancellation of the regional public fireworks displays will result in significant economic harm to the many small businesses in each community that rely on these yearly events to support their establishments. For example, in La Jolla, hotel rooms overlooking the ocean and restaurants with a view of the fireworks are booked months in advance of the Fourth of July Fireworks and represent a significant economic event for these businesses. Thousands of people come to the village of La Jolla to watch the fireworks and spend the day shopping and patronizing local restaurants. As noted by the San Diego Lodging Industry Association in their letter to Mayor Sanders, "Without question, the loss of Fourth of July fireworks displays will result in a loss of visitors to those communities that must cancel them. Tourists have a multitude of choices and can easily and quickly alter travel plans... Visitors who would have filled hotel rooms in La Jolla will find an alternate location that fits their needs. In this case, La Jolla's (and the City of San Diego's) loss will be Oceanside's, Coronado's, or perhaps Palm Springs' gain."

This sentiment was reiterated by San Diego City Councilmembers Sherri Lightner and Kevin Faulconer in their declarations made in connection with the litigation filed against the La Jolla fireworks display. Both Councilmembers estimated that the La Jolla fireworks display attracts 13,000 to 20,000 spectators at various La Jolla venues and supports many La Jolla businesses. The loss of these important economic events to the San Diego region would likely have a significant impact on the ability of many small business to continue to remain profitable, especially in light of the recent economic downturn.

C. The Tentative Order Threatens Free Speech Rights

There are significant free speech issues associated with Fourth of July fireworks displays which the terms of the Tentative Order either ignore or dismiss. In their declarations filed with the court in the La Jolla litigation, San Diego City Councilmembers Kevin Faulconer and Sherri Lightner both noted the importance of Fourth of July fireworks to the civic fabric of their communities. Likewise, John Adams wrote to his wife Abigail after the signing of the

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6 This information can be found at http://www.nfpa.org/assets/files/pdf/os.fireworks.pdf.

7 This information can be found at http://www.cityoftemecula.org/Temecula/Residents/PublicSafety/fireworkssafety.htm.

Declaration of Independence on July 4, 1776 that this day "ought to be solemnized with pomp and parade, with shows, games, sports, guns, bells, bonfires, and illuminations, from one end of this continent to the other, from this time forward forever more." Fourth of July celebrations are a form of free speech that cannot be suppressed by the Regional Board here.

It is not subject to dispute that Fourth of July fireworks are the fulfillment of these festivities and are deeply ingrained into the fabric of the entire nation as an important declaration of our collective freedoms. The burdensome regulations of this Tentative Order threaten those basic freedoms.

D. The Tentative Order Invites Additional Third-Party Litigation

The complexity of the issues presented by the Tentative Order and unsupported, yet definitive, statements in the Tentative Order that, for example, fireworks debris constitute "waste" make third-party litigation over compliance with the Tentative Order highly inevitable. If the Tentative Order is approved, local community groups and municipalities will be required to fight, one by one, with regards to whether or not they have complied with the terms of the Tentative Order. This litigation is likely to be extremely costly and would further erode the ability of these agencies and groups to produce future community fireworks events.

We also note that the Tentative Order would now require that any fireworks displays must "be located a sufficient distance" away from those areas designated by the State Water Resources Control Board as Areas of Special Biological Significance ("ASBS"). Tentative Order, at IV.C. In light of the recent litigation filed against our client, this requirement appears to be directed specifically at the La Jolla community fireworks display. But Scripps Park-based fireworks fall well outside of ASBS 29 (one-quarter to one-half mile), and are thus now "located a sufficient distance away." Any Tentative Order would need to make an explicit factual finding (with sufficient evidentiary support) that certain fireworks displays like the Scripps Park fireworks are exempt from this requirement. Otherwise, imprecise and unclear measurements such as "sufficient distance" will only provide litigation fodder for opponents to litigate the La Jolla community display out of existence.

The evidence shows that there are no demonstrable water quality issues associated with occasional public displays of fireworks, yet the proposed Tentative Order will likely enhance the litigation position of opponents of public fireworks displays by providing a platform for citizen lawsuits and force many celebratory displays to be cancelled by regional communities who are wary of risking a costly lawsuit.

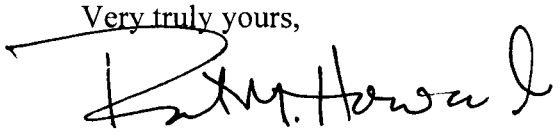
VII. CONCLUSION

In sum, the Tentative Order is a direct threat to Fourth of July community celebrations and other regional fireworks displays. The burdensome monitoring and reporting requirements will drive up overall costs such that most, if not all, local community displays will likely be cancelled. Further, the Regional Board lacks the statutory authority to regulate this activity as a "point source" discharge under the CWA, and all available scientific data supports a finding that occasional fireworks events should be treated as a de minimis source.

We therefore request that the Regional Board withdraw this Tentative Order, or alternatively issue a General NPDES permit exemption for public fireworks displays that occur from the same coastal location between four to ten times a year and/or fall below a reasonable and substantially defensible threshold for the volume of pyrotechnic material to be detonated.

Please feel free to contact me at (619) 236-1234 if you have any questions.

Very truly yours,



Robert M. Howard
of LATHAM & WATKINS LLP

Item No. 6
Supporting Document No. 5

Michelle Mata - Downtown SD fireworks

From: Mike Weinberg <mike.weinberg@cox.net>
To: <mmata@waterboards.ca.gov>
Date: 12/9/2010 11:03 PM
Subject: Downtown SD fireworks

From: Mike Weinberg <mike.weinberg@cox.net>
To: <mmata@waterboards.ca.gov>
Date: 12/9/2010 11:03 PM
Subject: Downtown SD fireworks

Th 12/9/10

To: Michelle Mata
San Diego Regional Water Quality Control Board

I was informed that the Board was accepting comments on fireworks discharges over water.

My wife and I and two small dogs live in a high-rise condo in downtown San Diego's Marina District.

Each summer, we are subjected to fireworks noise and smoke pollution nearly every Friday, Saturday, & Sunday, and sometimes on other days, from the San Diego Pops concerts held at the South Embarcadero behind the Convention Center. These fireworks are launched from a barge on the bay.

On hot summer evenings, we have to keep our windows closed until the smoke dissipates. On occasion, we have to clean up “accidents” in the house from our frightened puppies.

We also see and hear the fireworks from Petco Park after Padres games. On the 4th of July, we can witness 6 or more huge fireworks displays around San Diego Bay, followed by an inevitable cloud of pollutants that hovers for hours. On several other occasions throughout the year, we also see or hear fireworks.

Enough already! Please consider banning or limiting this outrageous excess of fireworks displays in downtown San Diego.

Perhaps laser light shows would be a suitable, non-polluting alternative.
Sincerely,

Mike Weinberg
San Diego, CA
mike.weinberg@cox.net

CITY OF MURRIETA

December 2, 2010

Mr. Dave Gibson
Executive Officer
California Regional Water Quality Control Board
San Diego Region
9174 Sky Park Court, Suite 100
San Diego, CA 92123-4353

RE: COMMENTS TO PROPOSED TENTATIVE ORDER NO. R9-2010-0124, PUBLIC DISPLAY OF FIREWORKS

Dear Mr. Gibson:

The City of Murrieta is pleased to have this opportunity to comment on the Tentative Order R9-2010-0124, regulating public display of fireworks. We have reviewed the draft permit and outlined our comments and concerns below:

1. The City of Murrieta has one firework show around the July 4th holiday at a park site. We do not believe there is any evidence gathered which concludes one firework show per year has a measurable effect on water quality.
2. Public entities with one firework show should be not be subject to the permit requirements. It is our understanding that one cause of the Tentative Order was Sea World which has been allowed to present 150 firework events per year! The Regional Board should not compare cities that have one firework event to a private enterprise that can have 150 events per year. Rather, require a permit for users who have multiple firework events per year.
3. The newly adopted Order R9-2010-0016 (MS4 Permit) already regulates illicit discharges and pollutants. It also requires street sweeping which the City already does. There is no need for another permit which consumes more staff time and revenue.
4. There are a number of the proposed BMP's in the Tentative Order that are related to skimming debris from the shore lines, use of barges, and docks which would not apply to firework displays over land. The other BMP's such as transporting fireworks safely, picking up debris, properly disposing of duds, inspecting the firing range are already required. A permit to detonate fireworks is required and the Fire Department inspects the firing during and after the event. The firework operator searches the firing area twice. Once immediately after the event at night and in the morning the following day. Duds and debris are removed during these two post inspections. **We feel that the Tentative Order should only apply to firework displays over large water bodies** which the City of Murrieta does not have.

If the Board will not exempt cities which have one firework event per year, then we ask that the following be incorporated as a worst case scenario:

- The permit fee should be for the permit term and not annually. This would be $1,452 and not the number of years of the permit term times this amount.
- The language in the Tentative Order should be revised so a city can easily read and know without a doubt if the permit applies or not.
- The Tentative Order should list the exemptions in the permit.

The City of Murrieta is opposed to the Tentative Order. We have not seen scientific evidence that proves one firework event over land is a significant threat to water quality. There should be an exemption for firework events over land, and the current MS4 Permit already provides a mechanism to regulate pollutants.

Respectfully,

CITY OF MURRIETA

RICK DUDLEY
CITY MANAGER

CC: City Council
David Barker, Michelle Mata, San Diego Regional Board Staff

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SAN DIEGO, CALIFORNIA 92106
www.sdcoastkeeper.org
IMPACT

December 9, 2010

Via Electronic Mail to MMata@waterboards.ca.gov
Michelle Mata
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123-4340

Re: Tentative Order No. R9-2010-0124, NPDES Permit No. CAG999002
General Waste Discharge Requirements for Discharges Associated With Public Displays of Fireworks to Surface Waters in the San Diego Region

Dear Ms. Mata and Regional Board members:

San Diego Coastkeeper respectfully submits the following comments on the Regional Water Quality Control Board's proposed general permit for discharges associated with the detonation of fireworks over surface waters in the San Diego Region. Fireworks pose a threat to the region's water quality because they contain harmful chemicals and other pollutants, which are released when detonated over water. The Clean Water Act prohibits discharges of pollutants from point sources into waters of the United States without a permit. 33 U.S.C. § 1311(a). San Diego Coastkeeper supports a strong permit—including monitoring provisions to ensure water quality is protected—for all fireworks discharges over or near waterways.

I. The Clean Water Act Regulates the Discharge of Fireworks into Waters of the United States.

Because fireworks displays occurring over or adjacent to surface water release harmful chemical and solid wastes from a discrete conveyance into those waters, persons seeking to discharge fireworks over or near waterways must first obtain a National Pollutant Discharge Elimination Systems ("NPDES") permit under the Clean Water Act. Section 303(a) of the Clean Water Act prohibits the discharge of any pollutant from a point source into waters of the United States without first acquiring a permit. *See* 33 U.S.C. § 1311(a).

1. Fireworks Are a "Pollutant" Regulated Under the Clean Water Act.

The Clean Water Act defines "pollutants" to include solid waste, chemical wastes, munitions, and municipal waste. *See* 33 U.S.C. § 1362(6). Fireworks are a pollutant because they are chemical waste; they typically contain chemicals such as aluminum, magnesium,

strontium, barium, sodium, potassium, iron, copper, sulfate, nitrate, and perchlorate. *See* California Reg'l Water Quality Control Bd., Addendum No. 1 to Order No. R9-2005-0091, NPDES No. CA0107336 for the Discharge of Waste from SeaWorld Aerial Fireworks Displays to Mission Bay, San Diego. Many of these chemicals pose serious risks to human health and wildflife. *See* N. Irving Sax & Richard J. Lewis, Sr., Dangerous Properties of Industrial Materials (Van Nostrand Reinhold, New York, 1989, 7th ed.); *see also* Richard T. Wilkin, Dennis D. Fine, and Nicole G. Burnett, *Perchlorate Behavior in a Municipal Lake Following Fireworks Displays*, 41 Environmental Science & Technology at 3966 (2007).

Fireworks are also a pollutant because they create solid waste. The cardboard casings, fuses and wires that comprise the fireworks may fall into water as trash after the munitions explode. California Reg'l Water Quality Control Bd. Order No. R9-2005-0091, NPDES No. CA0107336 for the Discharge of Waste from SeaWorld Aerial Fireworks Displays to Mission Bay, San Diego.

Furthermore, fireworks fired by, or on behalf of, a municipality constitute "municipal waste." And because fireworks are shot into the air and explode, they are "munitions" regulated as a pollutant under the Act. Fireworks, therefore, fit at least four definitions of "pollutant" regulated under the Clean Water Act.

2. Fireworks Are Launched from a Point Source.

The Clean Water Act defines "point source" as "any discernible, confined and discrete conveyance…from which pollutants are or may be discharged." 33 U.S.C. § 1362. Any discernable point of land or floating vessel discharging pollutants constitutes a point source under the Clean Water Act. Because fireworks are launched from a ship, boat, or discrete point on land, the detonation of fireworks is regulated under the Clean Water Act.

3. Any Fireworks Displays Over or Near Water Require a Permit.

The Clean Water Act regulates discharges of pollutants from a point source into waters of the United States. The term "waters of the United States" is used to describe most surface waters within the United States, including any waterway used for navigation, rivers, streams, wetlands, and waters affected by the tide. *See* 40 C.F.R. § 230.3(s). Coastal waters and bays such as San Diego Bay and Mission Bay are jurisdictional waters under the Clean Water Act. Therefore, any fireworks discharge over or near jurisdictional waters must be covered by an NPDES permit.

II. The Regional Board Has Acknowledged that the Clean Water Act Applies to Firework Displays over Surface Waters.

In 2007, the Regional Board issued a NPDES permit to Anheuser-Busch Inc., the owners of SeaWorld San Diego, for the discharge of fireworks-related pollutants. *See* California Reg'l Water Quality Control Bd. Order No. R9-2005-0091, NPDES No. CA0107336 for the Discharge of Waste from SeaWorld Aerial Fireworks Displays to Mission Bay, San Diego. The SeaWorld permit acknowledges that the fireworks release pollutants that adversely impact Mission Bay. *Id*. The permit requires SeaWorld to regularly monitor pollutant levels in both water and sediment in the vicinity of the fireworks displays. *Id*.

Water quality monitoring data collected by SeaWorld in its fireworks deposition zone show elevated chemical concentrations. *See SeaWorld Aerial Fireworks Displays 2008 Annual Report Executive Summary* (March 2009) and *SeaWorld Aerial Fireworks Displays 2009 Annual Report and 2010 Work Plan* (January 2010). These elevated pollutant levels can be attributed to SeaWorld's fireworks displays. *Id*. The SeaWorld fireworks permit illustrates that the all fireworks detonation over or adjacent to surface waters falls within the ambit of the Clean Water Act and requires a permit.

III. San Diego Coastkeeper Supports a Protective Discharge Permit Requiring Best Management Practices and Water Quality and Sediment Monitoring.

San Diego Coastkeeper applauds the efforts of the Regional Board to prepare a NPDES general permit for fireworks displays over or adjacent to the region's surface waters. San Diego Coastkeeper appreciates that fireworks shows are a popular way to celebrate holidays such as Independence Day and New Year's Eve, and Coastkeeper does *not* seek to eliminate all fireworks in the region. On the contrary, San Diego Coastkeeper seeks a permitting process that will ensure that fireworks displays over jurisdictional waters do not threaten water quality and that all potential dischargers fully comply with the law.

Coastkeeper urges the Regional Board to resist the pressures of the fireworks purveyors and local politicians to weaken the permit conditions or to eliminate the permit altogether.

Instead, we urge this Board to issue its general permit for fireworks-related discharges into the region's surface waters. Coastkeeper supports protective general permit provisions requiring the implementation of best management practices and mandatory water and sediment monitoring before and after the displays. Thank you for your attention to this matter.

Sincerely,

Nate Hausman
Legal Intern
San Diego Coastkeeper

Jill Witkowski
Staff Attorney
San Diego Coastkeeper

SAN DIEGO PORT TENANTS ASSOCIATION

December 9, 2010

San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123
Attn: Michelle Mata

Via email: mmata@waterboards.ca.gov

RE: Tentative Order No. R9-2010-0124, General Permit No. CAG99902
General Waste Discharge Requirements for the Display of Fireworks

Dear Ms Mata:

The San Diego Port Tenants Association (SDPTA) is a non-profit organization of businesses who lease land along the tidelands of San Diego Harbor. The two underlying objectives of the SDPTA are to collectively represent and promote a united forum of port tenants and advance and promote the common and mutual business interests of its members.
The SDPTA hereby provides the following, in regard to the San Diego Water Board's December 16, 2010 workshop allowing interested persons the opportunity to comment on Tentative Order No. R9-2010-0124:

1. The initial and annual application fee of $1,452 applies to an event sponsor whether one event or multiple events are performed in a yearly period. This seems very unfair to sponsors conducting only one event per year.

2. Clarify that the tentative order does not require or intend to require surface water quality monitoring on a per event basis.

3. The tentative order requires dischargers to either form or join a regional water body-monitoring coalition or conduct individual monitoring. Can the RWQCB provide specific examples of existing regional water body-monitoring coalitions that are currently conducting monitoring and the goals and objectives of these coalitions?

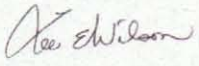
4. The State Water Resources Control Board's Water Quality Control Plan for Enclosed Bays and Estuaries – Part 1 Sediment Quality (Effective August 25, 2010), Section V, Benthic Community Protection guidance is identified as the

5. assessment tool for monitoring surface water (referred to as the Sediment Quality Objective [SQO] guidance). As is stated in the guidance document in Section C. Applicable Waters, "Part 1 does not apply to ocean waters* including Monterey Bay and Santa Monica Bay, or inland surface waters*." This fact needs to be clarified in the tentative order.

6. The tentative order indicates that the number of fireworks displays at Sea World averages between 110 and 120 events per year. The tentative order also states "SeaWorld's sediment chemistry monitoring results show higher levels of pollutants associated with fireworks events within the sediment in the fireworks deposition zone (FDZ), particularly for barium, chromium, cobalt, copper, silver and titanium. Results from short-term survival sediment toxicity sampling were highly variable spatially and temporally within the FDZ, and temporally within the reference sites. Samples in the reference sites and FDZ range from non-toxic to highly toxic making it difficult to detect any difference in short term toxicity. Discharges from SeaWorld's public fireworks events represent the highest level of water and sediment effects because 1) Mission Bay is unique due to the restricted circulation of waters within the bay, the shallow depth of the bay in the vicinity of the fireworks events, and 2) the high frequency of repeat fireworks events throughout the year at the same location results in maximum pollutant loading." Based upon the inconclusive results to date for the multi-year Sea World monitoring program, which is conducted within an area that the RWQCB describes as having the highest potential for fireworks-related water and sediment effects, it is unclear how the proposed integrated SOQ program would be able to identify fireworks-specific impacts to the benthic community in locations that experience a small fraction of the events that Sea World conducts per year, over a wider expanse (e.g., San Diego Bay). Please clarify.

Thank you for your kind attention to our comments. We look forward to meeting you at the workshop.

Sincerely,



Lee E. Wilson
Chairman of the Board

CC: Sharon Cloward, Jim Unger, Sandy Purdon, Craig Anderson, John Lormon, Barry Snyder, Dr. Ray Ashley, Pat Pfohl, Jennifer Ringle, David Gibson

December 5, 2010

David W Gibson
Executive Officer
California Regional Water Quality Control Board
9174 Sky Park Court - Suite 100
San Diego CA 92123

Dear Mr. Gibson,

The San Diego Symphony is asking the Water Quality Control Board to reconsider Tentative Order R9-2010-1024. As you might know, each of the 22 subscription performances of our Summer Pops series concludes with an approximately three-minute choreographed fireworks display over San Diego Bay. As an organization, we are concerned about the financial effect the Tentative Order will have on the cost to our performances.

The San Diego Symphony is the oldest orchestra in the state of California; currently celebrating our Centennial Season. As a non-profit organization, we rely heavily on public contributions, as we only receive less than 30% of our operating budget from ticket sales. More than one third of our ticket sales are generated during the 11 week Summer Pops series.

The fireworks display is a key aspect of the total Summer Pops experience. Feedback from our patrons indicates that many who purchase tickets to the summer season are swayed by the experience of enjoying the fireworks display in conjunction with a beautiful night of orchestral music.

Tentative Order No R9-2010-0124 includes a monitoring program that would increase the overall cost of a fireworks display to the point that we fear that the San Diego Symphony would be fiscally unable to continue to include them as part of our series.

We believe that discontinuing pyrotechnics as part of our concerts would change the Summer Pops experience and result in a sizable drop in ticket sales, potentially putting the Summer Pops series itself at risk. Therefore, we are asking that the Water Board reconsider Tentative Order R9-2010-1024.

Respectfully,

SAN DIEGO SYMPHONY

Edward B. Gill

Edward B. Gill
Executive Director

1245 SEVENTH AVENUE, SAN DIEGO, CA 92101
TEL. 619.235.0800 FAX 619.235.0005 WWW.SANDIEGOSYMPHONY.COM

B. Kelley SW

Item No. 6
Supporting Document No. 5

SAN DIEGO REGIONAL WATER QUALITY CONTROL BOARD

2010 DEC 13 A 9:34

December 10, 2010

Mr. David Gibson, Executive Officer
San Diego Regional Water Quality Control Board
9174 Sky Park Ct., Ste. 100
San Diego, CA 92124

Subject: Response to Tentative Order No. R9-2010-0124, NPDES No. CAG999002
General Waste Discharge Requirements for the Public Display of Fireworks

Dear Mr. Gibson:

The Laguna Beach City Council voted unanimously on November 16, 2010 to seek an exception to a potential prohibition of a municipal firework displays each Fourth of July over Heisler Park. Some of the key reasons that a once-a-year exception should be supported by the Regional Board are:

- ***Facilitates fire safety.*** The public fireworks display lasts about 20 minutes each year to celebrate Independence Day. The public event provides an alternative to private parties discharging fireworks elsewhere in the City.

- ***Reduces the risk of more serious discharges.*** Public fireworks displays reduce the risk of private events sparking fires that result in discharges to ocean waters. The runoff from a single structure fire or wildfire is much more problematic than a brief annual fireworks show.

- ***No evidence of significant impacts.*** There is no evidence to demonstrate that the 20 minute annual fireworks show has ever caused in any measureable or significant difference in ocean water quality.

- ***Narrow Exception Requested.*** Laguna Beach is a small coastal community that is surrounded by open space. In 1993, wildfires destroyed more than 360 homes in town. Although Heisler Park is near an Area of Special Biological Significance, it is also the only location in town that provides a safe venue for fireworks be providing a safe distance from homes, businesses, and more than 12,000 acres of open space. The City is only asking for a narrow exception, once-a-year, for Heisler Park.

The City of Laguna Beach fully supports the Regional Board and has worked consistently and diligently to improve coastal water quality. Fireworks have been launched annually at Heisler Park for more than 20 years, yet ocean water in Laguna Beach is among the highest quality in the State. For all of the aforementioned reasons, the City of Laguna Beach requests that the Regional Board develop an annual exception for public agencies to launch fireworks solely on the Fourth of July.

Sincerely,

Toni Iseman

Doc Scanned On: 12/14/10
A.Ernst Time: 10:40

Toni Iseman, Mayor

505 FOREST AVE. • LAGUNA BEACH, CA 92651 • TEL (949) 497-0378 • FAX (949) 494-1864

22ND DISTRICT AGRICULTURAL ASSOCIATION
State of California

December 13, 2010

Mr. David Gibson, Executive Director
Regional Water Quality Control Board
9174 Sky Park Ct # 100
San Diego, CA 92123-4353

Re: 22nd District Agricultural Association / Del Mar Fairgrounds Comments on Proposed Tentative Order R9-2010-0124 General Waste Discharge Requirements Associated with The Public Display of Fireworks to Surface Waters Within The San Diego Region

Dear Mr. Gibson:

On behalf of the 22nd District Agricultural Association / Del Mar Fairgrounds, thank you for the opportunity to comment on proposed Tentative Order R9-2010-0124 regarding the public display of fireworks. The Del Mar Fairgrounds conducts only a single fireworks display on the Fourth of July every year during the San Diego County Fair, which is held every summer and draws over one million visitors annually. The Del Mar Fairground's annual Fourth of July fireworks display has been held continuously for over 62 years. We are concerned that the Tentative Order threatens to end an important tradition thrilling thousands of San Diego County citizens every year.

The Del Mar Fairgrounds is unaware of any evidence that its annual show has caused any degradation in water quality. The fireworks display does not occur over a surface water body regulated by the Regional Board. In fact, the nearest water bodies regulated by the Regional Board are more than 1,000 feet away, exceeding the 'fall out range' of any debris. [1]

Nonetheless, the extensive permitting, monitoring and BMPs proposed in the Tentative Order has the potential to make the economics of the Del Mar Fairgrounds' single annual fireworks display cost- prohibitive. We are advised that several people have estimated the water and sediment monitoring alone could cost between $30,000 and $100,000 per event, depending on the amount of testing required. Because the Del Mar Fairgrounds' annual fireworks display costs approximately $25,000, the proposed Order's Requirements have the potential to at least double the cost.

[1] Although there are two small inland lakes at the Del Mar Fairgrounds, it is our understanding these are not regulated by the Regional Board.

2260 Jimmy Durante Boulevard • Del Mar, California 92014-2216
TELEPHONE: (858) 755-1161 • FAX: (858) 755-7820
www.sdfair.com

Mr. David Gibson, Executive Director
Regional Water Quality Control Board
December 13, 2010

We respectfully request that the Regional Board reconsider Tentative Order R9-2010-0124 in light of this information or, alternatively, amend the tentative order to exempt entities hosting only one or two annual fireworks displays conducted by licensed professionals.

Sincerely yours,

22nd District Agricultural Association

Rebecca Bartling,
COO/Deputy General Manager, 22nd DAA

Item No. 6
Supporting Document No. 5

CLEAN WATER NOW! COALITION
P.O. Box 4711, Laguna Beach, CA 92652 - 949.280.2225 - www.cleanwaternow.com

"The Clean Water Now! Coalition is dedicated to the protection, restoration and preservation of aquatic and riparian ecologies worldwide."

To: Michelle Mata
Water Resource Control Engineer
Core Regulatory Unit
SDRWQCB

Date: December 14, 2010

Re: NPDES Regulation of Public Display of Fireworks

Michelle:

The Coalition welcomes the opportunity to provide comments regarding its position in this matter.

We have followed this issue for several years, and wish to go on the record now as deeply appreciative of the incredible work product on this topic already, as provided by Marco Gonzalez of Coast Law Group. Per usual, Marco continues to be a beacon and lightning rod for water quality impairment and compliance remedies.

Our concerns presently are multitudinous, but we feel that there is a definite inextricable nexus between the NPDES permitting process, the MLPA State Marine Reserve areas (SMR) and SWRCB designated Areas of Special Biological Significance (ASBS). Fireworks displays must be viewed with these constructs in the foreground.

We wish to point out how acutely we feel the imminent announcement of re-configured or newly designated SMR listings by Cal Fish & Game in the MLPA now being considered by the State should affect the San Diego Board's decision-making process in the fireworks matter. When a SMR also enjoys ASBS status then the proposed protection and enforcement levels become markedly greater.

Here in our hometown of Laguna Beach, Heisler Park (Main Beach) carries both designations, ergo in or near that zone the SDRWQCB should prohibit fireworks. Due to the air and water borne migration of contaminants, the inherent technical difficulties for containment of said pollutants, an enlarged buffer zone should be part of the enjoinment.

When and where endangered and/or threatened species are present the protection should be more prominent and strident. If the public fireworks displays performed presently are within SMR and/or ASBS then they should sunset, be phased out, that is cease. Period.

For the past several years we've attempted to convince SDRWQCB staff that the presence at the Point of Discharge or in the oceanic migration corridor for Endangered Species Act aquatics, or even those listed as threatened, should automatically trigger an amendment to that watershed's Basin Plan Objectives (BPO). Contemporaneously we have petitioned the SWRCB staff contact for ASBS to integrate our reasoning into future ASBS designation considerations.

We have primarily focused upon ***O. mykiss***, the Southern Steelhead. It is our position that ALL tributaries and their POD vicinities that are part of the Southern California Designated Population Segment for the steelhead should get the highest priority, the greatest level of protection.

There is a definite need for a master plan or at least mandatory inter-agency collaboration, a systematic approach to create consistency and uniformity, that honors or bundles all of the concepts mentioned. The Coalition is very frustrated that our petitions are hitting walls or being held up due to the unnecessary AND non-compliant truncated/segmented situation occurring presently. The SDRWQCB has created a BPO Task Force or TAC recently but it is populated by a dominant, 99% chronic violator constituency, hence neutered at its inception.

We didn't conjure the idea of such a master management plan approach out of thin air, it is an integral element in CF&G laws and ESA requirements themselves, embedded in the ASBS prescriptions too. We do feel that the SWRCB needs to close the loop, connect the dots as it were to be consistent and in compliance with both State and federal regulations. It makes no sense, legally or biologically, to NOT make it an immediate priority, post haste.

In the late 1990's the SDRWQCB initiated the first steps in such a strategy by amending the BPOs for San Mateo and San Juan Creeks when the steelhead entered the ESA listing. Unfortunately, the Board, its parent SWRCB and CF&G failed to take the next logical step: All PODs for tributaries that are designated steelhead habitat (or those that are considered for recovery/recolonization) should be immediately listed as SMR and carry the ASBS protection status.

The Coalition profoundly believes and will continue to petition the Board and staff to amend ALL of the BPOs in its jurisdiction to comply with the conditions that are optimally conducive for said species presence and/or return. The SDRWQCB, as the local agency for the SWRCB, is the logical choice to assist, to facilitate a joining of prescriptions regionally. Just as watersheds are continuums and treated

as such by EPA, so too should near-tidal habitats be seen as a gestalt, as a coherent totality.

As for the fireworks displays, it would seem a no-brainer that at any POD or in the immediate vicinity of such a POD that is identified ESA or threatened habitat should also enjoy ASBS. Moreover, it should be axiomatic that there be a 100% ban on any and all fireworks displays as well in or near such SMR/ASBS zones.

As an enviro-consultant specializing in hydrology and water quality I know about the negative effects that Pollutants of Persistence (POP) and Contaminants of Concern inflict. Fireworks are not just a short-term, insignificant impairment, insular incidences or impairments. Their adverse impacts, especially upon sediment, are long-term, they cascade throughout marine eco-systems and water columns, not to mention impose sustained bio-magnification effects.

Respectfully submitted,

Roger von Bütow

Roger von Bütow Founder & Executive Director
Clean Water Now! Coalition (Established 1998)
P.O. Box 4711 Laguna Beach CA 92652
Home Office: (949) 715.1912 (Voicemail AFTER 6 rings)

Cal Coastal Commission Volunteer Beach Cleanup Information/Messages (949) 280.2225
Website: www.cleanwaternow.com
"The Clean Water Now! Coalition is dedicated to the protection, restoration and preservation of aquatic and riparian ecologies worldwide."

"Friends of the Aliso Creek Steelhead": www.alisocreeksteelhead.org

A Proud Community Affiliate
of "KEEP CALIFORNIA BEAUTIFUL": www.keepcaliforniabeautiful.org

COASTAL ENVIRONMENTAL RIGHTS FOUNDATION

December 15, 2010

Attn: Michelle Mata
San Diego Regional Water Quality Control Board
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Via Electronic Mail
mmata@waterboards.ca.gov

Re: Tentative Order No. R9-2010-0124, NPDES Permit No. CAG999002
General Waste Discharge Requirements For Discharges Associated With Public Displays of Fireworks To Surface Waters In The San Diego Region

Dear Ms. Mata and Regional Board members:

Please accept the supplemental documents submitted on behalf of the Coastal Environmental Rights Foundation (CERF) in strong support of the proposed water quality protections to be provided upon adoption of Tentative Order No. R9-2010-0124 by the Regional Water Quality Control Board.

Thank you for your inclusion of these additional materials relevant to the discussion at tomorrow's public workshop and consideration of the proposed permit conditions.

Sincerely,

COASTAL ENVIRONMENTAL RIGHTS FOUNDATION

SARA S. HONADLE
Programs Director

Encl.:

- "Changes in Forced Expiratory Flow Due to Air Pollution from Fireworks" Environmental Research 9, 321-331 (1975)
- "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization" EPA Office of Research and Development (NCEA-1-0503) Jan. 16, 2002
- "Perchlorate: Not Only Rocket Science" The Environmental Form, Vol 27, No. 3 May/June 2010
- "Particulate Oxidative Burden Associated with Firework Activity" Environmental Science & Technology, 2010, 44 8592-8301
- "Weighing fireworks' effects" Concord Monitor July 3, 2010

ENVIRONMENTAL RESEARCH 9, 321–331 (1975)

Changes in Forced Expiratory Flow Due to Air Pollution From Fireworks

Preliminary Report

RICHARD MERRILL SMITH

Department of Physiology, University of Hawaii, School of Medicine, Honolulu, Hawaii 96822

AND

VU-DINH DINH

Department of Geography, University of Hawaii, Honolulu, Hawaii 96822

Received February 6, 1974

Spirometry in humans and air sampling have been performed during a brief but intense bout of air pollution due to exploding fireworks on New Year's Eve in Honolulu, Hawaii. An integrating nephelometer recorded a peak concentration of respirable particles in excess of 3.8 mg/m³. X-ray dispersive analysis, combined with scanning electron microscopy of particles collected with an Anderson cascade impactor, indicated that most of the small particles were probably crystals of KCl. Conditions were ideal for a possible SO_2–KCl aerosol synergism which may have led to the measured changes in maximal midexpiratory flow ($FEV_{25-75\%}$). Two male subjects with a history of chronic respiratory disease experienced an average decrease of 26% in $FEV_{25-75\%}$ when compared to that measured the previous night. Three normal males experienced a 4.7% decrease approaching statistical significance ($0.1 > P > 0.05$), but the overall difference in $FEV_{25-75\%}$ in healthy male and female subjects combined was not statistically significant. Thus, while susceptible people may be measurably affected, the general population of Honolulu probably experienced little, if any, change in $FEV_{25-75\%}$ with the air pollution levels reported here.

INTRODUCTION

Each New Year's Eve the people of Honolulu are exposed to a brief but intense bout of air pollution characterized by increased SO_2 and particulate matter of unknown composition as a result of the legal burning of fireworks on private property. Especially under conditions of low wind velocity, the smoke from the possibly more than $1 million worth of fireworks markedly decreases the visibility in Honolulu (Bach *et al.*, 1972). In a recent survey of 175 patients suffering from chronic respiratory disease, 68% reported that they took special precautions at home to avoid the smoke and 73% found it more difficult to breathe during the fireworks.[1] In the only published study of possible adverse health effects due to one of these severe air pollution episodes, Bach *et al.* (1972)

[1] Houghtby, G. Director, Health Education. Hawaii Thoracic Society. Personal Communication, 1974.

reported that while mortality data were unchanged, five Honolulu hospitals experienced a 113% increase in emergency room visits due to respiratory distress in people with a history of chronic respiratory disease.

Other than hospital admissions, no data exist concerning possible adverse effects of fireworks air pollution on people with or without a history of chronic respiratory disease. The present study was performed on the New Year 1972–1973 to determine if acute changes in forced expiratory flow occurred in healthy residents of Honolulu exposed to the fireworks smoke. In addition, the instantaneous respirable particle concentration and 18 hour average concentration, size distribution, and composition of the particulate matter produced by burning fireworks are presented, together with selected scanning electron microscope (SEM) photomicrographs of the particles.

MATERIALS AND METHODS

Pulmonary Function

Some physical characteristics of the subjects used in this study are presented in Table 1. Of the nine acceptable subjects, all except one smoker (subject 5) were near normal limits on control tests of forced vital capacity (FEV), one sec-

TABLE 1
PHYSICAL CHARACTERISTICS AND RESPIRATORY DISEASE HISTORY OF SUBJECTS

Subject	Sex	Age	Height (meters)	Weight (kg)	Control measurements (Percent of predicted)[a] FEV	$FEV_{25-75\%}$	FEV_1	History of adult respiratory disease and comments
1	M	38	1.58	61.4	104.9	82.8	94.0	Allergic asthma (Drixoral medication). Gas trapping in control spirogram.
2	M	27	1.82	79.5	108.8	128.2	94.2	Hospitalized for recurrent bronchitis at age 21. Gas trapping in control spirogram.
3	M	23	1.90	79.5	131.2	124.0	99.3	None
4	M	24	1.81	75.0	121.5	146.3	98.1	None, competitive swimmer.
5	M	44	1.72	70.0	107.2	62.5	82.9	None, smokes pipe and 1–10 cigarettes per day for 17 years.
6	F	26	1.90	68.2	114.0	118.6	99.1	None, childhood asthma.
7	F	18	1.52	42.3	95.7	—	100.0	None, childhood asthma.
8	F	26	1.70	56.8	99.4	111.2	94.3	None
9	F	27	1.73	56.8	118.5	165.0	113.0	None, smokes infrequently.

[a] Calculated from the following equations obtained from normal standing subjects (Altman and Dittmer, 1971): FEV: 0.052 Height—0.022 Age—3.60 (Males); 0.052 Height—0.018 Age—4.36 (Females); $FEV_{25-75\%}$: 5.85—0.0523 Age (Males); 5.63—0.0549 Age (Females); and FEV_1: 0.201 Height—0.400 Age + 129 (Males); 0.253 Age + 93 (Females).

ond forced expiratory volume (FEV_1), and maximal midexpiratory flow rate ($FEV_{25-75\%}$), measured on January 20, 1973. However, a detailed personal history filled out by each subject indicated that male subjects 1 and 2 had a history of chronic respiratory disease persisting to adulthood. Both of these subjects had control spirograms indicative of gas trapping at the end of forced expiratory maneuvers. Since none of the other subjects (3–9) exhibited marked gas trapping or a history of chronic respiratory disease, they were treated separately from subjects one and two.

Measurements of pulmonary function in the standing position were performed between 11:35 PM and 12:30 AM on three occasions. The first two took place in the open garage of a home in residential Manoa Valley (about two miles from the H-1 highway). This site is about 200 feet above sea level and is 80 feet above the valley floor. Two Collins spirometers (9 and 13 1) and the air sampling instruments were set up about 15 and 20 feet from the curb, respectively. Unfortunately, between the first control measurements on December 30 (called Control I) and the experimental measurements on December 31, a cold front passed over Hawaii and the air temperature (T_a) measured at the experimental site fell from 20 to 15°C. Therefore, we performed a second control experiment (Control II) at a T_a of 15°C well after the air pollution episode in order to separate possible temperature effects from air pollution effects. We used a large climate-controlled chamber (Forma Scientific, Inc.) to produce the 15°C air.

On all three testing occasions the subjects arrived at the measurement site at least 30 min before testing began. The subjects' activity before arrival was not restricted except to refrain from heavy drinking or exercise. No smoking was allowed for 30 min before testing began. Each subject performed three to six forced expiratory maneuvers into the same Collins spirometer on each occasion under the direct supervision of one of us. Encouragement was given to ensure that each expiratory maneuver began at total lung capacity and ended at residual volume, and the suggestions of Hyatt, Schilder, and Fry (1958) were followed. All of the curves obtained on each occasion were analysed for FEV, FEV at 1, 2 and 3 seconds, and $FEV_{25-75\%}$. The FEV measure used for each subject is the largest of the several recorded on each occasion and the $FEV_{1,2,3}$, are taken from the same curve and are expressed as % FEV. The $FEV_{25-75\%}$ is the maximal value obtained from several efforts so long as this FEV was at least 90% as large as the largest FEV recorded on that occasion. In exactly one-half of the FEV curves used in this paper, the maximal FEV curve also yielded the maximal $FEV_{25-75\%}$.

All gas volumes were measured at ambient temperature (T_a) and converted to BTPS assuming a body temperature of 37°C. Since T_a changed significantly between testing dates, we tested that the gas in the spirometer bells after several forced expirations at 20 and 15°C was at T_a, using small YSI thermistors in the bells. In both 15 and 20°C air, repeated rapid FEV maneuvers did not cause the bell air temperature to deviate significantly from T_a.

Air Sampling

The air-borne mass concentration of particulate matter was determined with an Andersen (nonviable) cascade impactor (Anderson 2000, Inc.). Air was sucked through the eight stage sampler at 1.5 ft^3/minute (cfm) and the particles were

collected on preweighed thin aluminum foil disks 3.125 in. in diameter and 0.25 mil in thickness. The foil disks were handled only with forceps and were weighed on a Cahn electrobalance with a stated accuracy of ± 2 μg within the required weighing range of 20 mg. After the gravimetric determination, the aluminum disks were cut into small sections, mounted on blocks, and shadowed with gold or palladium for observation with a Cambridge S_{24}-10 SEM. Of the some 400 impaction deposits per stage, 4 to 5 deposits were scanned and representative areas were photographed. The SEM is equipped with an EDAX X-ray dispersive analysis attachment which allowed us to determine the partial elemental composition of the smoke particles.

Instantaneous air particulate concentrations were estimated with an integrating nephelometer (Meteorology Research, Inc.) which measures the concentration of "respirable" particles from 0.2 to 1.2 μm in diameter (roughly comparable to stages 5, 6, and 7 of the Andersen sampler). The amplified nephelometer output was continuously recorded on a strip chart recorder (Hewlett-Packard, Model 680) with a chart speed of 0.07 in./minute. The air was sampled at 10 cfm. The nephelometer was zeroed immediately before each run, using filtered dry air, and the manufacturer's conversion was used to convert scattering coefficient to average mass concentration. The relative accuracy of the scattering coefficient is about 4% (Porch, Charlson and Radke, 1970), but we did not attempt an error estimate of the absolute concentrations reported here.

RESULTS

Air Sampling

The time course of the air pollution due to fireworks on New Year's Eve as measured by the nephelometer in Manoa Valley, Honolulu is shown in Fig. 1. At 8 PM the average mass concentration was already about 150 $\mu g/m^3$ due to sporadic explosions of fireworks (Fig. 1,a). At about 11:35 PM, when we began the pulmonary function tests, the concentration increased sharply, exceeding the 380 $\mu g/m^3$ level, and forcing the change of scale on the nephelometer (Fig. 1,b). During the fifteen minutes before midnight the pollution level increased from less than 1 mg/m^3 to 3.8 mg/m^3 and continued beyond the nephelometer measurement capability. Fifteen minutes after midnight the concentration fell low enough to be measurable and continued to fall rapidly so that the lower scale could be used at 12:30 AM, by which time we had completed the pulmonary function tests. One hour later the concentration of respirable particles in the air had returned to near normal values for Honolulu. During the half hour around midnight when most of our pulmonary function testing was done, the average concentration was at least 2.15 mg/m^3.

The 18 hour average concentration and size distribution of suspended particulate matter in the air on Control I and New Year's Eve is shown in Table 2. The Control I run of the Andersen Sampler which started at 6 PM on December 30 and ended at 12 AM on December 31 yielded an average concentration of 20 $\mu g/m^3$, with a fairly even distribution among the stages except for stage 2 which contributed relatively more than the others. The experimental run covering the New Year's celebration which started at 8 PM on December 31 and ended at 1 PM on January 1, yielded a value of 113 $\mu g/m^3$. This increase of more than five times

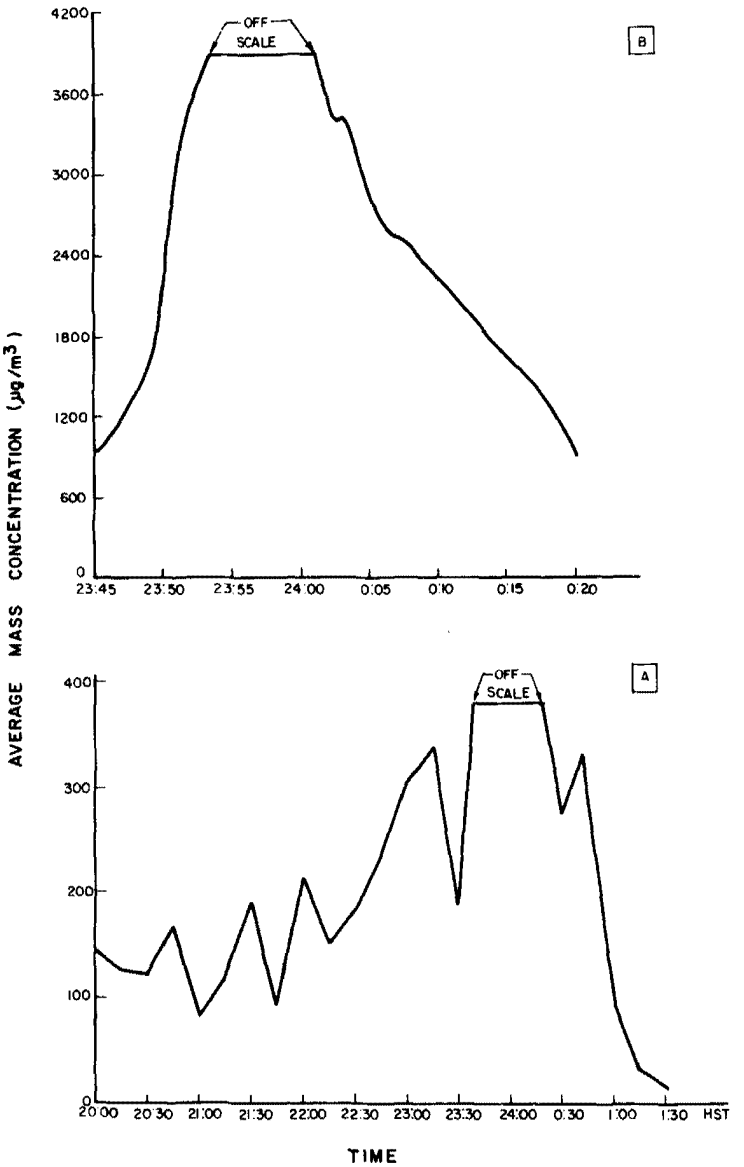


FIG. 1. Fifteen (a) and five (b) minute average mass concentration of respirable particles measured on December 31, 1972–January 1, 1973 with an integrating nephelometer.

over the previous day's value was mainly due to a sharp increase in the smaller particles collected on stages 5, 6, and 7. This result, which tends to corroborate the nephelometer result, indicates that most of the air pollution due to fireworks is composed of particles between 0.3 and 2.0 μm (or smaller); these particles are known to penetrate and be retained by the lung (Dennis, 1971).

All stages of the Andersen samples collected on December 31–January 1 were viewed with the scanning electron microscope, but only selected results from stages 0, 2, 4, 6 and 7 are presented in Fig. 2. Stages 0, 2, and 4 (Fig. 2: a, b, c)

TABLE 2
SIZE DISTRIBUTION OF SUSPENDED PARTICULATE MATTER IN HONOLULU ON THE 1972–1973 NEW YEAR

Andersen impactor stage	Aerodynamic particle diameter by stage[a] (μm)	Weight ($\mu g/m^3$) Sample 1 December 30–December 31	Weight ($\mu g/m^3$) Sample 2 December 31–January 1
0	>8,8	2	5
1	5.6 -8.8	1	4
2	3.8 -5.6	7	7
3	2.7 -3.8	3	10
4	1.7 -2.7	2	7
5	0.84–1.7	1	14
6	0.52–0.84	2	40
7	0.34–0.52	2	28
	Total	20 $\mu g/m^3$	113 $\mu g/m^3$

[a] Normal sampler flow rate is 1 cfm. We used 1.5 cfm and estimated size distribution for an impaction efficiency of 50% by stage at this flow rate from a calibration chart provided by Carl H. Erickson, Director of Engineering, Anderson 2000, Inc.

show that most of the larger particles collected (about 28% by weight of the total collected) are unidentified fungal spores, and explains why stages 0–4 did not show a marked weight change from December 30 to December 31. In addition to the large biological particles, large crystals were seen in stages 2–4 (Fig. 2, b).

Most of the material collected on stages 5–7 was composed of crystals and unidentified amorphous material (Fig. 2: d, e). X-ray microanalysis of single crystals from stage 7 (Fig. 2, f) indicated the presence of four major energy peaks representing from left to right the following elements: aluminum, gold, chlorine, and potassium. The flash powder used in fireworks is composed of about 25% aluminum powder.[2] However, the particles are deposited on aluminum disks, so it is impossible to know how much of the aluminum peak is due to particulate aluminum from the air sample and how much is background due to the aluminum disk. The gold peak is due to coating the particles with gold. The chlorine and potassium peaks indicate that much of these crystals is KCl. However, energy emission counts from two different crystals from stage 7 (shown as solid bars and dots respectively in Fig. 2, f) indicate that the potassium and chlorine do not always appear in constant proportions, so that other salts of potassium may be present. The crystals were hygroscopic since they deliquesced in an atmosphere saturated with water vapor at 24°C.

Pulmonary Function

The results of the pulmonary function tests are shown in Table 3. Two subjects with a history of adult respiratory disease showed a significant decrease in

[2] Miller, D. C. Bureau of Compliance, Consumer Product Safety Commission. Washington, DC 20016. Personal Communication, 1973.

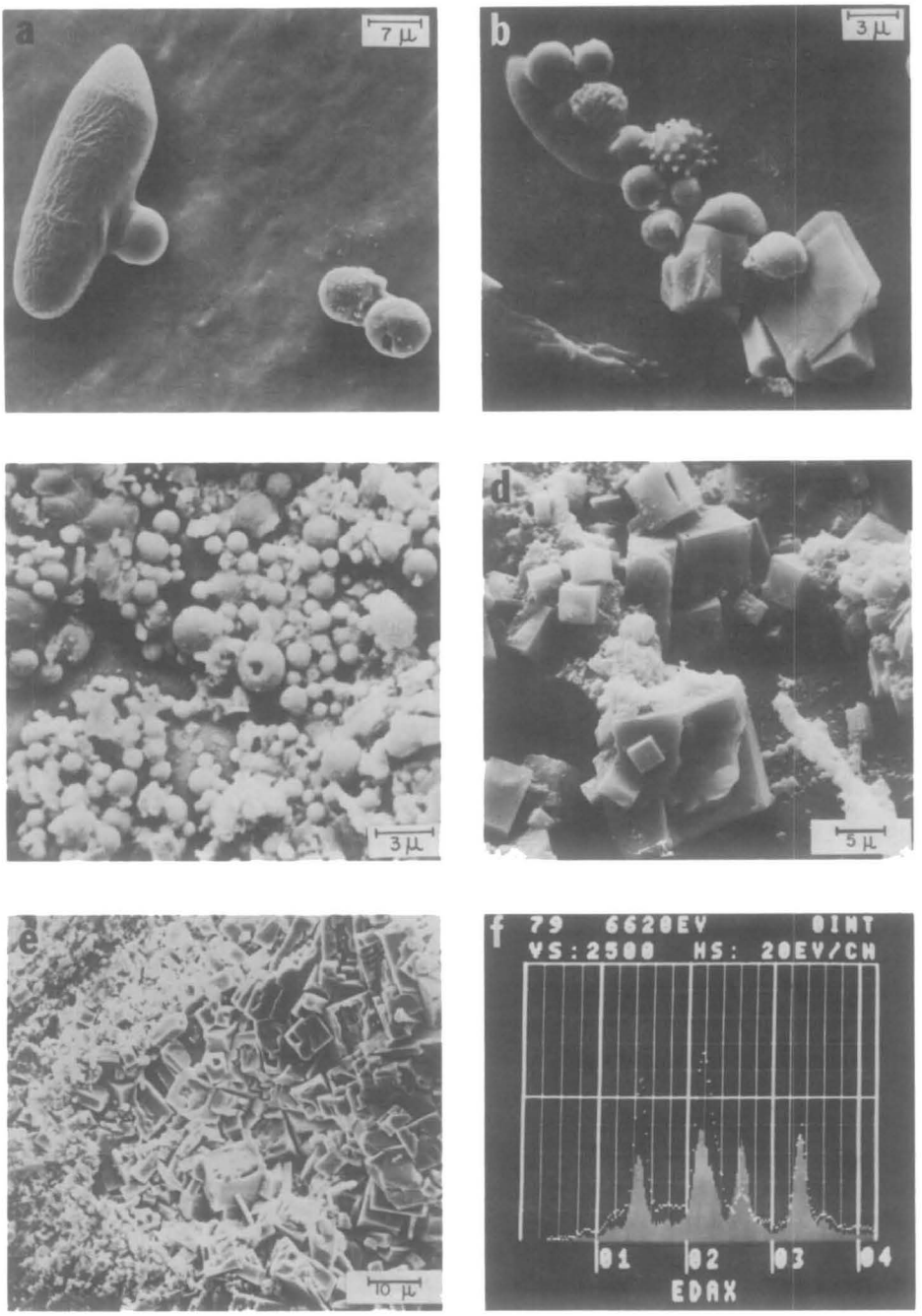


FIG. 2. Photographs of particles collected with an Andersen sampler in Honolulu on December 31, 1972—January 1, 1973, and viewed with a scanning electron microscope; a, × 1440; b, × 3240; c, × 3000; d, × 2000; e, × 1125. Fig. 2, f is an energy count from crystals shown in 2, e. See text for description.

TABLE 3
COMPARISON OF FEV MEASUREMENTS BEFORE AND DURING FIREWORKS

Subjects and analysis[a]	December 30–31, 1972			December 31–January 1, 1972–73		
	FEV (1)	FEV_1 (% FEV)	$FEV_{25-75\%}$ (1/sec)	FEV (1)	FEV_1 (% FEV)	$FEV_{25-75\%}$ (1/sec)
History of disease						
1	3.770	76.4	2.818	3.699	74.1	2.366
	3.754	77.0	2.423	3.504	74.8	2.297
2	4.560	77.0	5.108	4.318	75.6	3.290
	4.438	80.5	4.193	4.264	59.1	2.807
$\bar{x}$	4.131	77.7	3.636	3.946	70.9	2.690
$\bar{d}$	0.184	6.83	0.945			
P	$0.025 > P > 0.01$	$0.15 > P > 0.1$	$0.05 > P > 0.025$			
Normal males						
3	6.271	80.9	5.326	5.906	79.1	5.208
4	6.469	81.4	6.678	6.203	85.1	6.277
5	4.096	63.7	2.197	3.947	68.0	2.058
$\bar{x}$	5.612	75.33	4.734	5.352	77.40	4.514
$\bar{d}$	0.260	−2.07	0.219			
P	$0.025 > P > 0.01$	NS	$0.10 > P > 0.05$			
Normal females						
6	3.858	85.6	4.237	3.783	86.2	4.552
7	2.889	88.3	3.542	2.728	84.7	3.140
8	4.217	81.5	4.335	4.044	85.5	4.448
9	4.471	96.8	5.724	4.560	88.8	5.580
$\bar{x}$	3.859	88.05	4.460	3.779	86.3	4.430
$\bar{d}$	0.080	1.75	0.030			
P	$0.15 > P > 0.1$	NS	NS			
Combined normal males and females						
$\bar{x}$	4.610	82.6	4.577	4.453	82.5	4.466
$\bar{d}$	0.157	0.114	0.111			
P	$0.025 > P > 0.01$	NS	$0.15 > P > 0.1$			

[a] One-tailed paired design t test ($df = n - 1$); NS, $P < 0.15$; P values > 0.15 are indicated.

the two highest FEV and $FEV_{25-75\%}$ measurements between control and New Year's Eve. The FEV_1 did not show a significant change. $FEV_{2,3}$ were nearly identical on the two nights for all subjects and have not been included in the table.

Seven normal subjects had a significant reduction in the FEV, but no change in the FEV_1. The $FEV_{25-75\%}$ decreased but not to generally accepted levels of statistical significance. While each of the three normal males showed a reduction in $FEV_{25-75\%}$ which approached statistical significance ($0.10 > P > 0.05$), two of the four females showed an increase. Among the normal males, it is interesting to note that the fit young swimmer (subject 4) and the older sedentary smoker (subject 5) both experienced a 6% decrease in $FEV_{25-75\%}$.

DISCUSSION

Forced Expiratory Volume

We suspected that our most consistent finding, a decrease in FEV, was due to the 5°C drop in environmental temperature between December 30 and December 31, since very high concentrations of particulate matter are known to have very little effect on pulmonary compliance (Dubois and Dautrebaude, 1958). We tested this hypothesis on January 20, 1973 in an environmental chamber preset to 15°C. A 48-hour run with the Andersen sampler indicated no measurable particulate matter in the chamber. The two maximal FEV efforts for the two subjects with a history of adult respiratory disease were the same on New Year's Eve and on Jan. 20 ($\bar{d} = 0.0026$, $t = 0.05$). Similarly, the seven normal subjects showed no significant difference between these two test dates ($T_a = 15$°C): $\bar{d} = 0.0442$, $t = 1.120$, $.4 > P > .3$. However, for all 9 subjects combined, there was a nearly statistically significant decrease from Control I (20°C): $\bar{d} = 0.111$, $t = 1.78$, $.1 > P > .05$). Thus, we conclude that the significant change in FEV between December 30 and December 31 (Table 3) was probably due to temperature and not fireworks smoke. The effect of cold to reduce the FEV is explicable if peripheral vasoconstriction induced a blood shift of about 150 ml into the pulmonary circulation, displacing an equal volume of gas. While no subjects felt cold in 20°C air, most complained of the cold in 15°C air.

Forced Expiratory Flow

This study presents the first direct evidence that fireworks air pollution in Honolulu can alter pulmonary function in susceptible people (Table 3, subjects 1 and 2). This result was not due to the 5°C decrease in T_a between Control I and New Year's Eve since the $FEV_{25-75\%}$ was also significantly ($P < 0.05$) larger on Control II than on New Year's Eve. Moreover, we know of no data which would indicate that the slight change in RH between December 30 and December 31 (75% and 82% RH, respectively) could have caused the 26% reduction in $FEV_{25-75\%}$ seen in subjects 1 and 2. Thus, the most likely explanation for this observation is the smoke from burning fireworks. Such a result was anticipated in light of clinical observations and a published report on hospital admissions for chronic pulmonary patients on New Year's Eve (Bach *et al.* 1972).

Although five of the seven healthy subjects tended to show a decrease in $FEV_{25-75\%}$ from December 30 to December 31, the average change was not significant (Table 3). Thus, the population of Honolulu unburdened by chronic respiratory disease probably experiences little, if any, change in $FEV_{25-75\%}$ with the pollution levels reported here. However, Frank *et al.* (1962) exposed humans to 4–15 ppm SO_2 and also found a negligible change in FEV_1 and only a 7% decrease in peak flow rate while the resting tidal flow resistance increased 89%. They suggest that the deep inspiration required for all FEV tests may reverse the small airway narrowing detectable during quiet breathing. Thus, the possibility exists that a more sensitive test of airway resistance would have disclosed a significant change in the healthy subjects in the present study.

At 80 feet above the valley floor, our subjects may not have been exposed to the highest concentration of smoke, which tends to settle in the valleys in the absence of trade winds. The wind velocity at 11 PM on December 31 was about 4 mph, (anonymous, 1972) which is well below the average of 8–11 mph for Honolulu. Bach *et al.* (1972) used the same nephelometer the previous year on the floor of Palolo Valley, adjacent to Manoa Valley. They found the lower scale limit (380 $\mu g/m^3$) was exceeded before 9 PM and for most of five hours remained above this limit. That year the wind velocity was 2 mph or less and the RH about 90%. Thus, the smoke concentrations reported here were probably less than those of the previous year and may have been less than that in other areas during the 1972–73 New Year. However, even assuming that higher smoke concentrations may have existed and that many susceptible people were affected, the significance of an acute reduction in $FEV_{25-75\%}$ with respect to longer-term health remains unknown.

The combustion products of fireworks taken alone are probably not highly toxic. The typical compostion of the flash powder formulated in Hong Kong and used in fireworks exploded in Hawaii is: aluminum powder, 25%; potassium chlorate, 45% (potassium perchlorate may also be used); and sulfur, 30%.[2] Thus, we expected SO_2 gas, KCl crystals, and oxides of aluminum to be produced. The average SO_2 level from 10 PM to 2 AM December 31–January 1 was 108 $\mu g/m^3$ at the fifth story level in downtown Honolulu.[3] These levels of SO_2 (0.04 ppm) and particulate matter (up to 4.0 mg/m^3) are below those found to cause acute effects in humans (Frank *et al.*, 1962; McDermott, 1962). However, the SO_2 level in Manoa Valley around midnight was probably much higher than 0.04 ppm.

Amdur and Underhill (1968) reported that 10 mg/m^3 of KCl aerosol, while having no effect on airway resistance in guinea pigs, caused a 3.3-fold potentiation of the effect of 2.3 ppm SO_2. They related this effect to the solubility of SO_2 in various aerosols. Potassium chloride was found to be 1.4 times as effective as NaCl in potentiating the response to SO_2. Recently, McJilton, Frank, and Charlson, (1973) confirmed Amdur and Underhill's results in guinea pigs using low concentrations of SO_2 and NaCl. They found that 1 ppm SO_2 in combination with 1 mg/m^3 NaCl aerosol increased pulmonary flow resistance in guinea pigs by 45%, but only when the RH was high (80%). They argued that if the RH is high enough the hydration of the aerosol particles and hence their uptake of SO_2 proceed too rapidly for the SO_2 to be scrubbed from the air by the nose. The particles then carry the irritating SO_2 into the lung. The RH at the time of the New Year fireworks was 82% at the Honolulu airport. Thus, it seems probably that the 26% decrease in $FEV_{25-75\%}$ in susceptible people was the result of an SO_2–KCl aerosol interaction. However, the pollution was apparently subthreshold for spirometric effects to be seen in subjects without a respiratory disease history. Nevertheless, the potential exists for nearly one million people to be affected in future years should the use of fireworks continue to grow or should Honolulu experience very calm wind conditions on New Year's Eve.

[3] Data provided by the Hawaii State Health Department, Division of Air Pollution. Normal 24 hour average concentration of SO_2 at this site is 15–20 $\mu g/m^3$.

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EPA

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization

External Review Draft
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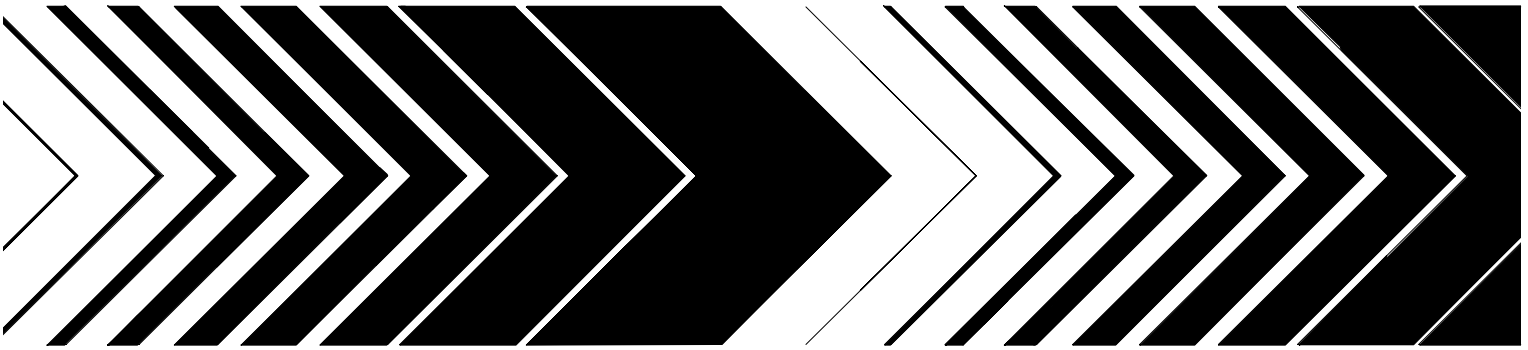

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Internal Review Draft

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization

National Center for Environmental Assessment
Office of Research and Development
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Washington, DC

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1998 External Peer Review Draft

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Preface

The purpose of this review is to provide scientific support and rationale for hazard identification and dose-response assessments based on the emerging data for both human health and ecological effects caused by exposures to perchlorate. It is not intended to be a comprehensive treatise on the chemical or the toxicological nature of perchlorate.

In Chapter 10, the U.S. Environmental Protection Agency (EPA) has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response (U.S. Environmental Protection Agency, 1995) for both the human health and ecotoxicological effects of perchlorate. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the individual assessments and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

Development of these hazard identifications and dose-response assessments for perchlorate have followed the general guidelines for risk assessments set forth by the National Research Council (1983). Other EPA guidelines that were used in the development of this health risk assessment include the Assessment of Thyroid Follicular Cell Tumors (U.S. Environmental Protection Agency, 1998a), Guidelines for Neurotoxicity Risk Assessment (U.S. Environmental Protection Agency, 1998b), 1996 Proposed Guidelines for Carcinogen Risk Assessment (Federal Register, 1996), Guidelines for Reproductive Toxicity Assessment (U.S. Environmental Protection Agency, 1996a), Use of the Benchmark Dose Approach in Health Risk Assessment (Crump et al., 1995), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. Environmental Protection Agency, 1994), Proposed Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicology Studies (Whalan and Redden, 1994), Guidelines for Developmental Toxicity Risk Assessment (Federal Register, 1991), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. Environmental Protection Agency, 1988), The Risk Assessment Guidelines of 1986 (U.S. Environmental Protection Agency, 1987), and the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

The document presents the hazard identification or dose-response assessment for noncancer toxicity for each route of exposure, either the oral reference dose (RfD) or the inhalation

reference concentration (RfC). The RfD and RfC are meant to provide information on long-term effects other than carcinogenicity, although more recently, the value of mode-of-action information to inform the potential for a continuum from noncancer toxicity as precursor lesions to carcinogenicity presented as tumors has been recognized (Federal Register, 1996; Wiltse and Dellarco, 1996). Consideration of this continuum is especially pertinent to the evaluation of the potential toxicity of perchlorate. When such a continuum can be characterized, the dichotomous approaches to "noncancer" versus "cancer" toxicity can be harmonized into one route-specific estimate. The objective is to select a prominent toxic effect or key event that is pertinent to the chemical's key mode of action, defined as a chemical's influence on molecular, cellular, and physiological functions (Wiltse and Dellarco, 1996). A harmonized approach to the neurodevelopmental and neoplastic effects of perchlorate is proposed herein.

In a default characterization without mode-of-action information, the RfD typically is based, in part, on the assumption that a threshold exists for certain toxic effects, both for the individual and the population; whereas, a threshold may not exist for other carcinogenic effects. Thus, if the critical toxic effect is prevented, then all toxic effects are prevented. In general, the RfD or RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure or continuous inhalation exposure to the human population (including sensitive subpopulations) that is likely to be without deleterious noncancer effects during a lifetime. The oral RfD is expressed in units of milligrams per kilogram per day. The inhalation RfC considers toxic effects for both the respiratory tract as the portal of entry, as well as for effects remote to the respiratory tract (extra-respiratory or systemic effects). The RfC is expressed in units of milligrams per cubic meter.

The carcinogenicity assessment is meant to provide information on three aspects of the carcinogenic risk assessment for perchlorate: the EPA classification and quantitative estimates of risk from both oral and inhalation exposure. The classification reflects a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed.

The screening-level ecological risk assessment of environmental contamination by perchlorate follows the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

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EXECUTIVE SUMMARY

The purposes of this document is to present an assessment that updates previous provisional values issued by the U.S. Environmental Protection Agency (EPA) for an oral reference dose (RfD) for perchlorate and revises the assessment previously released as a draft external review document (U.S. Environmental Protection Agency, 1998d). The objective of this assessment is to derive a human health risk estimate, based on an evaluation of its potential to cause toxicity or cancer, and to provide a screening-level ecological risk assessment for perchlorate based on all toxicity data that recently have become available to the Agency as of fall 2001. Another important objective was to evaluate the evidence for indirect exposures, i.e., those exposures not by direct ingestion of contaminated water. This revised assessment incorporates data from new studies and analyses in response-level to recommendations made at a previous peer review of the 1998 draft (Research Triangle Institute, 1999). Most of these data were obtained as results of a testing strategy that was designed with knowledge of the mode of action for perchlorate toxicity that identified major data gaps in the data available prior to 1997. This executive summary concisely presents key findings from the present assessment.

SUMMARY FINDINGS

Sources of Perchlorate Contamination and Occurrence

- Perchlorate is an oxidizing anion that originates as a contaminant in ground and surface waters from the dissolution of ammonium, potassium, magnesium, or sodium salts. Perchlorate is exceedingly mobile in aqueous systems and can persist for many decades under typical ground and surface water conditions.
- Ammonium perchlorate is manufactured for use as the oxidizer component and primary ingredient in solid propellant for rockets, missiles, and fireworks. Because it is a reducing agent, it can undergo a variety of intramolecular redox reactions that lead to the release of gaseous products. Through such reactions, it acts as a thrust booster.
- Perchlorate salts are also used on a large scale as a component of air bag inflators. Perchlorate salts are also used in nuclear reactors and electronic tubes, as additives in lubricating oils, in

tanning and finishing leather, as a mordant for fabrics and dyes, in electroplating, in aluminum refining, and in rubber manufacture, as a mordant for fabrics and dyes, and in the production of paints and enamels. Chemical fertilizer had been reported to be a potential source of perchlorate contamination, but new investigations by the Agency have determined that this is not an issue for agricultural applications.

- Large-scale production of perchlorate-containing chemicals in the United States began in the mid-1940s. Because of its shelf life, perchlorate must be washed out of the United States' missile and rocket inventory to be replaced with a fresh supply. Thus, large volumes have been disposed of in various states since the 1950s.
- Perchlorate began to be discovered at various manufacturing sites and in well water and drinking water supplies within the months following the April 1997 development of an ion chromatography analytical method that achieved a method detection limit (MDL) of approximately 1 ppb and a minimum reporting limit (MRL) of 4 ppb. There are 20 states with confirmed releases in ground or surface water. There are 40 states that have confirmed perchlorate manufacturers or users based on EPA Information Request responses.
  In California, most of the locations where perchlorate has been detected are associated with facilities that have manufactured or tested solid rocket fuels for the Department of Defense or the National Aeronautics and Space Administration.
- To date, there has not been a systematic national survey of perchlorate occurrence and a National Primary Drinking Water Regulation for perchlorate does not currently exist. Perchlorate was placed on the Contaminant Candidate List (CCL) in March 1998. The CCL lists priority contaminants (defined as either known or anticipated to occur in public water systems) in need of research, guidance development, regulatory determinations, or monitoring by the states. Perchlorate was listed as a contaminant that required additional research and occurrence information before regulatory determinations could be considered.
- Perchlorate was placed on the Unregulated Contaminants Monitoring Rule (UCMR) in March 1999 (Federal Register, 1999) to gather needed exposure information. Under the UCMR, all large public water systems and a representative sample of small public water systems were required to monitor for perchlorate beginning in January 2001. This effort does not extend to investigating potential sources in ground and surface water that have not migrated into public water supplies. Identification of the magnitude and extent of perchlorate occurrence in the

environment is important in assessing the routes of exposure to humans and for determining the different types of organisms and ecosystems that may be affected.

- In early 2000, an analytical method to detect perchlorate in drinking water (EPA Method 314.0) using ion chromatography was published as a direct final rule (Federal Register, 2000). The EPA Method 314.0 was also approved as a monitoring method for the UCMR (Federal Resister, 2000). The MDL for the method is 0.53 ppb and the MRL is 4 ppb. Improvements developed commercially in the analytical capabilities may lower the MRL to the sub-part per billion level in the near future.
- Adequate exposure characteristics of transport and transformation in the environment are also absent. Preliminary biotransport studies at six contaminated sites indicate a potential for uptake into plant and animal tissues in ecosystems. Extension of analytical methods to detect perchlorate in plant and animal tissues awaits validation before a conclusive determination can be made.

An Integrated Approach to Comprehensive Risk Characterization

- Perchlorate is of concern for several reasons. First, there were uncertainties in the toxicological database available that could be used to evaluate the potential for perchlorate to produce human health effects when present at low levels in drinking water. The purpose of the targeted toxicity testing strategy was to develop a database to address key data gaps. Secondly, the actual extent of the occurrence of perchlorate in ground and surface waters is not known at this time. Additionally, the efficacy of different treatment technologies for various water uses (such as drinking water or agricultural applications) and different scales (i.e., large or small volumes) is still being determined. Finally, the extent and nature of ecological impact or transport and transformation phenomena in various environmental media have only, as yet, been studied superficially.
- To adequately and comprehensively characterize the risks posed by perchlorate contamination and to develop scientifically-based management strategies that effectively mitigate the potential risks posed by perchlorate contamination, several advances are essential. The analytical methods used to characterize various exposures must be accurate and precise. The exposure estimates cannot be gauged with respect to their risk unless robust health and ecological risk estimates are available. Treatment technologies should be targeted to levels of concern and

tailored to the intended water use. Technology transfer is necessary so that all affected parties and concerned citizens are apprised of accurate and reliable information that is up to date with the evolving state of the science.

- The toxicity testing strategy was expedited through a unique partnership between the Department of Defense and EPA, together with members of an Interagency Perchlorate Steering Committee (IPSC), which includes other governmental representatives from the National Institute for Environmental Health Sciences (NIEHS) and affected state, tribal, and local governments.
- The charge of the IPSC is to facilitate and coordinate accurate accounts of related technological issues (occurrence surveys, health assessment, ecotoxicology assessment, treatability, waste stream handling, and analytical detection). This assessment is intended to address the need for evaluation of perchlorate's potential to cause human health effects or impact on ecological systems, based on currently available data.

Physicochemical Characteristics

- As an oxidant, perchlorate is kinetically nonlabile. This means the reduction of the central chlorine atom from an oxidation state of +7 (perchlorate) to −1 (chloride ion) occurs extremely slowly. Sorption is not expected to attenuate perchlorate because it absorbs weakly to most soil minerals. Natural chemical reduction in the environment is not expected to be significant. These two factors account for perchlorate being both very mobile in aqueous systems and persistent for many decades under typical ground and surface water conditions.
- The activation energy to perchlorate reduction is so high that it cannot be expected to act as an oxidant under human physiological conditions (i.e., dilute solution, unelevated temperatures, neutral pH). This is supported by absorption, distribution, metabolism, and elimination studies that show perchlorate is excreted virtually unchanged in the urine after absorption.

Hazard Identification and Mode of Action Testing Strategy

- The health effects and toxicity database available in the spring of 1997 was determined to be inadequate for quantitative risk assessment by an independent (non-EPA) peer review. A testing strategy was developed based on a hazard identification using the available data and the suspected mode of action for perchlorate to target testing on potential effects of perchlorate.

Data from this effort was used to support the previous EPA draft assessment and this revised assessment in 2002.

- To design a testing strategy based on the mode of action for a chemical, it is necessary to understand its toxicokinetics and toxicodynamics. Perchlorate is readily absorbed from the intestinal tract, and oral uptake is considered to be the major route of exposure. Because of its high charge, perchlorate does not pass readily through the skin. Exposure via inhalation is expected to be negligible because the vapor pressure of perchlorate salts and acids is expected to be low at room temperatures. Droplet size during showering likely would preclude inhalation of perchlorate-contaminated water as an aerosol. Perchlorate is known to inhibit the uptake of iodide in the thyroid at the sodium (Na+)–iodide (I-) symporter, or NIS, thereby causing a reduction in the hormones thyroxine (T4) and triiodothyronine (T3). When these hormones enter the blood circulation, they are bound to plasma proteins. There may be other locations of inhibition of iodide transport in the gland. Perchlorate itself is not metabolized in the thyroid or peripheral tissues.
- Control of circulating concentrations of these hormones is regulated primarily by a negative feedback known as the hypothalamic-pituitary-thyroid axis or feedback system involving three organs: (1) the thyroid, which produces T4 and T3; (2) the pituitary gland which produces TSH; and (3) the hypothalamus, which also responds to and helps to maintain optimal T4 and T3 levels. The hypothalamus stimulates the pituitary gland through thyrotrophic-releasing hormone (TRH) to produce thyroid stimulating hormone (TSH), which then prompts the thyroid to produce T4 and T3. Cells in the hypothalamus and pituitary gland respond to the levels of circulating T4 and T3, such that when thyroid production levels are low, there is a signal to increase the output of TRH and TSH. Circulating hormone levels (T4, T3, and TSH) can be monitored readily to serve as biomarkers of exposure and effect of agents that disrupt the status of this negative feedback system.
- The hypothalamic-pituitary-thyroid feedback system for regulation of thyroid hormones is conserved across species. Differences in plasma protein binding between rats and humans account for differences in the circulating half-life of the hormones and in thyroid response to TSH between the species. New studies since 1999 have confirmed that the inhibition of iodide uptake by perchlorate at the NIS is essentially the same sensitivity across species. This is

important when considering decrements in T4 as important to neurodevelopmental effects versus neoplasia that results in the gland due to stimulation by TSH.

- Given its mode of action as an inhibitor of iodide uptake that results in disturbances of the hypothalamic-pituitary-thyroid axis, concerns arose about the potential for perchlorate to cause carcinogenic, neurodevelopmental, developmental, reproductive, and immunotoxic effects. Further, there is concern for ecotoxicology effects on various aquatic and terrestrial plants and animals.
- The human health testing strategy for perchlorate developed in 1997 originally included eight different recommended studies to address data gaps and enhance the mechanistic information on the mode of action. The goal of these studies was to provide a comprehensive database on which to arrive at a revised human health risk assessment with greater confidence than previous recommended provisional values. These studies are described briefly below.
  (1) A 90-day oral bioassay to identify other target tissues in young adult rats; to provide data on the effects of repeated exposures to perchlorate on T3, T4, and TSH levels; to evaluate recovery of effects after 30 days; and to screen for some reproductive parameters. A genotoxicity assay also was performed on rats from the terminal sacrifice.
  (2) A neurodevelopmental study in rats to evaluate the potential for functional and morphological effects in offspring from the mother exposed during pregnancy and lactation.
  (3) A Segment II developmental study in rabbits to evaluate the potential for perchlorate to cause birth defects and to provide data on thyroid hormone effects in a second species other than the rat.
  (4) A two-generation reproductive toxicity study to evaluate the potential for perchlorate to cause deficits in reproductive performance in adult rats and for toxicity in the young offspring.
  (5) Absorption, distribution, metabolism, and elimination (ADME) studies to characterize the pharmacokinetics of perchlorate in laboratory animals and humans and to provide data necessary to allow construction of models for quantitative description of different internal dose metrics and interspecies extrapolation.

(6) Mechanistic studies that characterize the effects of perchlorate on the iodide uptake mechanism across species as a link with the ADME studies to aid in the quantitative extrapolation of dose across species.

(7) A battery of genotoxicity assays to evaluate the potential for carcinogenicity by evaluating the potential for direct effects on deoxyribonucleic acid (DNA).

(8) Immunotoxicity studies to evaluate the potential for perchlorate to disrupt immune function, including cell-mediated and humoral toxicity.

- After the External Peer Review in 1999, additional studies were performed to replicate the neurodevelopmental study (i.e., changes in brain morphometry and motor activity); determine the developmental toxicity potential in rats versus rabbits; investigate additional aspects of immunotoxicity; and develop a consistent nomenclature and scoring system for the histopathological lesions in the thyroid gland. Additional pharmacokinetic data was also developed into physiologically-based pharmacokinetic (PBPK) models of perchlorate and iodide distribution.
- A battery of ecological screening tests as part of the 1997 testing strategy was conducted as part of the 1997 testing strategy in laboratory organisms representative of ecological receptors across soil, sediment, and water to evaluate dose-response relationships. These were considered to be a tier of tests to give an idea of gross toxicity that would determine the need and types of tests to be performed in the next tier. The tests did not measure the amount of perchlorate in the tissues of the species being tested. Based on stakeholder input and the need for a more focused battery of tests, lettuce was substituted for duckweed because of Tribal concerns regarding the sizable lettuce crop along the Colorado river. The following species were selected for the first round of testing:

  (1) *Daphnia magna* (water flea) to represent an aquatic invertebrate

  (2) *Ceriodaphnia magna* (water flea) to represent an aquatic invertebrate

  (3) *Lactuca sativa* (lettuce) to represent a vascular plant

  (4) *Pimephales promelas* (fathead minnow) to represent an aquatic invertebrate

  (5) *Eisenia foetida* (earthworm) to represent a soil invertebrate

  (6) *Microtus pennsylvanicus* (meadow vole) to represent an herbivore
- Other studies in the set of tests included the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) and a phytoremediation study to examine uptake, distribution, and degradation in

experimental systems with rooted cuttings of woody plants, including willow, Eastern Cottonwood, and eucalyptus.

- Additional studies, some of chronic duration, on effect levels in aquatic animals, an aquatic plant, a terrestrial plant, and a soil invertebrate have been performed since 1999. A study of perchlorate occurrence in six selected sites with known or suspected contamination also examined perchlorate concentrations in site media and in various ecological receptors.

Human Health Assessment

- The testing strategy confirmed that the target tissue for perchlorate toxicity was the thyroid gland. Anti-thyroid effects included iodide uptake inhibition, perturbations of T3, T4, and TSH hormones, and thyroid histopathology in adult, fetal, and postnatal rats across studies with a range of experimental design. Thyroid weight in these studies was also effected. Other than effects in the thyroid, no effects were observed in rabbits of the developmental study, but the developmental study in rats identified 30 mg/kg-day as the lowest observed adverse effect level (LOAEL).
- Competitive inhibition of iodide uptake at the NIS by perchlorate is the key event leading to both potential neurodevelopmental and neoplastic sequelae. The decrement in iodide uptake leads to subsequent drops in T4 and T3 that can lead to permanent neurodevelopmental deficits. Because of strong correlations between changes in iodide uptake inhibition with decrements in T3 and T4; between T3 and T4 with changes in TSH; and between changes in T3, T4, or TSH with thyroid histopathology, an assessment model was proposed that used the changes in T3, T4, and TSH as the precursor lesions to subsequent effects that potentially could lead to thyroid tumors or to altered neurodevelopment. This assessment approach essentially harmonizes noncancer and cancer approaches because it is presumed that the no-observed-adverse-effect-level (NOAEL) for the precursor lesions will preclude any subsequent sequelae at higher doses.
- Thyroid tumors were observed in previous studies in rats exposed in long-term bioassays at high doses. Thyroid tumors were more recently also diagnosed in the first-generation (F1) adults (second parental generation [P2]) at 19 weeks in a two-generation reproductive study. Both the latency and incidence of these tumors were significant relative to the entirety of the National Toxicology Program data base for this type of tumor and in this strain of rat. These

effects and the demostration of a progression with duration of effects on hormones and thyroid histopathology in the 90-day study raised the concern that extended exposures to perchlorate may change the hypothalamic-pituitary-feedback system or the cellular sensitivity and demand for thyroid hormones.

- The rat model is considered relevant yet conservative for human health risk assessment of potential thyroid neoplasia because of the differences in thyroid structure and hormone half-lives. Perchlorate was demonstrated to be nongenotoxic in the testing battery employed, suggesting the antithyroid effects are an indirect mode of action for thyroid tumor formation.
- Due to the age- and time-dependent nature of the key event of perchlorate toxicity and its anti-thyroid effects, the revised RfD was based on weight-of-the-evidence approach to the entire data base. The RfD is proposed to be protective of both neurodevelopmental and neoplastic sequelae. An administered dose of 0.01 mg/kg-day was supported as a lowest-observed-adverse-effect level (LOAEL) based on effects on brain morphometry in pups from a PND21 sacrifice in a neurodevelopmental study that repeated similar observations made in a similar 1998 study, hormonal effects indicative of hypothyroidism (decreased T4 and increased TSH) in the dams of those same pups on GD21, thyroid histopathology and hormone changes in these same pups at various developmental stages (GD21, PND4, PND9, and PND21), thyroid histopathology and hormone changes at the 14- and 90-day sacrifice dates in a subchronic study, and indications of immunotoxicity (dermal contact hypersensitivity).
- A human equivalent exposure (HEE) was calculated using physiologically-based pharmacokinetic (PBPK) models for interspecies adjustment based on the area under the curve (AUC) of perchlorate in the serum as the dose metric. The HEE for the maternal dams was chosen for operational derivation because brain morphometry effects may have been programmed *in utero* and because the dams of effected pups were hypothyroid.
- A composite uncertainty factor of 300 was used to address uncertainties in the extrapolations required for the RfD derivation. A three-fold factor for intraspecies variability was retained due to the variability observed in the data and PBPK modeling for the adult humans and because the subjects used to develop the models did not provide kinetic data for the potentially susceptible population. There was also uncertainty in the parallelogram approach to extending the adult structures to predict doses for different life stages in the human. A full factor of ten was applied to extrapolate the LOAEL for the adverse effects (brain morphometry, colloid

depletion and hormone changes) observed in various studies at the 0.01 mg/kg-day dosage level. A three-fold factor for duration was applied due to the concern for the biological importance of the statistically significant increase in tumors observed in the F1-generation pups (second parental, P2 generation) at 19 weeks and the evidence for progression of effects with extended exposure in the 90-day study. The finding of tumors at 19 weeks raised concern for *in utero* programming, i.e., that disruption of thyroid hormones in the developing fetus may predispose the developing neonate and adult to future insults to the thyroid gland. This factor can also be viewed as part of a data base deficiency since there are no adequate long-term bioassays of perchlorate. Finally, a three-fold factor was applied for inaccurate characterization of immunotoxicity since recent studies reinforced concern for this potential endpoint. Because the test article was ammonium perchlorate, an adjustment factor of 0.85 was made for the percent of molecular weight of the salt from ammonium (15.35%), so that the RfD is expressed for perchlorate as the anion alone. This was done to be compatible with the analytical methods that measure the anion in environmental samples and because most perchlorate salts readily dissolve in water. The resultant revised RfD value for perchlorate is 0.00003 mg/kg-day. Confidence in the principal study, the data base and the RfD were all designated as medium.

Screening Ecological Risk Assessment

- A secondary acute value of 5 mg/L (as perchlorate) was derived to be protective of 95% of aquatic organisms during short-term exposures with 80% confidence. The secondary chronic value of 0.6 (as perchlorate) likewise was derived to be protective of 95% of aquatic organisms during short-term exposures with 80% confidence. These values were derived based on sodium perchlorate and are probably protective even if ammonium perchlorate is the contaminant released. Calculated ammonia-nitrogen concentrations corresponding to those values are below the acute and chronic ambient water quality criteria for ammonia, regardless of pH.
- For terrestrial plants, the quartile inhibitory concentrations for growth in soil and sand were 78 mg/kg (293 mg/L) and 41 mg/kg (160 mg/L), respectively. A factor of 10 was applied to account for interspecies variance to obtain a screening benchmark of 4 mg/kg.

- Because of limited data on effects for soil invertebrates, a conservative estimate of a threshold for soil community effects was derived at 1 mg/kg. The equivalent aqueous phase benchmark is 2.8 mg/L.
- A factor of 10 for interspecies variance and LOAEL to NOAEL extrapolation was applied to the human health risk LOAEL estimate based on rat data (0.01 mg/kg-day) to obtain a screening benchmark of 0.001 mg/kg-day for the representative herbivore (meadow vole) because it also is a rodent. The population-level implications of this effect are unknown, but it seems likely that such effects on the thyroid could diminish survivorship and fecundity, which would diminish population production.
- Data are available showing that perchlorate accumulates in the tissues of exposed fish, amphibians, and invertebrates. However, data are insufficient to determine whether perchlorate is concentrated in those tissues to levels exceeding the levels of exposure. By contrast, several studies have shown that perchlorate is taken up and concentrated in aerial plant parts, especially leaves, although studies designed for the purpose of quantifying plant concentration factors have not yet been conducted.

Uncertainties and Assessment Research Needs

- Accurate exposure information is a requisite for risk characterization for both human and ecological assessments. These data should include transport and transformation processes, notably the fate of perchlorate in irrigated soils because of the potential for evaporative concentration.
- Research concerning the human health risks of perchlorate needs to better characterize the dose-response for perchlorate inhibition of iodide uptake in adults, fetuses, and neonates. More definitive studies linking iodide uptake inhibition and the degree of perturbation of the hypothalamic-pituitary-thyroid axis (i.e., changes in T3, T4, and TSH levels) and association with neurobehavioral problems, thyroid changes, and neoplastic sequelae may continue to improve the confidence in the assessment. Understanding the relative sensitivity of laboratory animal assays of neurodevelopmental effects versus epidemiological studies of neuropsychological development also needs to be advanced. Research on potential factors influencing sensitivity is critically requisite. Animal models of thyroid impairment such as iodide deficiency and “womb to tomb” exposure designs should be explored.

- Because only a screening tier of tests has been performed, the major uncertainty derives from data gaps. Data on bioaccumulation in aquatic biota would allow evaluation of exposure of organisms that feed on fish and other aquatic organisms. Effects of perchlorate on algae and aquatic macrophytes are required to estimate risks to aquatic primary producers. Data on bioaccummulation in aquatic plants are necessary to assess direct impact to primary consumers (i.e., planktonic and benthic invertebrate communities). Data on accumulation in terrestrial vascular plants also should be investigated further. The factor applied for the use of subchronic data in fish could be addressed by chronic effect testing. Effects also should be determined in nondaphnid invertebrates and of dietary exposure in birds and herbivorous or litter-feeding invertebrates.

Risk Characterization

- As noted above, the lack of exposure information precludes comparison with the human health and ecological toxicity assessment for accurate characterization of risk. Indirect human exposure pathways can be addressed best by a new EPA document, Methodology for Assessing Health Risks Associated with Multiple Pathway of Exposure to Combustor Emissions, which is scheduled for final release in January 2002.
- Noncancer neurobehavioral effects have been shown at lower doses. The estimate for perchlorate has been based on precursor effects considered protective for both the thyroid neoplasia and neurodevelopmental effects. It is appropriate for comparison against direct oral exposures. The frequency and magnitude of exposure are key attributes for characterization compared with those assumptions of continuous lifetime exposure assumed in the derivation. The degree to which the particular suspected population at risk fits with the assumptions used in the RfD derivation should be kept in mind when performing any risk characterization. Further, RfD estimates are not intended to serve as a “bright line” because, by definition, there is an order-of-magnitude uncertainty around the estimate. This typically translates into a range of threefold below to threefold above the RfD.
- Ecological risk could not be precluded nor accurately characterized because of the significant data gaps described above.

1. INTRODUCTION

The purpose of this document is to revise the previous human health and ecological risk assessment external review draft (ERD) document (U.S. Environmental Protection Agency, 1998d). This revision is based on recommendations made at the 1999 external peer review (Research Triangle Institute, 1999). The peer review panel recommended some alternative analyses and several additional studies. This revised assessment addresses these recommendations and is based on all data made available to the Agency as of Fall 2001; including new studies from the perchlorate testing strategy. The purpose of this chapter is to provide background information on the current status of perchlorate (ClO_4^-) contamination in the United States and an historical perspective on how certain issues of concern have evolved to prominence. The role of this risk assessment will be placed in context with respect to the overall integrated approach to addressing the perchlorate contamination and regulatory readiness.

1.1 PRODUCTION USES AND SOURCES OF PERCHLORATE CONTAMINATION

Perchlorate is an oxidizing anion that originates as a contaminant in ground and surface waters from the dissolution of perchloric acid and of the salts including ammonium, potassium, magnesium, or sodium. With the exception of potassium perchlorate, each of these compounds is extremely soluble. Potassium perchlorate is regarded as sparingly soluble; however, it may dissolve completely under the appropriate environmental conditions.

Ammonium perchlorate is the oxidizer and primary ingredient (by mass) in solid propellant for rocket motors. For example, ammonium perchlorate (NH_4ClO_4) makes up 69.7% of the propellant for the space shuttle rocket motors and 65 to75% of the Stage I motors of the Minuteman III and 68% of the Titan missile motors (Rogers, 1998). Because the ammonium ion is a reducing agent, ammonium perchlorate can undergo a variety of intramolecular redox reactions that lead to the release of gaseous products. The explosive decomposition shown in Equation 1-1 is induced thermally and occurs at temperatures below 300 °C (Schilt, 1979a).

$$4\ NH_4ClO_4(s) \rightarrow 2\ Cl_2(g) + 3\ O_2(g) + 2\ N_2O(g) + 8\ H_2O(g) \qquad (1\text{-}1)$$

Through such reactions, ammonium perchlorate also acts as a thrust booster. Even after such decomposition, the dichlorine and dioxygen thus produced remain capable of engaging in subsequent redox reactions with fuels.

Specific uses of various perchlorate salts include: solid rocket fuel oxidizer, flares, and pyrotechnics (potassium); solid rocket fuel oxidizer, explosives, chemical processes and pyrotechnics (ammonium); precursor to potassium and ammonium perchlorate and in explosives (sodium); and military batteries (magnesium) (Rogers, 1998). Perchlorate salts also are used on a large scale as a component of air bag inflators. Other industrial or commercial applications of perchlorate salts include their use in nuclear reactors and electronic tubes; as additives in lubricating oils; in tanning and finishing leathers; as a mordant for fabrics and dyes; in electroplating, aluminum refining, and rubber manufacture; and in the production of paints and enamels (Siddiqui et al., 1998). A 1998 report raised the concern that chemical fertilizer is a potential source of perchlorate contamination (TRC Environmental Corporation, 1998). More recent studies limit concern to certain types of fertilizer containing Chilean caliche (Urbansky, 2000; U.S. Environmental Protection Agency, 2001a,b; Urbansky and Collette, 2001); however, production practices have been changed to address that issue. Besides their large-scale commercial uses, perchlorate salts often are employed on a small scale in laboratory chemical studies as ionic strength adjustors or as noncomplexing counterions. Some still are used in medical diagnostics in thyroid function tests. Perchloric acid is used for various laboratory applications requiring a strong acid. Wet ashing organic matter with perchloric acid still is performed today as a means of preparation for certain samples. Anhydrous magnesium perchlorate ($Mg(ClO_4)_2$) is a strong desiccant; however, historically, Anhydrone®, a slightly hydrated form of $Mg(ClO_4)_2$, has been used to collect the water formed in combustion analyses.

The large-scale production of perchlorate-containing chemicals in the United States began in the mid-1940s. The approximate percentages sold for specific end uses are 92% as an oxidizer, 7% as an explosive, and 1% other uses (American Pacific Corporation, 1998). The typical volume of production ranged from 1 to 15 million lb per year (Rogers, 1998) although production in the 1980's was generally 20 to 30 million pounds per year (Kerr-McGee Chemical LLC, 1998; American Pacific Corporation, 1998). Solid rocket fuel inventories are growing at a

significant rate as systems reach the end of their service life and as treaties mandate motor disposal. The current disposal method for these motors is open burning or open detonation, both of which are becoming increasingly difficult to perform under intense public and regulatory pressure based, in part, on concern over incomplete destruction via these methods. Currently, the large solid rocket motor disposal inventory shows 55 million lb of propellant awaits disposal, and this number is expected to be over 164 million lb by the year 2005 (Siddiqui et al., 1998). A significant portion of this inventory contains ammonium perchlorate, which now can be reclaimed and recycled into new motor propellants. The accepted method for removal and recovery of solid rocket propellant from rocket motors is high-pressure water washout. This method generates large amounts of aqueous solution containing low concentrations of ammonium perchlorate. Although ammonium perchlorate can be recovered from these aqueous solutions, it is cost-prohibitive to remove it entirely. Most of the locations where perchlorate has been detected in ground or surface waters are in areas associated with development, testing, or manufacture of aerospace materials. Explosives and fireworks manufacturing and disposal operations have also been implicated in a number of environmental releases. Laboratory activities and fertilizer operations are potential sources of contamination in relatively few known instances. Perchlorate contamination also may occur where mining activities use explosives extensively (Siddiqui et al., 1998).

When ammonium perchlorate is released to water, the salt is highly soluble and dissociates completely releasing ammonium (NH_4) and perchlorate (ClO_4^-):

$$NH_4ClO_4(s) \xrightarrow{H_2O} NH_4^+(aq) + ClO_4^-(aq). \qquad (1\text{-}2)$$

Its high solubility is not affected by pH or temperature. It is likely that most of the ammonium has been biodegraded, and the cation in the environment is best viewed as mostly sodium (Na^+) or possibly hydrogen (H^+), especially where contamination levels are below 100 ppb; nevertheless, those regions with high concentrations of perchlorate ion probably retain a small fraction of ammonium ion (Urbansky, 1998a). At those sites where contamination has occurred for decades, very little (if any) ammonium ion has been found. To date, there has been no quantitative determination of the cations responsible for the charge balance.

As an oxidant, perchlorate is kinetically nonlabile. This means that reduction of the central chlorine atom from an oxidation state of +7 (perchlorate) to −1 (chloride ion) occurs extremely slowly. This will be elaborated on in Chapter 2 in the discussion of physicochemical characteristics. Sorption is not expected to attenuate perchlorate concentrations because it absorbs weakly to most soil minerals. Natural chemical reduction in the environment is also not expected to be significant. Together, these two factors account for perchlorate's high mobility and persistence for many decades under typical groundwater and surface water conditions. Figure 1-1 summarizes the various pathways through which perchlorate can reach ground and surface water sources.

1.2 EVOLUTION OF ANALYTICAL DETECTION METHODS AND EMERGING OCCURRENCE DATA

The Region 9 Office of the U.S. Environmental Protection Agency (EPA) first became aware of the potential contamination issues with perchlorate in 1985 when samples measured with a colorimetric method reported contamination in 14 wells ranging from 0.11 to 2.6 ppm (Takata, 1985). The Region 9 office requested assistance from the Centers for Disease Control and Prevention (CDC) to evaluate the potential health effects of these levels of perchlorate (Takata, 1985). In response the CDC recommended validation of the colorimetric measures but could not address the potential for toxicity of the chemical because of toxicity data insufficiencies (Margolis, 1986). The CDC also recommended additional testing to determine potential target tissues and the effects from long-term, low-level exposures. The absence of a valid analytical method to measure low concentrations of perchlorate and the lack of data with which to characterize the risk of toxicity led Region 9 of EPA to focus on chemicals other than perchlorate at these sites. By the early 1990s, however, perchlorate at detectable levels (>1 mg/L) was found in monitoring wells at a California Superfund site, and EPA Region 9 increased its effort to establish a human-health-based reference dose (RfD) in order to help gauge the risk of the contamination that was beginning to be characterized. In 1997, after perchlorate was discovered in a number of California water supplies, the California Department of Health Services (CA DHS) adopted 18 ppb as its provisional action level.

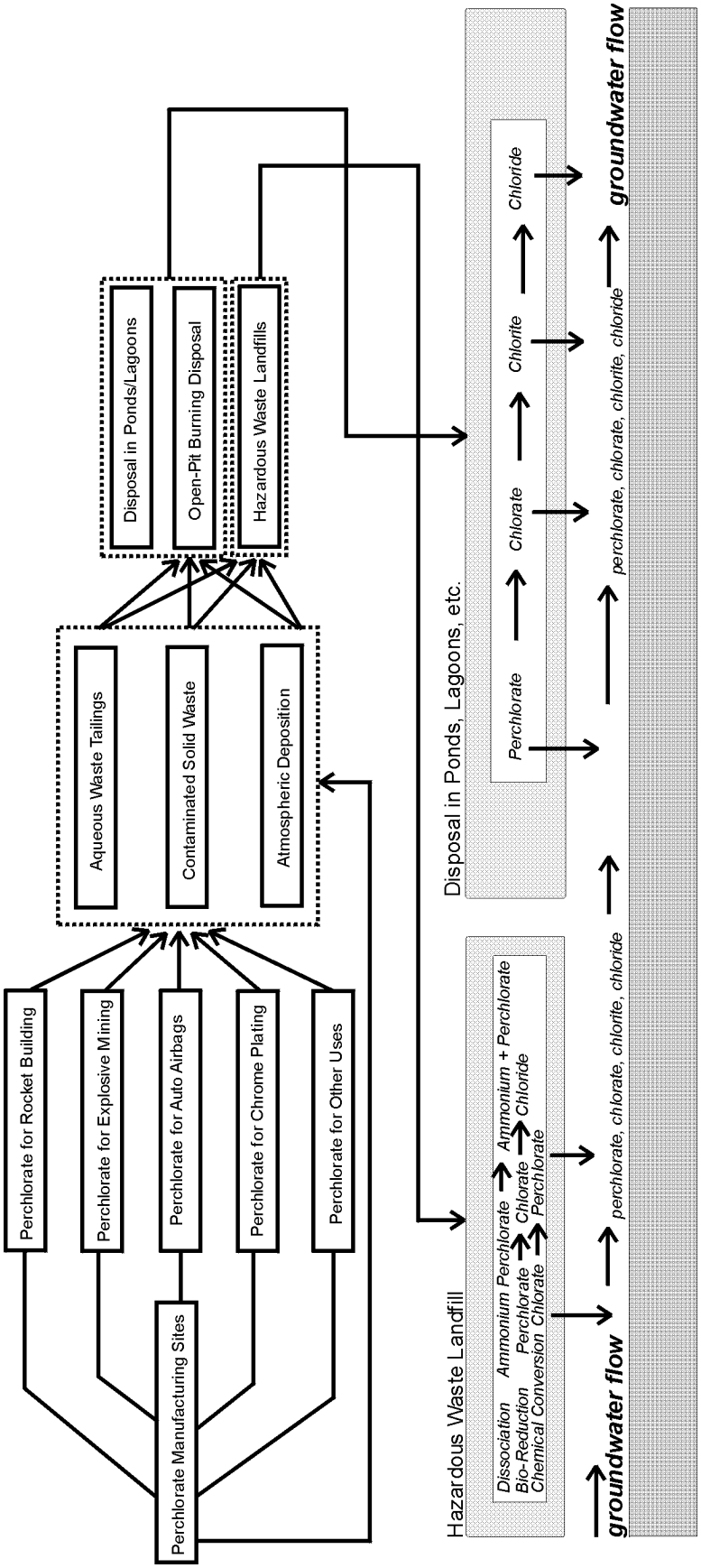

Figure 1-1. Sources and pathways of groundwater contamination for perchlorate. (Modified from Siddiqui et al., 1998.)

In January 1997, the California Department of Health Services' Division of Drinking Water and Environmental Management requested the Sanitation and Radiation Laboratory Branch (SRLB) test for perchlorate in drinking water wells potentially affected by groundwater migrating from the Aerojet facility near Sacramento. Based on its provisional action level, Region 9 of EPA indicated that a reporting limit of at least 4 ppb would be necessary. However, procedures to measure perchlorate at such low levels were not available. An ion chromatographic (IC) method was capable of detecting 400 ppb; and, during the previous year, Aerojet had improved the method to detect 100 ppb. By March 1997, SRLB and an analytical equipment manufacturer had developed an IC method that achieved a method detection limit of approximately 1 ppb and a reporting limit of 4 ppb. Within several months following the March 1997 development of the low-level (4 ppb) IC detection method, perchlorate was discovered at various manufacturing sites and in well water and drinking water supplies in California, Nevada, and Utah.

Efforts in several additional laboratories helped improve the IC method (Eldridge et al., 1999; Urbansky, 2000). Although IC is the dominant analytical method used at this time, a variety of additional techniques are being refined for perchlorate analysis, including: mass spectrometry, Raman spectrometry, capillary electrophoresis, and others (Urbansky, 2000). Recent publications have reported detection of perchlorate in tap water at levels as low as 0.1 ppb (Handy et al., 2000; Koester et al., 2000).

In March 1999, EPA included perchlorate in the Unregulated Contaminant Monitoring Rule (UCMR) (Federal Register, 1999). Under the UCMR, all large public water systems and a representative sample of small public water systems were required to monitor for perchlorate beginning in January 2001. The EPA Method 314.0 for the analysis of perchlorate in drinking water using IC methods was published in early 2000 as a direct final rule (Federal Register, 2000). The EPA Method 314.0 was also approved as a monitoring method for the UCMR (Federal Register, 2000). However, this effort does not extend to investigating potential sources in groundwater and surface water that have not migrated into public water supplies. Additional information about the UCMR is available at the web site http://www.epa/gov/safewater/ucmr.html.

The CA DHS adopted 18 ppb as its provisional action level in 1997 after perchlorate was discovered in a number of California water supplies. The CA DHS also added perchlorate to the list of unregulated chemicals for which monitoring is required in 1999 (Title 22, California Code

of Regulations §64450). By September 2001, over 2,800 sources of public water supply had been sampled in California by water supply agencies responding to CA DHS requirements. Most of these sources represent water supply wells. Of the sources sampled, 206 (over 7 percent) had perchlorate concentrations greater than 5 ppb in at least two samples (Figure 1-2). Most of these wells have as their source groundwater plumes that have spread as far as nine miles from the site of original release.

At this time, there has not been a systematic national survey of perchlorate occurrence. Several states and EPA regions are taking significant steps to test water supplies for perchlorate, notably the states of Arizona, Utah, and Texas, EPA Regions 6 and 7, and Suffolk County, New York. Figure 1-3 indicates states with confirmed perchlorate manufacturers or users, and Figure 1-4 indicates those states in which facilities have, in response to reported releases, directly measured perchlorate in groundwater or surface water. Table 1-1 describes these locations. The data published in Siddiqui et al., 1998 (drinking water systems in New Mexico, Indiana, Pennsylvania, and Iowa) are displayed in Figure 1-3 and in Table 1-1, but they have not been independently confirmed.

Information on other potential sites across the country is being gathered from the Department of Defense (DoD) and National Aeronautics and Space Administration (NASA) searches and from EPA information requests made to perchlorate manufacturers. The EPA has notified state, tribal, and local governments when it has evidence of perchlorate manufacture and use in these governmental jurisdictions. The American Water Works Association Research Foundation is coordinating a survey to characterize possible perchlorate contamination of drinking water sources in areas of high risk. The EPA will build on these survey data and other information to discover potential sources and evaluate threats to water resources.

Region 9 officials have collected information concerning detected perchlorate releases in 20 different states (Table 1-1). For two of these states, Pennsylvania and Indiana, the only reported releases have not been confirmed by a state or federal agency and should be considered questionable until the detections can be independently validated. In Washington State, propellant was observed scattered around open burn/open detonation sites although results of solid rocket chemical analyses of groundwater samples are not yet available. In California, most areas where perchlorate has been detected are associated with facilities that have manufactured, tested, or disposed of solid rocket fuels and propellants for DoD or NASA. Two facilities that

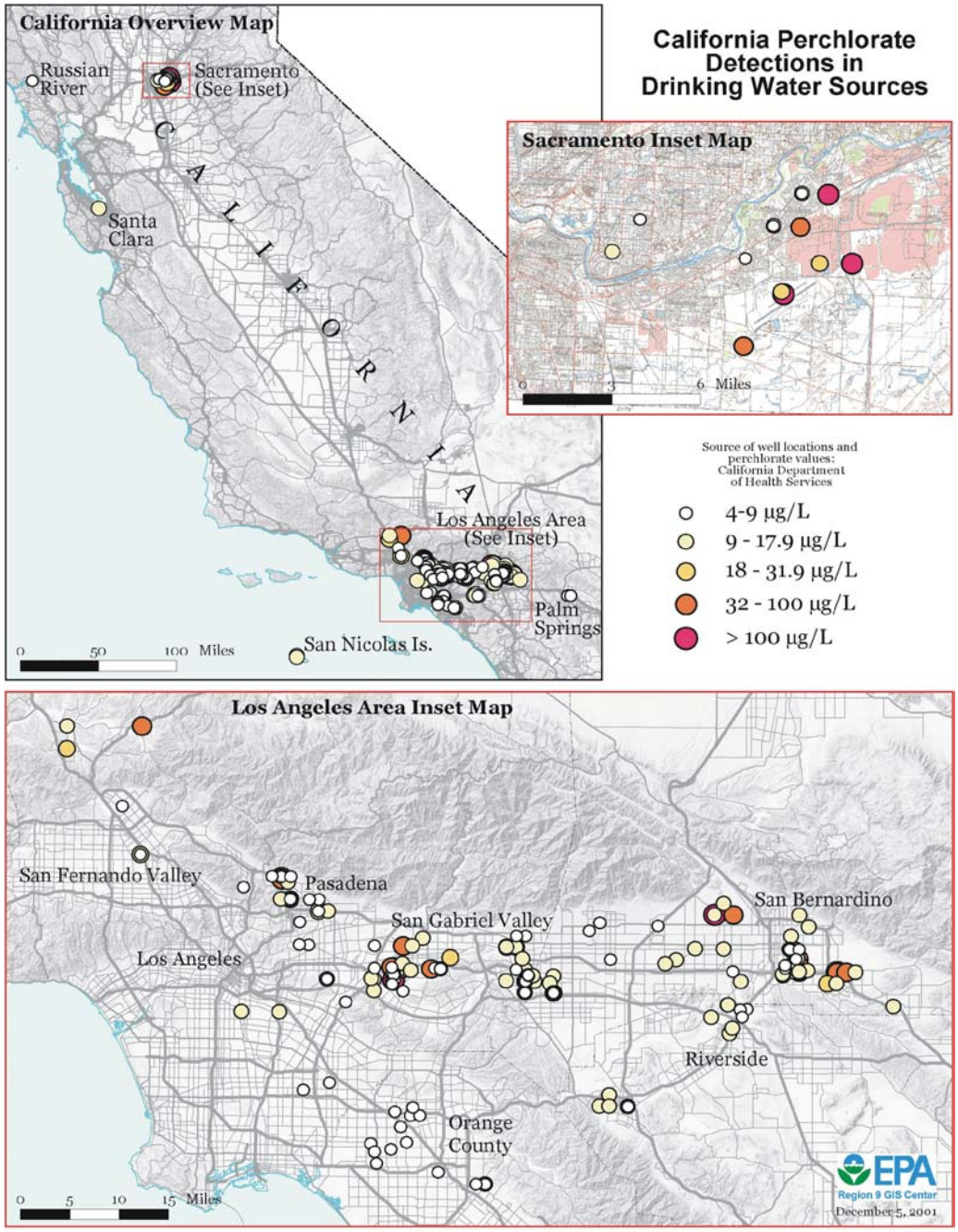

Figure 1-2. Distribution of perchlorate detected in public water supply sources in California. Also noted are several large sites of groundwater contamination that include perchlorate releases (Mayer, 2001).

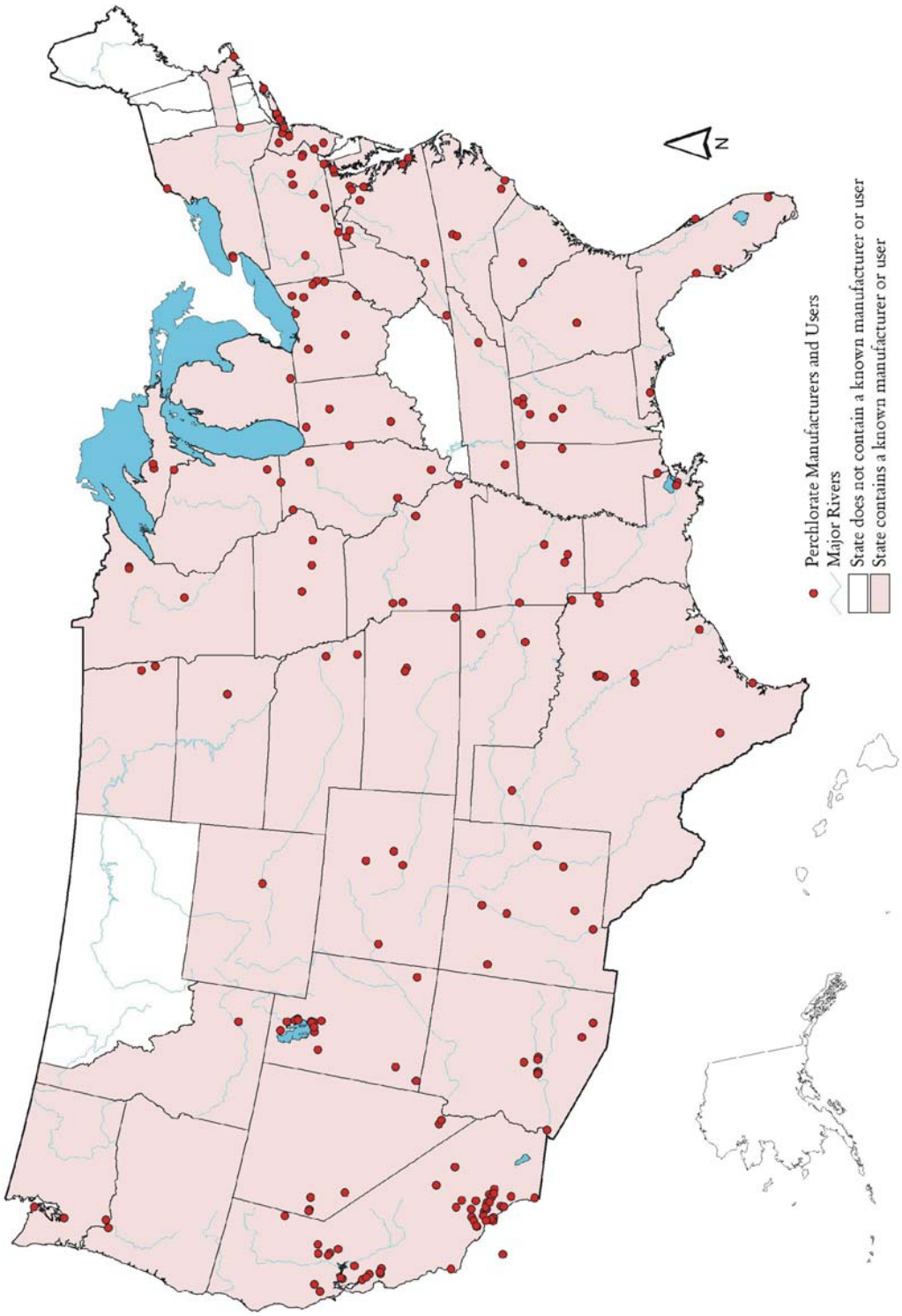

Figure 1-3. Locations of specific perchlorate manufacturers or users identified through responses to EPA Information Requests from current manufacturers (identifying shipments of at least 500 pounds in any year) and through investigations by state and local authorities (Mayer, 2001).

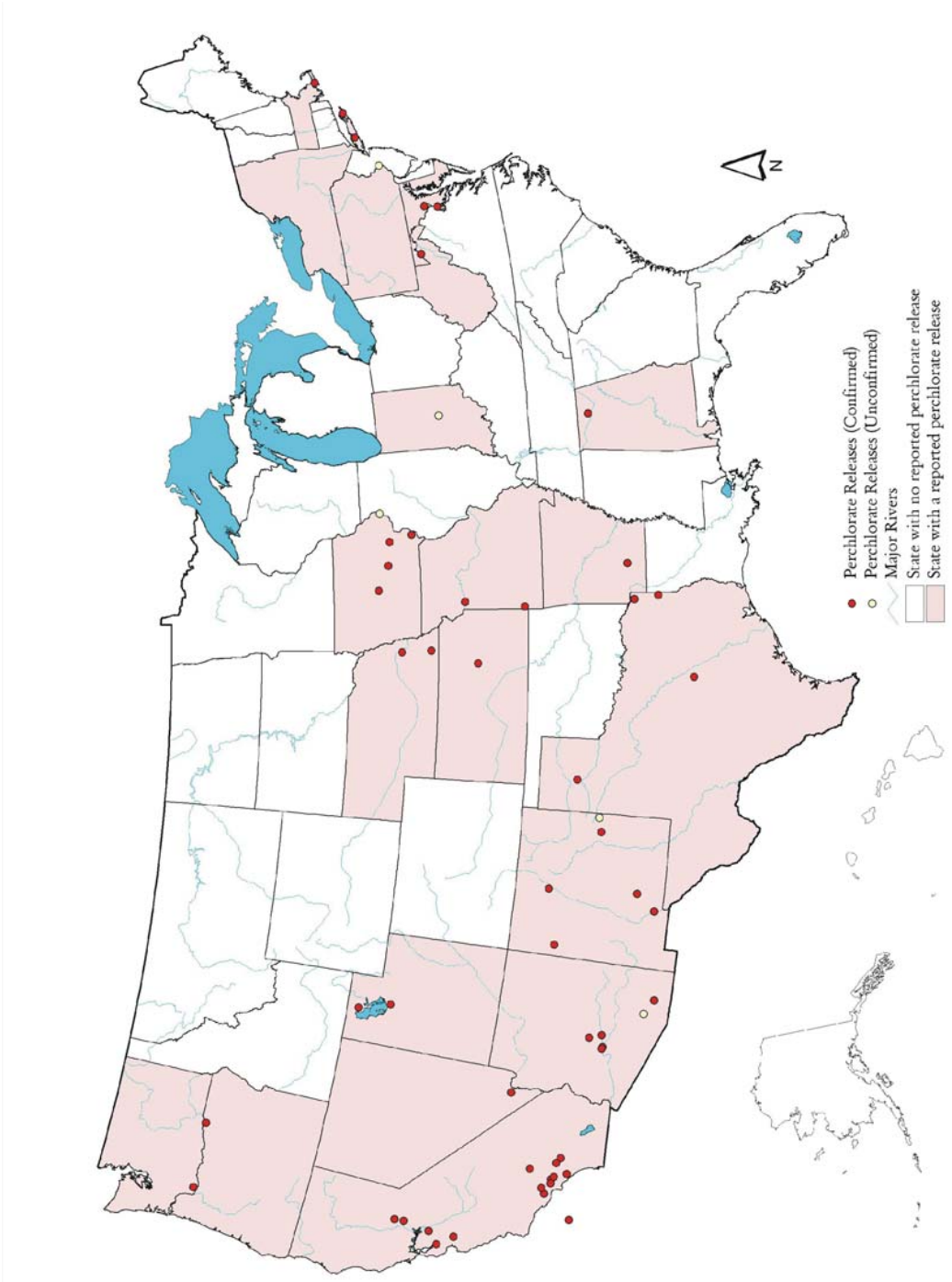

Figure 1-4. Locations of reported environmental releases of perchlorate to groundwater, surface water, or soil. Perchlorate measured in four water supplies in New Mexico, Iowa, Indiana, and Pennsylvania has been published in Siddiqui et al., 1998, but has not been confirmed independently by EPA or state authorities. Monitoring for perchlorate releases in most states is very limited or nonexistent (Mayer, 2001).

TABLE 1-1. OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2OO1[a] (Mayer, 2001)

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
AL	Redstone Army Arsenal - NASA Marshall Space Flight Center Huntsville, AL	Propellant Manufacturing, Testing, Research, Disposal	Monitoring Well Springs/Seeps	19,000 37
AZ	Apache Nitrogen Products Benson, AZ	Explosives Manufacturing	Monitoring Well	670
AZ	Aerodyne Gila River Ind. Res., Chandler, AZ	Propellant Testing	Monitoring Well	18
AZ	Davis Monthan AFB Tucson, AZ	Explosives/Propellant Disposal	Soil	Not confirmed
AZ	Unidynamics Phoenix Inc. Phoenix Goodyear Airport, Goodyear, AZ	Explosives/Ordnance Manufacturing	Monitoring Well	80
AZ	Universal Propulsion Phoenix, AZ	Rocket Manufacturing	Soil	—
AZ	Unidynamics Phoenix Inc. Whiter Tanks Disposal Area Maricopa County, AZ	Explosives/Ordnance Disposal	Public Water Supply Well (Unconfirmed Report) Soil	(4) —
AR	Atlantic Research East Camden, AR	Rocket Manufacturing Disposal - Open Burn/Open Detonation	Monitoring Well Surface Water Soil	1,500 480,000 —
CA	Aerojet General also affects Mather AFB Rancho Cordova, CA	Rocket Manufacturing	Public Water Supply Well Monitoring Well	260 640,000
CA	Alpha Explosives Lincoln, CA	Explosives Manufacturing	Monitoring Well Reported in Surface Water	67,000
CA	Boeing/Rocketdyne, NASA at Santa Susana Field Lab U.S. DOE Santa Susana, CA	Rocket Research, Testing and Production	Monitoring Well	750
CA	Edwards AFB Jet Propulsion Lab, North Base Edwards, CA	Rocket Research	Monitoring Well	300
CA	El Toro Marine Corps Air Station Orange Co., CA	Explosives Disposal	Monitoring Well	380
CA	Lawrence Livermore National Laboratory Site 300 Tracy, CA	U.S. DOE Explosives Research	Monitoring Well	84

TABLE 1-1 (cont'd). OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2OO1[a] (Mayer, 2001)

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
CA	Lockheed Propulsion Upper Santa Ana Valley Redlands, CA	Rocket Manufacturing	Public Water Supply Well	87
CA	NASA - Jet Propulsion Lab Raymond Basin Pasadena, CA	Rocket Research	Public Water Supply Well	54
CA	Rialto, CA	Fireworks Facility (?) B.F. Goodrich (?) Rocket Research and Manufacturing	Public Water Supply Well (inactive)	811
CA	San Fernando Valley Glendale, CA	Grand Central Rocket (?) Rocket Manufacturing	Monitoring Well	84
CA	San Gabriel Valley Baldwin Park, CA	Aerojet Rocket Manufacturing	Public Water Supply Well Monitoring Well	159 2,180
CA	San Nicholas Island Ventura Co., CA	U.S. Navy Firing Range	Public Water Supply (Springs)	12
CA	Stringfellow Superfund Site Glen Avon, CA	Hazardous Waste Disposal Facility	Monitoring Well Private Well	682,000 37
CA	UTC (United Technologies) San Jose, CA	Rocket Testing	Monitoring Well	180,000
CA	Whittaker-Bermite Ordnance Santa Clarita, CA	Ordnance Manufacturing	Public Water Supply Well	47
CA	Whittaker Ordnance Hollister, CA	Ordnance Manufacturing	Private Well Monitoring Well	810 88
IN	American Water Works Service Greenwood, IN	Unknown Source	Public Water Supply Well (Unconfirmed Report)	(4)
IA	American Water Works Service Clinton, IA	Unknown Source	Public Water Supply Well (Unconfirmed Report)	(6)
IA	Ewart, IA	Unknown Source	Livestock Well	29
IA	Hills, IA	Unknown Source	Private Well	30
IA	Napier, IA	Agriculture (?)	Private Well	10
KS	Herington, KS	Ammunition Facility	Monitoring Well	9
MA	Massachusetts Military Res. Barnstable Co., MA	Disposal - Open Burn/ Open Detonation	Monitoring Well	300
MD	Naval Surface Warfare Center Indian Head, MD	Propellant Handling	Waste Discharge to Surface Water	>1,000

TABLE 1-1 (cont'd). OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2OO1[a] (Mayer, 2001)

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
MD	White Oak Fed. Research Center (Naval Surface Warfare Center) White Oak, MD	Propellant Handling	Monitoring Well	72
MO	ICI Explosives Joplin, MO	Explosives Facility	Monitoring Well	107,000
MO	Lake City Army Amm. Plant Independence, MO	Propellant Handling	Monitoring Well	70
NE	Lewiston, NE	Agricultural Chemical Facility	Shallow Private Well	5
NE	Mead, NE	Fireworks Facility	Monitoring Well	24
NV	Kerr-McGee/BMI Henderson, NV	Chemical Manufacturing	Public Water Supply Monitoring Well Surface Water	24 3,700,000 120,000
NV	PEPCON Henderson, NV	Chemical Manufacturing	Monitoring Well	600,000
NM	American Water Works Service Clovis, NM	Unknown	Public Water Supply Well (Unconfirmed Report)	(4)
NM	Ft. Wingate Depot Activity Gallup, NM	Explosives Disposal	Monitoring Well	2,860
NM	Holloman AFB Alamogordo, NM	Rocket Testing	Monitoring Well Seasonal Surface Water Soil	40 16,000 —
NM	Los Alamos National Lab Los Alamos, NM	U.S. Dept. of Energy Lab Chemicals	Public Water Supply Well Monitoring Well Deep Borehold Water	3 220 1,662
NM	Melrose Air Force Range Melrose, NM	Explosives	Public Water Supply Well	25
NM	White Sands Missile Range White Sands, NM	Rocket Testing	Monitoring Well Soil	21,000 —
NY	West Hampton Suffolk County, NY	Unknown Source(s)	Public Water Supply Well Monitoring Well	16 3,370
NY	Yaphank Suffolk County, NY	Fireworks	Private Well Monitoring Well	26 122
PA	American Water Works Service Yardley, PA	Unknown	Public Water Supply Well (Unconfirmed Report)	(5)

TABLE 1-1 (cont'd). OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2OO1[a] (Mayer, 2001)

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
TX	Longhorn Army Ammunition Depot Karnak, TX	Propellant Handling	Monitoring Well Reported in Surface Water Soil	169,000 — —
TX	McGregor Naval Weapons Plant McGregor, TX	Propellant Handling	Monitoring Well Reported in Surface Water Soil	91,000 — —
TX	PANTEX Plant (USDOE) Amarillo, TX	Explosives	Monitoring Well	5
TX	Red River Army Depot Texarkana, TX	Propellant Handling	Monitoring Well	80
UT	Alliant Tech Systems Magna, UT	Rocket Manufacturing	Public Water Supply Well	16
UT	Thiokol Promontory, UT	Rocket Manufacturing	Well Supply Well (Inactive)	42
WA	Camp Bonneville near Vancouver, WA	Explosives/Propellant Disposal	Soil	—
WV	Allegheny Ballistics Lab Rocket Center, WV	Rocket Research, Production, Open Burn/Open Detonation	Surface Discharge of Groundwater Extraction	400

[a]Data reported to EPA Region 9 as of November 2001. All reports have been confirmed by federal, state, or county agencies except where noted. Soil concentrations are not listed.

manufactured ammonium perchlorate in Nevada were found to have released perchlorate to groundwater resulting in low levels (4 to 24 ppb) in Lake Mead and the Colorado River. This water is used for drinking, irrigation, and recreation for millions of people in Nevada, California, Arizona, and by Native American tribes.

The concentrations reported in wells and surface water vary widely. At one facility near Henderson, NV, perchlorate in groundwater monitoring wells was measured as high as 0.37% (3.7 million ppb). The highest level of perchlorate reported in any public water supply well was 800 ppb in an inactive well in California. Few active public water supply wells have perchlorate greater than 100 ppb, and none are reported at this level outside of California.

Perchlorate was found in a number of water supply wells on Long Island, NY, including several downgradient from a fireworks facility. It has been speculated that the wide distribution pattern of the New York contamination could be a result of low levels of perchlorate contained in fertilizer imported from Chile (TRC Environmental Corporation, 1998; Urbansky, 2000; Suffolk County Department of Health Services, 2001a,b). Agricultural chemicals also have been implicated as a potential source of perchlorate contamination in Nebraska at a shallow well near a speciality fertilizer facility (Williams, 2000). After state and federal officials in Region 7 added perchlorate analyses in their program testing hundreds of rural wells for fertilizers and agricultural chemicals. Their results showed that fertilizer application to farmlands is an unlikely source of perchlorate in Midwestern states.

In addition to discoveries at facilities involved with rocket propellants, explosives, and fireworks, a number of perchlorate detections have been made at current or former military facilities where propellants and explosives were disposed of by detonation and burning. Cooperation from Department of Defense (DoD) and Department of Energy (DoE) officials will continue to be important for examining these types of potential sources.

In the past three years, the increasing interest in investigating the environment has resulted in increasing detections. It is likely that regional positive efforts at detection may largely explain the distribution of known areas of release to the environment (Figure 1-4) when compared to the potential distribution suggested in Figure 1-3. As the efforts for detection become more uniform nationwide, the occurrence of perchlorate in the environment may more closely resemble the pattern of perchlorate usage.

It is important to distinguish between minimum detection limit (MDL) and the minimum reporting limit (MRL), which is also called the practical quantitation limit (PQL). MDLs are calculated from the precision of replicate low level measurements and are assumed to reflect 99% confidence that a trace concentration above zero can be detected. MRLs are higher values that reflect actual quantifiable concentrations. The EPA calculated and published an MDL for Method 314 (Ion Chromatography) at 0.53 μg/L (Federal Register, 2000). This was derived through the analysis of 7 replicate samples fortified at 2.0 μg/L. Based upon this result, an MRL for perchlorate was established at 4.0 μ/L. Dionex, the manufacturer of the ion chromatography column, published an MDL of 0.2 μg/L and MRL of 2.0 μg/L (Dionex, 2000).

Method 314 does not represent the lowest possible MRL or MDL. Unpublished improvements in the ion chromatography method may lower the MRL to the sub-part per billion level (Yates, 2001). Several research and commercial laboratories have been developing mass spectrometry methods to detect sub-ppb levels of perchlorate (Urbansky et al., 1999; Magnuson et al., 2000 a,b; Urbansky, 2000; Handy et al., 2000; Koester et al., 2000; Winkler, 2001). It is reasonable to expect that a reliable sub-ppb MRL for perchlorate will be commercially available in the very near future. The Agency encourages development of these emerging methods (e.g., LC/MS/MS) to eliminate interferences that can be encountered by extending IC methods for low-level analysis in a variety of matrices (e.g., soil or plants and animal tissues). The market demand for this capability may determine the commercial availability and expense of this method. Regulatory pressure to ensure protection or water supplies and to maintain treatment process control is also a factor driving the development of lower reporting limits for perchlorate. Thorough method validation and quality assurance information must be complied to establish a standard analytical method in the sub-ppb range for various media.

1.3 HEALTH AND ECOTOXICOLOGY RISK ASSESSMENTS—HISTORICAL OVERVIEW

This section briefly summarizes how the assessments for the health and ecotoxicology risks of perchlorate contamination have evolved. This document represents the revised assessment that incorporates additional data and analyses recommended at the external peer review convened by the Agency in February, 1999 (Research Triangle Institute, 1999).

1.3.1 Overview of Perchlorate Health Risk Assessment

The EPA Region 9 office requested evaluation of the toxicology data from the EPA Superfund Technical Support Center (Stralka, 1992). The EPA Superfund Technical Support Center issued a provisional RfD in 1992 (Dollarhide, 1992) and a revised provisional RfD in 1995 (Dollarhide, 1995) based on a literature review (Environmental Resources Management, Inc., 1995) submitted by the Perchlorate Study Group (PSG). Ideally, an RfD is based on a database that evaluates an array of endpoints that address potential toxicity during various critical life stages, from developing fetus through adult and reproductive stages. The provisional RfD

values (1992 and 1995) were based on an acute study in which single doses of potassium perchlorate caused the release of iodide (I^-) from the thyroids of patients with Graves' disease, an autoimmune condition that results in hyperthyroidism. It was difficult to establish a dose-response for the effects on thyroid function from daily or repeated exposures in normal humans from the data on patients with Graves' disease because of a variety of confounding factors, including that the disease itself has effects; that often only a single exposure, rather than repeated exposures was tested; that only one or two doses were employed; and that often the only effect monitored was iodide release from the thyroid or control of the hyperthyroid state. Nevertheless, a no-observed-adverse-effect-level (NOAEL) was determined to be 0.14 mg/kg-day based on release of iodide in the thyroid, followed by incomplete inhibition of iodide uptake. Uncertainty factors that ranged from 300 to 1,000 were applied to account for data missing on additional endpoints and extrapolations required to calculate a lifetime human exposure level. The provisional RfD values issued are listed as such by EPA because they did not undergo the internal EPA and external peer review required of estimates available on the EPA's Integrated Risk Information System (IRIS). Standard assumptions for ingestion rate and body weight were applied to the RfD to calculate the reported range in the groundwater cleanup guidance levels of 4 to 18 ppb.

In recognition of the potential influence of the reduced analytical detection limit, a reevaluation of the provisional 1992 and 1995 RfDs that serve as the basis of the provisional action level was warranted. An external non-EPA peer review convened in March 1997 to assess an analogous RfD derivation by an independent organization (Toxicology Excellence for Risk Assessment, 1997) determined that the health effects and toxicity data were insufficient for a credible quantitative risk analysis (Toxicology Excellence for Risk Assessment, 1998a). The external peer review panel concluded that the limited database was insufficient to rule out effects of perchlorate on other organs, so it could not be determined unequivocally that the effect on the thyroid was the critical effect. In particular, the reviewers were concerned that developmental toxicity, notably neurological development affected by hypothyroidism during pregnancy, could be another critical effect of perchlorate that had not been examined adequately in studies to date. In response to the March 1997 external peer review of the provisional RfD value, a subsequent external peer review of experts was convened in May 1997 to recommend and prioritize a set of studies to address the key data gaps and to reduce uncertainties in various extrapolations

(Toxicology Excellence for Risk Assessment, 1998b). The objective of the new studies is to provide a comprehensive database that will support development of a robust RfD estimate that reduces the uncertainties inherent in the provisional values. The strategical basis of the new battery of toxicity studies is discussed in Chapter 3. These data featured prominently in the external peer review draft of the assessment issued by the EPA in December 1998. At the subsequent external peer review convened by the Agency in February 1999, recommendations were made for additional studies and analyses, including completion of those on studies that were only available as preliminary data at that time (Research Triangle Institute, 1999). The EPA committed to a second external peer review and a revised risk assessment in order to benefit from the additional insights that these data might bring to bear. The purpose of this current revised document is to incorporate all of the data from new studies and to respond to recommendations made at the previous external peer review.

Because the Agency is committed to utilizing the latest available science to support its human and ecotoxicological risk estimates, the Office of Research and Development (ORD) issued interim guidance in 1999 to its risk assessors and risk managers to be followed until this revised assessment became publicly available (Noonan, 1999). The recommendation was to continue using the standing provisional RfD range of 0.0001 to 0.0005 mg/kg-day for perchlorate-related risk assessment activities because of the significant concerns and uncertainties that remained to be addressed. This recommendation was based on the determination that important new analyses on emerging data would likely have an impact on the previously proposed health risk benchmark in the 1998 external review draft (U.S. Environmental Protection Agency, 1998d) and that, while the new estimates would reflect greater accuracy, the resultant revised risk estimate could be either higher or lower.

This recommendation helped to ensure that the Agency bases its risk management decisions on the best available peer reviewed science and was in keeping with the full and open participatory process embodied by the proposed series of external peer review workshops. It should be noted that, due to the uncertainty of whether the final proposed revised oral human health risk benchmark would increase or decrease based on the new data and analyses, the standing provisional RfD range was the more conservative of the estimates available at the time of the interim guidance and, therefore, more likely to be protective of public health. The recommendation was also consistent with Agency practice that existing toxicity estimates remain

in effect until the review process to revise them is completed. The steps necessary to complete this assessment are outlined in Section 1.4. Once completed, this assessment will be included on the Agency's Integrated Risk Information System (IRIS).

1.3.2 Overview of Ecotoxicology Screening Level Assessment

The mobility and persistence of perchlorate discussed in the beginning of this chapter also may pose a threat to ecological receptors and whole ecosystems either by direct harm to organisms or by indirectly affecting their ability to survive and reproduce. There were very limited data in 1997 with which to evaluate the effects of perchlorate on ecological systems; nor were there data about the possible uptake of perchlorate into agricultural products irrigated by contaminated water. Analytical tests had been derived to detect perchlorate in water, but little work had been done to extend these methods to testing plant and animal tissues or food crops for perchlorate.

Searches of available databases revealed minimal information on the ecological effects of ammonium perchlorate or any of perchlorate's other salts. Little data exist to describe perchlorate's ecological effects on various soil, sediment, or aquatic receptors, including aquatic vertebrates, aquatic or sediment invertebrates, and bacteria or plants. The data that were available suggested effects on thyroid-hormone-mediated development in the South African clawed frog, *Xenopus laevis*, in the range of 50 to 100 ppm, and 1,000 ppm had been shown to completely block the metamorphosis of tadpoles. Effects on development and population growth also had been indicated in the freshwater lamprey at 100 ppm and the freshwater hydra at 350 ppm. Mortality was observed in cold-water trout (6,000 to 7,000 ppm) and *Daphnia magna* (670 ppm). Effects on seed germination and growth of agricultural plants were reported at 10 ppm.

Under the auspices of the Ecological/Transport and Transformation Subcommittee of the Interagency Perchlorate Steering Committee (IPSC, see Section 1.5), the U.S. Air Force (USAF) Detachment 1, Human Systems Center, Brooks Air Force Base (AFB), in conjunction with EPA, developed a proposal for a battery of screening-level bioassays in laboratory-reared organisms representative of soil, sediment, and water column receptors, to evaluate dose-response relationships. The identified tests focus on identifying gross (direct) toxicity tests whose endpoints can include mortality, growth, and reproductive success. Bioassays with standard

protocols and general regulatory acceptance were chosen. Although these were screening-level tests and provided only an indication of gross toxicity, they provided the dose-response information required to make decisions about the need for a next tier of tests to be completed (e.g., bioavailability, bioaccumulation, histopathology).

Additional new studies were recommended at the 1999 external peer review in the ecotoxicology arena as well, and some additional data has become available that improves the information base somewhat. Most significantly, additional data are available on effect levels in aquatic animals, an aquatic plant, a terrestrial plant, and a soil invertebrate; and some of these data are for chronic durations. In addition, surveys have been conducted at several sites of known or suspected perchlorate contamination with analysis of environmental and biological materials for perchlorate levels. While these new data have been incorporated in the current revision and are described in Chapter 8, the knowledge in this arena requires that the ecological assessment must still be characterized as a screening level rather than definitive. The number of species is still quite low and the site surveys aimed only to describe the range of exposures at the sites. The ecotoxicological review will undergo the same peer review process as the health risk assessment that is described in Section 1.4.

1.4 RISK CHARACTERIZATION AND REGULATORY AGENDA

This section briefly describes pending regulatory activities that this evaluation and characterization will likely influence. Particular emphasis is placed on the revised health risk assessment and ecotoxicology assessments.

1.4.1 U.S. Environmental Protection Agency Regulatory Plans

The Safe Drinking Water Act (SDWA), enacted by Congress in 1974 and amended in 1986 and again in 1996 (U.S. Code, 1996), provides the basis for safeguarding public drinking water systems from contaminants that pose a threat to public health. The purpose of the SDWA is to protect public health by ensuring that public drinking water systems provide tap water that is safe for drinking and bathing. Within EPA, the Office of Ground Water and Drinking Water

develops National Primary Drinking Water Regulations (NPDWR) to control the levels of contaminants that may occur in public drinking water systems.

The 1996 amendments to the SDWA require EPA to publish a list of contaminants that are not currently subject to a NPDWR and are known or anticipated to occur in public water systems. This list, known as the Contaminant Candidate List (CCL), is the source of priority contaminants for research, guidance development, regulatory determinations, and monitoring by the states. The SDWA requires EPA to determine whether or not to regulate at least five contaminants from the CCL by 2001. The CCL also must be reviewed and updated every 5 years; the next review is scheduled for 2003.

With broad public input and consultation with the scientific community, a draft CCL was published on October 6, 1997. The draft CCL specifically requested comment on whether to include perchlorate on the CCL based on the limited information EPA had received on perchlorate's occurrence in drinking water supplies at the time of publication. As a result of the public comments and the obtainment of additional occurrence information, EPA determined that sufficient information exists to raise concern over perchlorate's potential public health impact and added perchlorate to the final CCL published on March 2, 1998.

The CCL consists of 50 chemical and 10 microbiological contaminants and is divided into two categories: (1) contaminants for which sufficient information exists to begin to make regulatory determinations in 2001, and (2) contaminants for which additional research and occurrence information is necessary before regulatory determinations can be made. Perchlorate falls into the latter category because of the need for additional research in the areas of health effects, treatment technologies, analytical methods, and extent of occurrence.

1.4.2 State Regulatory Plans

The CA DHS and the California EPA Office of Environmental Health Hazard Assessment (CA EPA OEHHA) reviewed the EPA risk assessment reports for perchlorate and established its action level at 18 ppb based on the provisional RfD values from the EPA Superfund Technical Support Center. The CA DHS advises water utilities to remove drinking water supplies from service if they exceed the 18-ppb action level. If the contaminated source is not removed from service because of system demands, and if drinking water provided by the utility exceeds the action level, the CA DHS advises the utility to notify its customers. On August 1, 1997, the CA

DHS informed drinking water utilities of its intention to develop a regulation requiring monitoring of perchlorate as an unregulated chemical. Legislative action to establish a state drinking water standard for perchlorate by January 2000 (California Senate Bill 1033 [California State Senate, 1998]) was vetoed by the governor after passage by both houses. The governor supported prioritizing the regulation of perchlorate in drinking water but objected to the strict time schedule required.

In July 2001, the CA EPA OEHHA posted a notice on its web site indicating that it was initiating a risk assessment for perchlorate in connection with the development of a public health goal (PHG) for a number of chemicals in drinking water (www.oehha.ca.gov/public_info/public/phgannounc.html). PHGs are concentrations of chemicals in drinking water that are not anticipated to produce adverse health effects following long-term exposures. These goals are non-regulatory in nature but are to be used as the health basis with which to update the state primary drinking water standards established by CA DHS for chemicals in drinking water subject to regulation. A 45-day public comment period will be provided after posting, followed by a public workshop. Scientific peer reviews are arranged through the University of California. The overall process will include time for revisions, further public comment, and responses to comments. The new PHGs are scheduled for publication in 2003.

New York, Arizona, and Texas also initially adopted the level of 18 ppb as their version of advisory levels for water supply systems. Texas and Arizona health departments revised their perchlorate advisory levels based on research presented in EPA's December 1998 External Review Draft Toxicity Assessment. In July 1999, Texas arrived at a value of 22 ppb in drinking water by calculating the exposure of a 15 kg child drinking 0.64 liter per day and using the reference dose proposed in the 1998 EPA ERD document. Texas revised this value to 4 ppb in October 2001 based in part on the interim ORD guidance (Noonan, 1999). Arizona derived a 14 ppb level in March 2000, based on a 15 kg child drinking 1 liter per day and using the proposed RfD in the 1998 EPA ERD document. New York State has continued to use 18 ppb as the advisory level for perchlorate in drinking water.

The Nevada Division of Environmental Protection (NDEP) has authority under Nevada Water Pollution Control Regulations to address pollutants in soil or groundwater. The state's Corrective Action Regulations direct NDEP to establish action levels for hazardous substances, pollutants, or contaminants, using drinking water standards such as a maximum contaminant

level (MCL), health advisories, or background or protective levels (determined by IRIS or the equivalent). In August 1997, Nevada determined that the action level of 18 ppb, as established by EPA, would be the recommended action level for cleanup, pending a more current risk assessment.

1.5 SUMMARY

Perchlorate contamination is a concern for several reasons. First, there are uncertainties in the toxicological database that is used to address the potential of perchlorate to produce human health effects when present at low levels in drinking water. Additionally, the actual extent of perchlorate occurrence in ground and surface waters and other media (soils or plant and animal tissues) is unknown—a problem compounded by limits to the analytical detection method. The efficacy of different treatment technologies for various water uses, including drinking and irrigation, is also not well established. Finally, the nature and extent of ecological effects and details about transport and transformation phenomenon in various environmental media have been studied only superficially. EPA aims to more comprehensively characterize the risks to human and ecological health from perchlorate contamination through the integrative approach presented in Figure 1-5.

Thus, a number of key pieces of information and scientific advances are essential to adequately characterize the risks of perchlorate contamination and to develop scientifically-based management strategies that effectively mitigate the potential risks posed by perchlorate contamination. Accurate characterization of exposures relies on reliable analytical detection methods. The exposure estimates cannot be gauged with respect to their risk unless a robust health risk estimate is available. Treatment technologies should be targeted to levels of concern and tailored to the intended water use. Technology transfer is necessary so that all affected parties and concerned citizens are appraised of accurate and reliable information that is up-to-date with the evolving state-of-the-science. The purpose of the revised risk characterizations presented in this document is to serve in this integrative approach by providing more robust risk estimates than those that currently exist provisionally in order to better gauge the potential human health and ecological impacts.

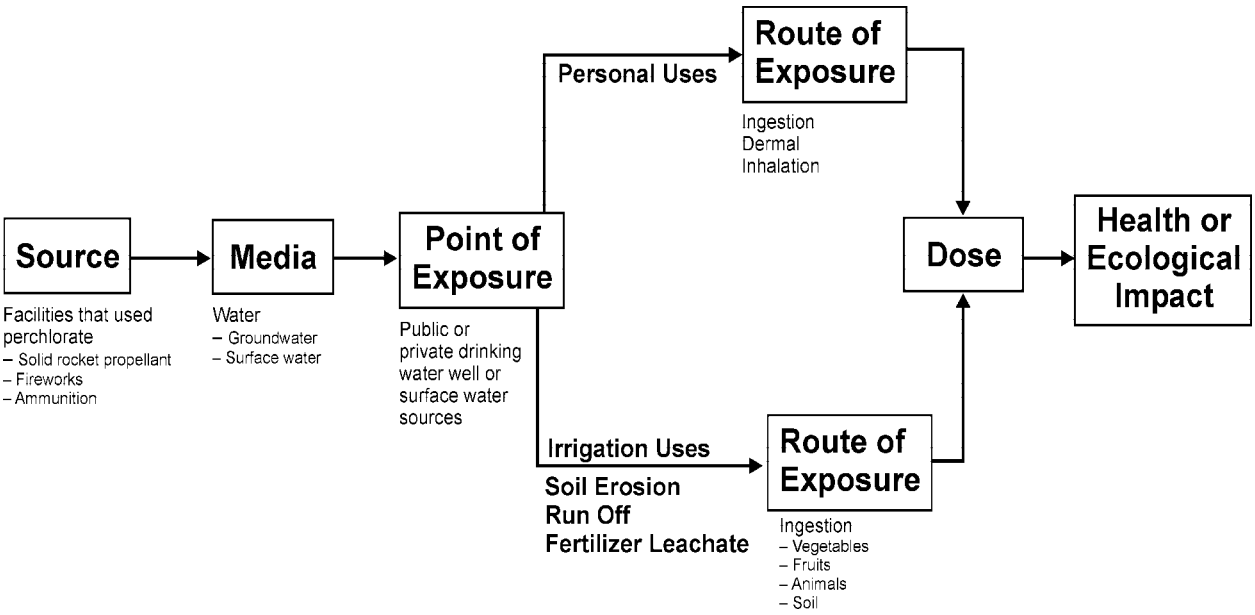

Figure 1-5. Considerations for comprehensive characterization of perchlorate contamination. (Modified from Underwood, 1998.)

The National Center for Environmental Assessment (NCEA) in the Office of Research and Development (ORD) of EPA first evaluated the emerging information and new human health/toxicity and ecotoxicity data from the testing strategy (see Chapter 3) and issued an external peer review draft in December 1998. In February 1999, an external peer review workshop was convened. The peer review panel endorsed the conceptual approach proposed by NCEA and recommended additional studies and analyses. This revised risk characterization document represents a response to those recommendations and includes data made available to the EPA as of Fall 2001.

As with any risk assessment, incorporation of new data is an iterative process. Because of regulatory schedule constraints, this assessment has gone forward with the recognition that new data may warrant further revision at a future date. Data in additional analyses that are warranted and which will be arriving in the period between the issuance of the external peer review draft and the external peer review workshop are identified herein and may be presented at that workshop.

Independent, external peer review of the study protocols, toxicity studies, and revised risk assessment for perchlorate will be critical to ensuring that future decisions will be protective of human health and that the potential for ecotoxicology is characterized appropriately. The IRIS program will oversee the external peer review and has tasked a qualified contractor to manage the peer review of technical issues related to the development of the human health and ecotoxicology assessments, including system design, conduct of toxicity studies, statistical analysis of data, designation of effect levels, selection of critical effect and uncertainty factors, and risk characterization. The peer review will be conducted by a panel of technical experts selected by contractors in ecotoxicology; neurotoxicology; developmental, reproductive, genetic, and general toxicology; endocrinology; pathology; biostatistics; dose-response modeling; and risk assessment.

The risk characterization assessment package, supporting studies, and study protocols for the new data will be distributed to the peer review panel in advance of the peer review meeting. The peer reviewers are charged with evaluating experimental protocols, performance, and results for any new studies since 1999 in addition to how the data are used in this risk assessment. Peer reviewers will independently review the risk assessment package and supporting studies and will submit their written comments to the IRIS contractor prior to the peer review meeting. The IRIS contractor will compile the peer reviewers comments and distribute them to all of the reviewers prior to the meeting which will be held on March 5 and 6, 2002. Sacramento was selected as a location for its accessibility to stakeholders and peer reviewers. The public will be invited to attend and observe the peer review meeting and may obtain pre-meeting comments at that time. Following the peer review meeting, the peer review panel will generate a report detailing their comments on the reference dose package and supporting studies. NCEA then will generate a responsiveness summary report that will discuss how comments made by the peer reviewers have been addressed. The revised risk characterization will be issued subsequently by EPA as a final IRIS assessment after Agency consensus review across offices and laboratories and a final IRIS program clearance.

It should be noted that this assessment effort was accomplished in an expedited time frame through the partnership and cooperation of a number of governmental entities. The IPSC was formed in January 1998 to bring together government representatives from EPA; DoD; the National Institute for Environmental Health Sciences (NIEHS); and affected state, tribal, and

local governments. Participation in IPSC also has been solicited from other governmental entities. The purpose of the IPSC is to facilitate and coordinate accurate accounts of related technological issues (occurrence, health effects, treatability, waste stream handling, analytical detection, and ecological impacts) and to create information-transfer links for interagency and intergovernmental activities regarding these areas of concern.

Figure 1-6 shows the structure of the IPSC, members of its executive committee, and co-chairs of the subcommittees. Note that a subcommittee exists for each of the outstanding controversial issues regarding perchlorate contamination. These are identified in the comprehensive characterization framework presented in Figure 1-5. Research to obtain additional data and the development of new methods and applications is underway in these human health and ecotoxicology areas, as well as in most of the others, to ensure that the state-of-the-science is brought to bear in addressing the unique issues of perchlorate contamination.

The IPSC collaborated in 1998 with EPA ORD NCEA on a draft report to a Congressional committee that assesses the state-of-the-science on the health effects of perchlorate on humans and the environment and the extent of perchlorate contamination. The report also contained recommendations for future research to address emerging issues (U.S. Environmental Protection Agency, 1998e). This report will be finalized and sent to Congress after the IRIS file is completed. Updates on activities of IPSC can be found on the EPA Office of Water (OW) web site at the following address: http://www.epa.gov./ogwdw/ccl/perchlor/perchlo.html. Discussion papers presented by the IPSC present additional information on the areas (e.g., analytical and treatment technology) that have not been discussed in detail herein.

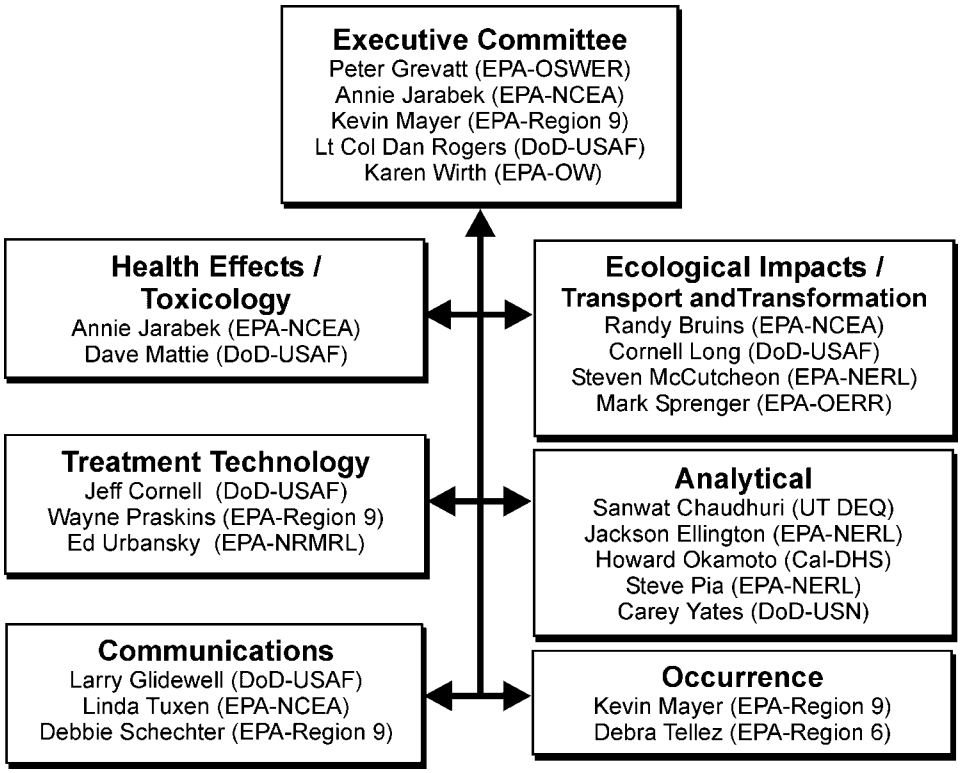

Figure 1-6. Structure and membership of the executive committee, subcommittees areas, and co-chairs of IPSC. The IPSC is designed to ensure an integrated approach to addressing the perchlorate contamination challenge and to provide accurate accounts of technical issues to stakeholders. (OSWER = Office of Solid Waste and Emergency Response, NCEA = National Center for Environmental Assessment, DoD = Department of Defense, USAF = U.S. Air Force, OW = Office of Water, NERL = National Exposure Research Laboratory, OERR = Office of Emergency Response and Remediation, NRMRL = National Risk Management Research Laboratory, Cal DHS = California Department of Health Services, USN = U.S. Navy, UT DEQ = Utah Department of Environmental Quality).

2. PHYSICOCHEMICAL CHARACTERISTICS

This chapter provides an overview of the physicochemical properties of perchlorate. These are important to understanding the persistence of perchlorate in the environment and to understanding how perchlorate is processed in various plants and animals. Additional toxicokinetic and toxicodynamic information can be found in Chapters 3 and 6; additional data on transport and transformation, including biotransport, are discussed in Chapters 8 and 9.

In the solid state, the perchlorate anion has been determined by X-ray diffraction to have a nearly perfect tetrahedral geometry with the four oxygen atoms at the vertices and the chlorine atom at the center as shown in Figure 2-1. In aqueous solution, the geometry is probably perfectly tetrahedral. The average chlorine-to-oxygen bond distance is 1.42 pm (Schilt, 1979b), and the oxygen-to-oxygen distance is 2.43 pm. The partial molar ionic volume is 44.5 cm^3/mol at 25 °C, compared with 36.7 for iodide.

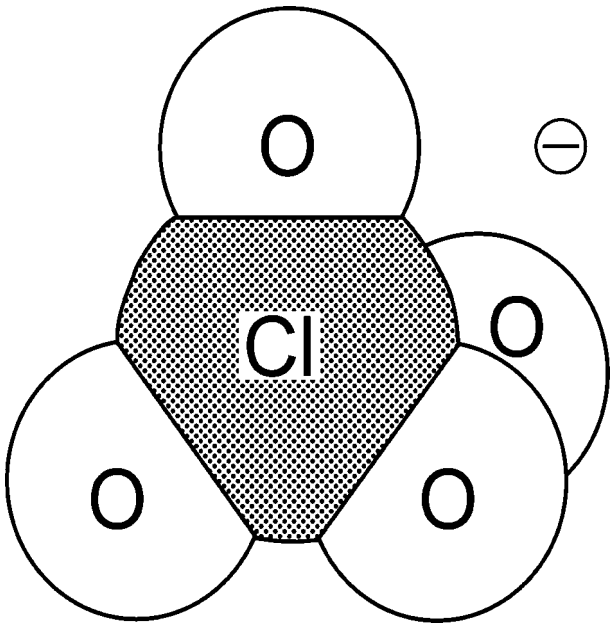

Figure 2-1. Chemical structure of perchlorate.

Perchlorate is widely known to be a very poor complexing agent and is used extensively as a counter anion in studies of metal cation chemistry, especially in nonaquous solution (Urbansky, 1998). In this application, it is comparable with other noncomplexing or weakly ligating anions, such as trifluoromethanesulfonate (triflate [$CF_3SO_3^-$]), tetrafluoroborate (BF_4^-), and, to a lesser extent, nitrate (NO_3^-). Some exceptions are known, but are rare, such as some copper compounds (Burke et al., 1982). All of these anions have a highly delocalized ($CF_3SO_3^-$, NO_3^-, ClO_4^-) or sterically blocked (BF_4^-) monovalent anionic charge and large volume. The low charge density reduces their affinity for cations and their extent of aquation (see Table 2-1).

TABLE 2-1. GIBBS FREE ENERGIES OF FORMATION FOR SELECTED ANIONS IN AQUEOUS SOLUTION (Urbansky, 1998)

Anion	$\Delta G_f^{"}$, kJ Mol^{-1}
BF_4^-	−1,490
PO_4^{3-}	−1,019
SO_4^{2-}	−744
HCO_3^-	−587
OH^-	−157
Cl^-	−131
NO_3^-	−109
Br^-	−104
ClO_4^-	−8.5
ClO_3^-	−8.0

This low association with cations is responsible for the extremely high solubilities of perchlorate salts in aqueous and nonaqueous media. As noted, the ammonium and the alkali metal salts of perchlorate generally are readily soluble in water. Salts of the smaller univalent cations (i.e., ammonium [NH_4^+], lithium [Li^+], and sodium [Na^+]) are very soluble; whereas, those of the larger univalent cations are less so (i.e., potassium [K^+], rubidium [Rb^+], and cesium [Cs^+]). Quaternary ammonium salts are less soluble still. The outstanding example is sodium

perchlorate, which is extremely soluble (>8 mol dm^{-3}). Table 2-2 lists these solubilities as well as other key physicochemical properties.

TABLE 2-2. PHYSICOCHEMICAL PROPERTIES OF AMMONIUM AND ALKALI METAL PERCHLORATES AT 25 °C (Schilt, 1979).

	Magnitude of Physicochemical Property of Perchlorate					
Physical Property	NH_4	Li	Na	K	Rb	Cs
Molecular Weight (g mol^{-1})	117.49	106.40	122.44	138.55		
Density	1.952	2.429	2.499	2.5298	2.9	3.327
Solubility (w/w %)						
Water	24.922	59.71	209.6	2.062	1.338	2.000
Methanol	6.862	182.25	51.36	0.105	0.000	0.093
Ethanol	1.907	151.76	14.71	0.012	0.009	0.011
n-Propanol	0.387	105.00	4.888	0.010	0.006	0.006
Acetone	2.260	136.52	51.745	0.155	0.095	0.150
Ethyl Acetate	0.032	95.12	9.649	0.001	0.016	0.000
Ethyl Ether	0.000	113.72	0.000	0.000	0.000	0.000
Thermochemical data						
$\Delta H_f^{"}$, kJ mol^{-1}	−290.4	−384.0	−385.7	−435.5	−434.7	−434.7
$\Delta G_f^{"}$, kJ mol^{-1}	−88.9[b]	−254[c]	−255[b]	−304	−306	−307
$\Delta S_f^{"}$, kJ mol^{-1}	186[b]	130[c]	142[b]	151	161	175
$\Delta H_{soln}^{"}$, kJ mol^{-1}	−26.6	26.1	14.7	50.6	56.8	55.6
Magnetic susceptibility ($\times 10^6$)	46.3	32.8	37.6	47.4	—	69.9
Molar refraction	17.22	—	13.58	15.27	—	—

[a]Thermochemical data converted from kcal/mol using 1,000 cal = 4.184 J.
[b]Weast (1989).
[c]Dean (1985).

Because of their large solubilities, the health risk assessment for perchlorate anion (ClO_4^-) is appropriate for perchlorate salts, including ammonium perchlorate *[CASRN* 7790-98-9*]*, sodium perchlorate *[CASRN* 7601-89-0*]*, potassium perchlorate *[CASRN* 7778-74-7*]*, and

lithium perchlorate *[CASRN* 7791-03-9*]*. The estimate is not appropriate to characterize the risk of effects of perchloric acid ($HClO_4$) *[CASRN* 7601-90-3*]* because it is a strong acid, and the dominant mode of toxicity is the irritating action of the hydrogen ion on skin and mucous membranes.

Perchlorate can be a strong oxidizing agent under certain conditions as indicated by its high reduction potential; therefore, the question has arisen as to whether or not it has the potential to behave as an oxidant in biological systems. The thermodynamics of the halogen oxoanions and oxoacids to participate in redox reactions are well understood. Under standard conditions in 1 M acid, where the species is reduced to chloride, the oxidizing strength and standard reduction potential ($E°$) increase as follows: $Cl_2 < HOCl < HClO_2 < ClO_3^- < ClO_4^-$. The reduction potentials for the oxoanions increase with increasing acidity or decreasing pH (i.e., they are stronger oxidizing agents in acidic solution). Consider, for example, the reduction of chlorine(VII) to chlorine(V) under both acidic and alkaline conditions. In 1.0 M $H^+(aq)$ solution (pH = 0),

$$ClO_4^- + 2\ H^+ + 2\ e^- \rightarrow ClO_3^- + H_2O, \qquad E° = 1.20\ V. \tag{2-1}$$

In 1.0 M $OH^-(aq)$ solution (pH = 14),

$$ClO_4^- + H_2O + 2\ e^- \rightarrow ClO_3^- + 2\ OH^-, \qquad E° = 0.37\ V. \tag{2-2}$$

The effect of pH can be explained in terms of Le Châtelier's principle. In Reaction 2-1, hydrogen ion is plentiful and acts a reactant; this drives the reaction forwards. In Reaction 2-2, hydroxide ion is a product of the reaction and is already present at 1 M. This reduces the driving force for this reaction to take place. The reaction is still spontaneous, as shown by the positive value of $E°$; nonetheless, the driving force is considerably smaller for this case. Thermodynamically, perchlorate is a stronger oxidant in the chlorine oxoanion series at the extremes of the pH scale; however, such extremes are difficult to achieve in vivo (Tsui, 1998).

In Chapter 1, perchlorate anion was described as a nonlabile oxidant. Although the driving force for reduction is very high, the activation energy required to start the process is also very high. With the chlorine oxoanions, kinetic lability runs counter to the thermodynamic stability.

That is, the most stable species, hypochlorite (ClO^-), reacts fastest; whereas, the least stable species, perchlorate (ClO_4^-), reacts the slowest. It is important to note that the activation energy required for the reduction of perchlorate to take place is a function not only of the perchlorate, but also of the chemical nature of the reductant. With common reducing agents (e.g., thiosulfate, sulfite, or ferrous ions), the activation energy is too high for any reaction to be observed. In fact, this property (lack of lability) is exploited routinely in chemical studies where perchlorate salts are used to control the ionic medium and strength, but do not themselves react.

An alternative way of expressing the thermodynamic driving force for a reaction is the Gibbs free energy function. Although the driving force for redox reactions is often conveniently expressed in terms of the potential, there are practical limitations to this approach. For example, in the decomposition reaction of ammonium perchlorate in Equation 1-1, an electric potential cannot be measured. The Gibbs free energy of reaction, ΔG°_{rxn}, is a measure of the energy available to do work when a reaction is performed under constant pressure at standard state conditions.[1] When ammonium perchlorate explodes, the gaseous products push against the surrounding air and thereby perform expansion work on the atmosphere.[2] ΔG°_{rxn} specifies the maximal nonexpansion mechanical work that can be obtained from a chemical reaction carried out at constant temperature and pressure.[3] If the nonexpansion work is the electrical work of a redox process, then an additional relationship applies (Equation 2-3), where n is the number of electrons transferred; F is the Faraday constant, 96,485 C (mol e)$^{-1}$; and E° is the electric potential for the reaction under standard state conditions.

[1]This is the case with reactions occurring exposed to the open air, rather than inside a sealed container. In a sealed container, where volume is constant and pressure changes, a different thermodynamic quantity, the Helmholtz free energy ΔA°_{rxn}, is used instead. The superscript circle indicates standard state conditions (i.e., solution concentrations of 1 mol dm^{-3} and gas pressures of 1 bar). All thermodynamic data are for a temperature of 298 K. All of the thermodynamic relationships herein apply at other conditions, and reference tables exist only for standard conditions. For other conditions, appropriate corrections must be made.

[2]Expansion work (W_{exp}) is significant only when a reaction has a net change in the number of gas molecules and can be calculated from the equation of state for a perfect gas: $W_{exp} = -P\Delta V = \Delta nRT$ (where P = pressure (atm), V = volume (L), n = number of moles, R = ideal gas constant (L atm k^{-1}), and T = temperature (K) and T and P are constant). For reactions occurring in the condensed phases, $W_{exp} \approx 0$.

[3]To obtain the maximal nonexpansion work, it is assumed that the process occurs reversibly so the loss of energy as heat is minimized. Although this is approximately true for an electrochemical cell, most chemical reactions do not take place under conditions that approach reversibility. For example, explosions are so irreversible because so much internal energy is lost as heat that the nonexpansion work is much smaller than ΔG°_{rxn}.

$$\Delta G^\circ_{rxn} = -w_{max} = -nFE^\circ \qquad (T, P \text{ constant}) \tag{2-3}$$

The negative sign is necessary because the work done on the environment represents a loss of free energy from the chemical system. Nonexpansion work includes, but is not limited to, causing an electric current to flow or lifting an object against gravity. Whenever a chemical reaction has the ability to do work on the surroundings, it will take place spontaneously.[4] ΔG°_{rxn} is calculated as follows using Hess's law:

$$\Delta G^\circ_{rxn} = \Sigma\, \Delta G^\circ_f \text{ (all products)} - \Sigma\, \Delta G^\circ_f \text{ (all reactants).} \tag{2-4}$$

The Gibbs free energy of formation, ΔG°_f, is calculated for the formation of a compound from its standard state as an element; consequently, $\Delta G^\circ_f = 0$ for $Cl_2(g)$ and $O_2(g)$. For Reaction 1-1,

$$\begin{aligned}\Delta G^\circ_{rxn} &= 2\Delta G^\circ_f\ [N_2O(g)] + 8\Delta G^\circ_f\ [H_2O(g)] - 4\Delta G^\circ_f\ [NH_4ClO_4(s)] \\ &= 2(104) + 8(-229) - 4(-89)\ \text{kJ} = -1{,}268\ \text{kJ}.\end{aligned} \tag{2-5}$$

This large negative value for ΔG°_{rxn} suggests that the decomposition of ammonium perchlorate is spontaneous and has a large quantity of energy available to do work. When 4 moles (468 g) of ammonium perchlorate decompose, enough energy is released to lift a 1 kg mass 130 km, heat and completely boil 0.5 kg of water (starting from 25 °C) , or power a 100-W light bulb for 3.5 h. Each molecule contains a large amount of potential chemical energy; however, a handful of ammonium perchlorate will not spontaneously explode. The free energy is not released because the reaction kinetics are too slow at room temperature—only an infinitesimal fraction of the molecules possesses enough energy to reach the activation energy of the transition state at any point. The activation energy for the reaction between an ammonium cation and a perchlorate anion also is too great for a reaction to occur.

[4]Readers who have studied thermodynamics will recall that the determining factor for the spontaneity of a chemical process is a net increase in the entropy of the universe (i.e., $\Delta S^\circ_{univ} > 0$). It can be shown that $\Delta G^\circ_{rxn} = -T\Delta S^\circ_{univ}$; therefore, $\Delta S^\circ_{univ} > 0$ means $\Delta G^\circ_{rxn} < 0$, and $\Delta S^\circ_{univ} > 0$ means $\Delta G^\circ_{rxn} < 0$ (because $T > 0$). As a consequence of these relationships, it can be stated definitively that negative free energy available to do positive nonexpansion work is a measure of the thermodynamic spontaneity of a chemical reaction. This implies that any chemical reaction capable of performing positive nonexpansion work will occur spontaneously. Conversely, positive free energy suggests that the reverse reaction is spontaneous.

The distinction between thermodynamic spontaneity and kinetic lability must be emphasized. A reaction with $\Delta G^\circ_{rxn} << 0$ and $E^\circ >> 0$ is thermodynamically favored, but may be so slow as to take virtually an infinite amount of time to occur (as is the case with most perchlorate reductions). On the other hand, a reaction that occurs very quickly may have a very small driving force. Reaction rates are fast when the combined internal energies of the reactants closely approach the activation energy required to form the transition state. In a similar case, the kinetic barrier (activation energy) explains why an open gas jet does not burst into flame until the heat of a match is applied.

It is well established that, in aqueous solution, chlorine(I), chlorine(III), and chlorine(V) species undergo their most facile reductions via nucleophilic attack at the chlorine atom rather than at the oxygen atom. When oxoanions are dissolved in water, the rate of net oxygen atom exchange (Equation 2-6) can be used to understand how reactions proceed:

$$OClO_n^- + H_2Ø \rightleftharpoons ØClO_n^- + H_2O, \quad Ø \text{ a labeled oxygen atom; } 0 \le n \le 3. \tag{2-6}$$

Reaction 2-6 proceeds through an associative mechanism in which the incoming water molecule attacks the central chlorine atom. Consider the simplest example, hypochlorous acid, for which the following mechanism is the accepted explanation (where Ø is again a labeled oxygen atom):

$$HOCl + H_2Ø \rightleftharpoons [HO\cdots Cl\cdots ØH_2]^\ddagger \rightleftharpoons OH^- + ClØH + H^+ \overset{H_2O}{\rightleftharpoons} H_2O + ClØH \tag{2-7}$$

The aquated species $[HO\cdots Cl\cdots ØH_2]^\ddagger$ represents the activated complex and is the transition state of Reaction 2-7; the proton is not directly transferred from the labeled water molecule to the hydroxide that is part of the HOCl molecule. Rather, a proton is lost to the bulk water of the solution form the activated complex, and another proton is gained. This activated complex may revert back to reactants or proceed to products.[5] As the number of oxygen atoms increases, the water has greater difficulty accessing the reaction site. The oxidation state of the chlorine increases by 2 with each additional oxygen atom; accordingly, the chlorine becomes more and

[5]Note that $\Delta G^\circ_{rxn} = 0$ because the reactants and products are chemically identical. This suggests a process at equilibrium in which the forward and reverse rates are balanced.

more electron-poor and holds the oxygen atoms closer to share their electrons. (This factor will be expanded on further when perchlorate is examined specifically.)

In perchlorate, which contains chlorine(VII), the central chlorine atom is sterically blocked from the attack of an incoming reducing agent by the tetrahedrally oriented oxygen atoms. As the oxidation state of the central chlorine atom increases, the strength of the chlorine-oxygen bonds also increases. The electron-deficient chlorine(VII) draws electron density from the oxygen ligands resulting in increased O($p\pi$)→Cl($d\pi$) back donation despite the high electronegativity of the oxygen atoms. Increased O-Cl bond strength thus further complicates oxoanion reduction by making oxygen-atom abstraction even more difficult.

Perchloric acid normally exhibits oxidizing behavior when heated and concentrated. When cold and dilute, $HClO_4$ acts only as a strong Brønsted-Lowry acid with no more oxidizing character than other mineral acids, such as sulfuric or hydrochloric acids. In the absence of free H^+, as in vivo, a reducer or a catalyst with a lot of free potential energy would be requisite to increase the rate (Tsui, 1998).

All observable perchlorate reductions reported in the literature are initiated via oxygen atom abstraction by air-sensitive transition metal species (Urbansky, 1998). The metal cations that react with perchlorate are all sensitive to atmospheric oxygen because they are strong (thermodynamically) and labile (kinetically facile) reductants. None of these metal ions would survive under human physiologic conditions. Certainly, any reductant capable of reacting with perchlorate, such as Ti^{III}(aq) (Earley et al., 2000), Ch_3ReO_2 (Abu-Omar et al., 1996), or certain Re^V complexes (Abu-Omar et al., 2000) would not survive in a physiologic environment. Thus, the activation energy to perchlorate reduction is so high that perchlorate cannot be expected to act as an oxidant under human physiological conditions (i.e., dilute solution, moderate temperatures, and nearly neutral pH). This is supported by absorption, distribution, metabolism, and elimination studies that show perchlorate is excreted virtually unchanged after absorption (see Chapters 3 and 6).

A catalyst increases the rate of chemical reactions by reducing the activation energy, increasing the number of collisions, or properly orienting chemical reactants. Many catalysts reduce the activation energy, but some have multiple effects. When a perchlorate ion collides with a reducing agent, the two entities can recoil unaffected or they can interact. If they interact, the entity they form is called an activated complex and is a transition state from which they can

separate or react. If they have sufficient internal energy (enough to overcome the activation energy), the species will react. For perchlorate, this means an oxygen atom is transferred to the reductant. If a catalyst is involved, it can act as an intermediate, removing oxygen atoms from the perchlorate and transferring them to the reductant. In the case of the rhenium (V) catalysts, the coordinated rhenium center accepts oxygen atoms from (and is therefore oxidized by) the perchlorate. This oxidized species (now containing Re^{VII}) then transfers an oxygen atom to (and is therefore reduced by) any reducing agent; however, the authors used thioethers and mercaptans for this purpose (Abu-Omar et al., 2000). Of particular interest in this work was that the conditions were not nearly so forcing as what is normally required for perchlorate reduction. The reaction took place at roughly neutral pHs and ambient temperatures.

Some bacteria have catalysts (i.e, enzymes known as reductases) that allow the microbes to use perchlorate as an oxidant in anaerobic metabolic pathways. Although oxygen is a stronger oxidant than perchlorate, bacteria will utilize perchlorate under low-oxygen conditions. For example, perchlorate-reducing monera use perchlorate reductases under conditions where conventional inorganic chemistry suggests that perchlorate reduction should be imperceptibly slow (Urbansky, 1998; Logan, 1998). Over the past few years, there has been a profusion of work in this area, mostly slanted towards bioremediation (Coates et al., 1999, 2000; Logan, 2001; Nzengung and Wang, 2000).

This chapter provides a brief summary of some physiochemical properties of the perchlorate anion, especially the salient features that might bear on its environmental and toxicological chemistry. Additional chemical issues are explored in some depth in Chapter 9 as related to analysis of environmental samples. Additional chemical-specific issues as related to the pharmacokinetics of perchlorate in organisms are discussed in Chapters 3 and 6.

3. TOXICOKINETICS/TOXICODYNAMICS AND MODE-OF-ACTION TESTING STRATEGY

This chapter explains the rationale that was the basis of the testing strategy which was designed to evaluate the potential critical targets for perchlorate and to establish a database robust enough to support a quantitative risk assessment. Aspects of the toxicokinetics and toxicodynamics of perchlorate and its interaction with the thyroid are discussed as the basis for the development of a testing strategy based on the mode of action of perchlorate. *Mode of action* is defined as a chemical's influence on molecular, cellular, and physiological functions (Federal Register, 1996; Wiltse and Dellarco, 1996). Understanding the mode of action helps to interpret the relevancy of the laboratory animal and human data to inform the most appropriate dose-response procedure (see Chapter 7).

As discussed in Chapter 2, perchlorate salts dissolve readily in water. The resultant anion is easily absorbed from the gastrointestinal tract. However, because of its high charge, neither perchlorate, nor other electrolytes applied from aqueous solution or aqueous media penetrate the skin readily (Scheuplein and Bronaugh, 1983). Uptake of inorganic ions such as perchlorate through the skin is typically less than 10% and frequently less than 1%. Exposure via inhalation of fumes or vapors is considered negligible because the vapor pressure of perchlorate salts and acids is low at room temperatures. The risk from exposure to particles would depend on the particle size distribution. Thus, the ingestion route is the major concern for the risk posed by the perchlorate contamination and is the focus of this characterization.

3.1 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION OF PERCHLORATE

Limited absorption, distribution, metabolism, and elimination (ADME) studies were in existence prior to the testing strategy discussed in Section 3.5. Although experimental studies in laboratory species and humans had been performed using radiolabeling techniques, most were at high concentrations, and the published data were expressed simply as thyroid:blood ratios of

radioactivity counts that provided no information on internal dose to biological tissues. Oral drinking water administration, the most relevant to the contamination issue, was not the norm. Time-course studies were very limited and essentially nonexistent for repeated administration. More importantly, no data existed on the co-administration of iodide (I^-) and perchlorate even though data were necessary to develop a physiologically based pharmacokinetic model (Fisher, 1998a). The following section describes the limited pharmacokinetic information that was considered when the data gap was highlighted during the development of protocols for the testing strategy. The development of physiologically-based pharmacokinetic models that describe ADME for perchlorate with data from the testing strategy will be discussed in Chapter 6.

Perchlorate appears to be eliminated rapidly, primarily in the urine (>90%), and virtually unchanged from both rats (Eichler and Hackenthal, 1962) and humans (Anbar et al., 1959). Durand (1938) measured urinary elimination from two human subjects who ingested 794 mg of sodium perchlorate in 100 g of water. Urinary elimination accounted for 50% of the administered dose within 5 hr and 95% within 48 hr. Half-lives have been reported for the rat ranging from <8 hr (95% in 60 hr) to ≈20 hr (Wolff, 1998). Stanbury and Wyngaarden (1952) reported that perchlorate appears in the urine within 10 to 15 min of oral dosing and that peak plasma levels occur within 3 hr. Perchlorate was reported to undergo a two-phased urinary elimination process in rats and calves. In rats, the first phase accounted for approximately 96% of the administered dose and had a half-life of 1 to 2 hr. The second phase accounted for 4% and had a half-life that ranged from 72 to 80 h. In calves, the first-phase half-life was reported to be 2 to 2.5 hr, and the second 23 to 27 hr (Selivanova et al., 1986, as cited in Allred, 1998). The kinetics of long-term administration of perchlorate have not been characterized. The distribution and metabolism of perchlorate and its relevance to potential toxicity in the thyroid will be discussed in greater detail in Section 3.3 following discussions of iodine metabolism and thyroid physiology in Section 3.2.

3.1.1 Human Studies

The majority of the human data on perchlorate ADME prior to the strategy was comprised of the therapeutic case and clinical studies of Graves' disease patients described in Section 4.2.2. These studies established the effect of perchlorate on the sodium (Na^+)-iodide (I^-) symporter (NIS) but were of limited use in establishing quantitative dose-response relationships.

Anbar et al. (1959) demonstrated that perchlorate was not metabolized in humans. Four patients were administered 200 mg (approximately 2.9 mg/kg using a default body weight of 70 kg) double-labeled $K^{36}Cl^{18}O_4$, and urine was collected 3 h after dosing. Perchlorate was found to be excreted at approximately 200 μg/min in the urine. Total urine radioactivity was distributed between ^{36}Cl , $^{36}Cl^{18}O_4^-$, $^{36}ClO_4^-$ and $^{36}Cl^-$ and indicated that perchlorate was excreted unchanged in the urine. No human data existed with which to adequately characterize the pharmacokinetics of perchlorate during steady-state, low-dose, repeated administration.

3.1.2 Laboratory Animal Studies

Although the perchlorate discharge test has been performed in rats (Atterwill et al., 1987), the procedure is very different than that used in humans and does not readily allow for comparison or extrapolation. Rats are dosed intraperitoneally (ip) with 100 μL (1 μCi) $^{125}I^-$, then dosed ip with potassium perchlorate at 5, 10, 25, or 50 mg/kg body weight from 1 to 6 h afterwards. Results are expressed as thyroid:blood ratios, which differ from how most human data are expressed. Additionally, the time points at which uptake is measured are highly dissimilar to those used in human studies.

Anbar et al. (1959) also attempted to confirm the lack of perchlorate accumulation and lack of metabolism in the thyroid in rats. White rats were injected ip with $^{36}KClO_4$, and the specific activity per gram of tissue was measured at 30 min, 4 hr, and 12 hr. The activity was greatest in the thyroid and peaked at 4 h. The salivary and adrenal glands also had high activity levels. Rabbits also were tested; the thyroid activity levels were again the highest of any tissue and peaked at 2 h. Rabbit testes had the next highest specific activities.

In one of the only co-administration studies, Anbar et al. (1959) simultaneously administered $^{131}I^-$ and $^{36}ClO_4^-$ in equimolar concentrations. The thyroid:blood specific activity for iodide was slightly higher than the ratio for perchlorate (1.80 and 1.69, respectively).

Halmi et al. (1956) examined iodide uptake in male Sprague-Dawley rats when active transport was completely blocked via sodium perchlorate. The rats were first administered 6 mg of propylthiouracil (PTU) subcutaneously to prevent iodide organification. Iodide uptake was prevented by administration of 100, 200, or 400 mg sodium perchlorate with half of each dose administered along with the PTU and the other half administered 45 min later with 5 to 50 μCi $^{131}I^-$. The rats were sacrificed 1.0 to 1.5 h after the iodide administration. Perchlorate reduced the

thyroid:blood ratio from 22.7 to 0.45; radioiodide was found to account for 30% of the thyroid gland volume when it entered the gland by diffusion alone. Rats sacrificed 4.0 to 4.5 h after iodide administration produced similar results, indicating that equilibrium is reached prior to 1.0 to 1.5 h. The distribution of radioiodide in other tissues also was examined. Perchlorate did not affect the organ:serum iodide ratios in the following organs: submaxillary salivary gland, parotid salivary gland, pituitary gland, adrenal glands, testes, spleen, kidneys, lung, skin, or diaphragm. However, perchlorate administration did affect the stomach wall:serum and gastric juice:serum iodide ratios (0.36 and 0.75, respectively) compared with the ratios for controls administered sodium chloride (1.45 and 15.8, respectively). This suggested a gastric iodide pump subject to inhibition by perchlorate and, as will be discussed in Chapter 6, the gastrointestinal tract is another tissue with NIS.

Goldman and Stanbury (1973) administered 0.1 μCi of the potassium salt of ^{36}Cl-labeled perchlorate ($K^{36}ClO_4$) by ip injection to male Sprague-Dawley rats that had been maintained on a low-iodine diet for 4.5 to 5.0 weeks prior to dosing (approximately 40 μg stable perchlorate per injection). The radionucleide retention in the thyroid, expressed as percent of dose per gram of tissue, was recorded at 2 h (6 rats), 4 h (5 rats), 8 h (6 rats), 24 h (6 rats), 48 h (6 rats), and 96 h (5 rats). The peak was reported to appear around 4 h and then to fall to approximately 5% of this peak value after approximately 96 h. An exponential function was used to estimate a half-life of 20 h. Urinary excretion data indicated that the disappearance rate from the plasma and thyroid and the appearance rate in the urine corresponded closely although the question was raised as to whether there is some curvilinearity to the urinary excretion, which may suggest limited saturation. The retained dose and its standard deviation in tissues at 96 h were reported as 0.142 ± 0.1, 0.125 ± 0.09, 0.098 ± 0.03, 0.048 ± 0.04, and background for the thyroid, kidney, spleen, liver, and brain, respectively.

Chow et al. (1969) compared the uptake of radiolabeled perchlorate and iodide ions with stable ions in normal and thyroid-impaired rodents. Intact male Sprague-Dawley rats were injected ip with 0.1, 0.2, or 5.0 meq/kg stable potassium perchlorate (14, 28, or 690 mg/kg, respectively) 2 h prior to sacrifice. The specific activity of the chlorine label ($^{36}Cl^-$) was 25.2 μCi/mmol. Thyroid impairment was affected by pretreatment with thyroid-stimulating hormone (TSH) (1 international unit [IU] TSH in 0.9% saline solution ip 18 h prior to perchlorate administration), hypophysectomization (removal of the pituitary), TSH and

hypophysectomization, or PTU (0.1% PTU in drinking water for 2 weeks prior to perchlorate administration). Perchlorate at the 0.1- and 0.2-meq/kg dose levels was found to preferentially concentrate in the rat thyroid as compared to the plasma, and the concentration was related inversely to dose. The high dose level did not result in the concentration of radiolabeled perchlorate in the thyroid. Rats pretreated with TSH or PTU also concentrated perchlorate at the lower dose levels. At the two lower levels, hypophysectomized rats were not able to concentrate perchlorate compared with intact rats, but the thyroid perchlorate concentration at the high dose level did not differ between intact and altered rats. In a second subset of the same study, rats were exposed to 0.005, 0.01, 0.02, 0.05, or 0.10 meq/kg perchlorate (0.69, 1.4, 2.8, 6.9, or 14 mg/kg, respectively) under the same general conditions. The concentration of radiolabeled perchlorate in the thyroid again was related inversely to perchlorate dose. Male albino guinea pigs also were exposed to the same doses. The guinea pigs displayed the same relationships as the rats, but concentrated more perchlorate in the thyroid compared to plasma levels.

Chow and Woodbury (1970) demonstrated that perchlorate is actively sequestered by the thyroid gland at low doses but that the capacity of the symporter to actively sequester perchlorate is exceeded at higher doses. Male Sprague-Dawley rats were functionally nephrectomized by ligating the renal pedicle of both kidneys 24 h before the rats were sacrificed. Perchlorate was administered as the radiolabeled potassium salt ($K^{36}ClO_4$) in solution by ip injection at 0.005, 0.1, or 2.0 mmol/kg stable potassium perchlorate (0.69, 14, and 280 mg/kg body weight, respectively, assuming 0.266 kg body weight; actual weight 226 ± 4 g) 2 to 240 min before sacrifice. A group of control rats received [^{14}C]-insulin, $^{35}SO_4^{-2}$ or $^{36}Cl^-$ 2 h prior to sacrifice to determine thyroid follicle volume and intrafollicular membrane potential. Concentrations of perchlorate in the thyroid and plasma were measured at 0.033, 0.067, 0.13, 0.2, 0.50, 1.0, 2.0, and 4.0 h after sacrifice. Again, perchlorate was actively sequestered by the thyroid gland at the low dose, but the capacity of the symporter to actively sequester perchlorate was exceeded at the higher doses (e.g., the thyroid:plasma [milligrams per gram:milligrams per liter] ratios at 15 min or 4 h post-dosing were 6.4, 0.69, and 0.36 or 13.8, 0.93, and 0.44 at the 0.5, 14.0, or 280.0 mg/kg doses, respectively). These data suggest that maximal inhibition by perchlorate of active uptake of iodide probably occurs below 14 mg/kg potassium perchlorate (10.0 mg/kg as perchlorate). If perchlorate-induced inhibition of active iodide uptake is substantial, iodide still may enter the thyroid by diffusion, but in a smaller amount. Likewise, if inhibition of iodide

uptake by perchlorate is incomplete, then iodide still may be actively sequestered into the thyroid, again in a smaller amount. Thus, perchlorate-induced thyroid hormone perturbations may plateau in adult rats dosed with perchlorate greater than approximately 5 to 10 mg/kg of perchlorate (Fisher, 1998a).

Wolff and Maurey (1962) demonstrated the competitive nature of the perchlorate inhibition in sheep thyroid tissue slices incubated at 37 °C for 100 min. This study showed that the K_m constants for anion accumulation and the K_i constants for inhibition of accumulation were identical within the error of the method.

Eichler and Hackenthal (1962) presented perchlorate elimination data for male and female Wistar rats dosed subcutaneously with 0.2, 1.0, or 6.0 of the $^{36}Cl^-$ sodium perchlorate salt ($Na^{36}ClO_4$) per 100 g body weight (2, 10, or 60 mg/kg). The elimination curves showed nearly linear, rapid excretion of perchlorate until 6 hr, at which time the curve slope started to decrease. The rate of excretion increased with dose. The elimination rates of the different doses prior to 24 h were significantly different from each other but were similar after 24 h. Over 60 hr, 93.4 to 97.4% of the administered dose was recovered, again suggesting that perchlorate was not metabolized.

In a recent review (Von Burg, 1995), perchlorate elimination curves in rats and calves were described as biphasic in both species. For rats, 96% of administered perchlorate is eliminated with a half-life of 1 to 2 hr. The second portion of the curve accounts for 4% of the dose, with half-life of 72 to 80 hr. Calves have a faster overall rate of elimination, but the initial elimination is slower. The first-phase half-life was 2.0 to 2.5 hr, and the second-phase half-life ranged from 23 to 27 hr.

An intravenous (iv) study performed at AFRL/HEST in Sprague-Dawley rats with perchlorate to characterize its inhibition of iodide uptake supports the conclusion that there is inhibition at low concentrations and there is a gradual plateau at higher concentrations (Meyer, 1998). Rats were dosed once by iv tail-vein injection with either 0.01, 0.1, 1.0, or 3.0 mg/kg of cold (i.e., not radiolabeled) ammonium perchlorate in saline. Perchlorate was administered as ammonium perchlorate, and the data are presented as milligrams perchlorate per kilogram body weight. Two hours after dosing with perchlorate, the rats were dosed again by iv tail-vein injection with 33 μg/kg ^{125}I dissolved in saline. Rats were sacrificed at selected times (n = 6 per time point) up to 24 h. Total and free ^{125}I were measured in serum, thyroid, and urine.

Perchlorate serum, thyroid, tissue, and urine analyses began in January 1999 and are reported in Chapter 6. For control comparison, rats were dosed once by iv tail-vein injection with 33 μg/kg nonradiolabeled iodide and ^{125}I mixed in physiologic saline. Rats (n = 6) were sacrificed at the same selected time points up to 24 hr.

Table 3-1 shows the percent of inhibition of ^{125}I uptake as measured by bound ^{125}I in the thyroid. Inhibition of ^{125}I uptake into the thyroid by perchlorate was measured by bound or free ^{125}I in the thyroid at various time points after the single-dose of perchlorate. Because the ^{125}I was administered 2 hr after dosing with ammonium perchlorate, these time points correspond to 4, 8, and 11 h after dosing. The most profound inhibitory effects were found at the 1.0- and 3.0-mg perchlorate/kg dose group; however, the trend for ^{125}I inhibition is evident at the 0.01- and 0.1-mg/kg levels (Meyer, 1998). By 24 h (26 h after dosing with perchlorate), inhibitory effects on ^{125}I uptake were still observed at the 1.0- and 3.0-mg/kg dose groups.

Recovery of ^{125}I in urine 24 hr after dosing with ^{125}I (26 h after ammonium perchlorate) was between 79 and 88% for control ^{125}I-dosed rats and perchlorate-dosed rats. The control ^{125}I-dosed rats excreted 79.5% (SD ± 5.50) of the ^{125}I dose over the 24-hr period; whereas, the perchlorate-dosed rats excreted 87% (SD ± 7.84), 86% (SD ± 4.47), 87.8 (SD ± 20.20) and 79.3 (SD ± 10.58) of the ^{125}I dose in urine at the 0.01-, 0.1-, 1.0-, and 3.0-mg/kg dose levels, respectively. The amount of ^{125}I in serum was elevated in the perchlorate-dosed animals compared to the control ^{125}I-dosed rats for up to 6 hr in all dose groups, suggesting that thyroid function was altered by perchlorate and that a transient "discharge" of organified ^{125}I occurred as reported in studies summarized in Chapter 3. Free ^{125}I levels in serum were similar between perchlorate-dosed and control ^{125}I-dosed rats (Meyer, 1998). These results are consistent with those of Chow et al. (1969) and Chow and Woodbury (1970). The pattern for the inhibition of iodide uptake, albeit only after a single dose, is strikingly similar to the patterns shown for the thyroid hormone decreases. Consequently, data on the species differences (i.e., rat versus human in particular) in perchlorate inhibition of the symporter will provide a basis for evaluating the degree of uncertainty that should be applied when utilizing laboratory animal data as the model for humans (see Chapter 7).

Repeated dose studies in rats (Fisher, 1998a) and in humans (Channel, 1998a) to establish the kinetics of perchlorate at steady-state performed by AFRL/HEST to further characterize the inhibition of iodide uptake by perchlorate are discussed in Chapter 6.

TABLE 3-1. PERCENT INHIBITION OF IODIDE UPTAKE IN THE THYROID GLAND OF SD RATS DOSED WITH PERCHLORATE (Meyer, 1998)

Time Points[a]	Dose (mg perchlorate/kg)	[Iodide] (μg/g)	Percentage of Inhibition
2 hr	Control[b]	24.4	—
	0.01	21.3	13
	0.1	18.6	24
	1	7.4	70
	3	2.99	88
6 hr	Control[b]	46.5	—
	0.01	36.7	21
	0.1	32.0	31
	1	19.2	59
	3	9.13	80
9 hr	Control[b]	55	—
	0.01	49.2	11
	0.1	39.2	29
	1	24.7	55
	3	10.0	82

[a]Time points correspond to dosing with ^{125}I and to 4, 6, and 11 hr after dosing with ammonium perchlorate.
[b]Dosed with only iodide (33 μg/kg).

3.2 IODINE METABOLISM AND THYROID PHYSIOLOGY

Iodine plays a central role in thyroid physiology as both a constituent of thyroid hormones and a regulator of thyroid gland function. Like perchlorate, iodine is absorbed efficiently from the gastrointestinal tract. Iodine in organic form is converted mostly to iodide before absorption (Cavalieri, 1997). The kidneys account for about two-thirds of the iodide cleared from plasma and more than 90% of the iodide cleared from the body. Sweat and breast milk account for various fractions of iodide loss, and fecal elimination constitutes approximately 1% of total body iodide clearance.

The thyroid gland concentrates iodide against an electrochemical gradient by a carrier-mediated mechanism driven by adenosine triphosphate (ATP). The activation energy required for perchlorate reduction is so high that it cannot act as an oxidant under physiological conditions (i.e., dilute solution, moderate temperatures, and neutral pH). Plasma membrane experiments indicate that the sodium cation (Na^+) and iodide cotransport are electrogenic, with a thermodynamically downhill transport of approximately two Na^+ ions driving one iodide ion against its electrochemical gradient into the cell. The transport is sensitive to ouabain, an inhibitor of ATPase. The molecule responsible for the transport of iodide has been named the *sodium (Nat)/iodide (I) symporter or NIS*. The thyroid thus has a specialized ability to concentrate iodide selectively from the surroundings where the concentration is very low (10^{-8} to 10^{-7} M) and where the concentration of chloride ions is in the order of 0.01 to 0.1 M. The transport is "active," not only by electrochemical criteria, but also by metabolic ones: it does not occur in the cold, it requires oxygen, and, as mentioned, it is a function of the ATP level. In addition to the thyroid, other organs that can concentrate iodide include the salivary glands, gastric mucosa, choroid plexus, mammary glands, and the placenta. Iodide secreted into the saliva and gastric juice is reabsorbed in the small intestine (Cavalieri, 1997).

Nevertheless, it is essentially only in the thyroid that the newly concentrated iodide can be metabolized further to form thyroid hormone; and, only in the thyroid, does TSH regulate the process. Thyroid hormones play numerous and profound roles in regulating metabolism, growth, development, and maintenance of homeostasis. It is generally thought that these actions result from the effects of the thyroid hormones on protein synthesis (Hill et al., 1989).

Figure 3-1 shows a schematic representation of thyroid hormone biosynthesis and secretion in a single thyroid follicular cell. The thyroid hormones are stored as amino acid residues in thyroglobulin (Tg), a protein constituting most of the colloid in the thyroid follicles. In situ, the follicular cell displays functional and structural polarity: the vascular space is at the bottom, and the lumen of the follicle is at the top. The striated circle straddling the basolateral membrane represents the iodide transporter. The process of thyroid hormone biosynthesis is first stimulated by TSH binding to the follicular cell TSH receptor and cyclic adenosine monophosphate (cAMP) activation (Hard, 1998). The protein portion of Tg is synthesized on rough endoplasmic reticulum (ER), and carbohydrate moieties are added by the Golgi apparatus (GA). Thyroglobulin proceeds to the apical surface in secretory vesicles (small open circles) that

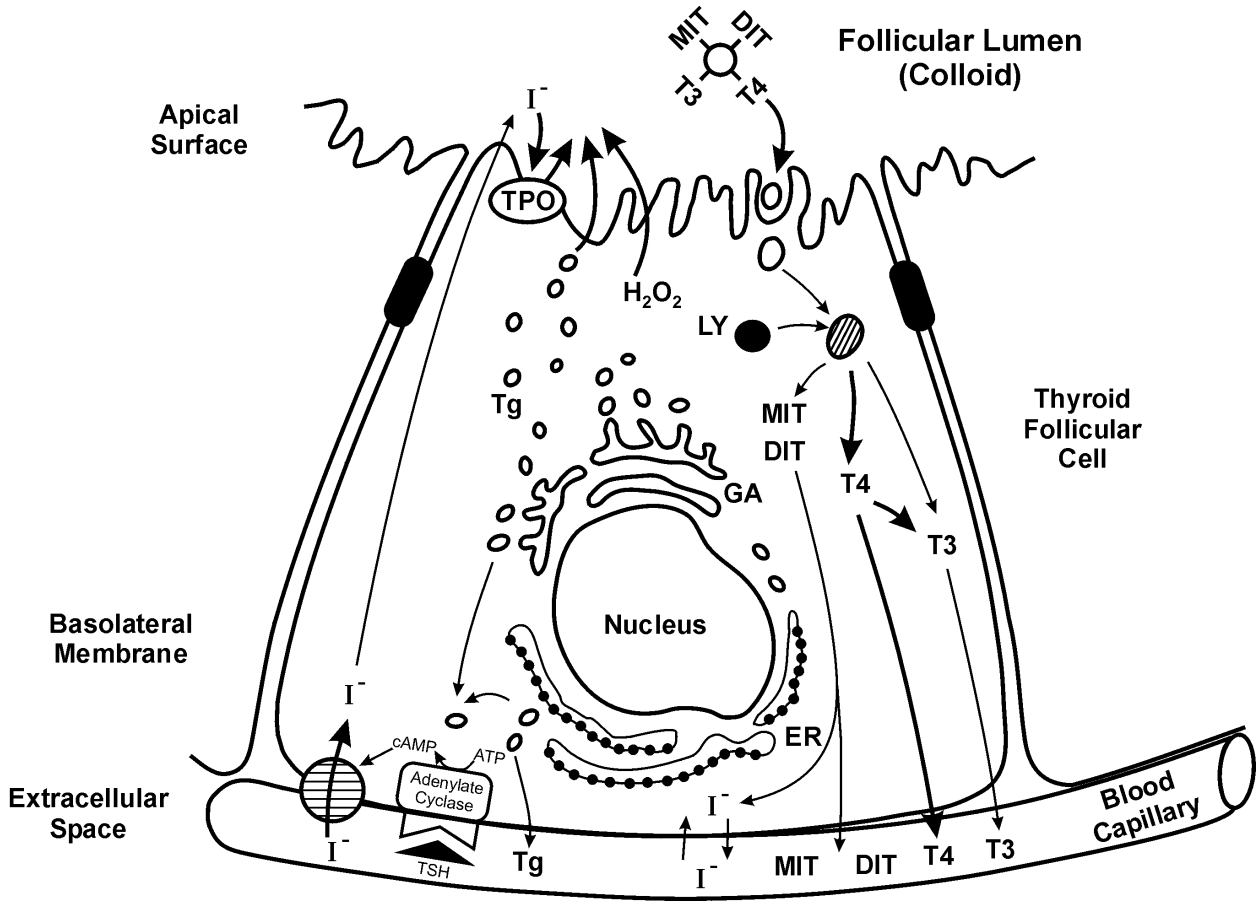

Figure 3-1. Schematic representation of thyroid hormone biosynthesis and secretion in a single thyroid follicular cell. (Modified from Hill et al., 1989; Cavalieri, 1997; and Fisher, 1996.)

fuse with the cell membrane and discharge their contents into the follicular lumen. Iodide enters the cell by active transport, and then, at the apical surface, is oxidized by thyroid peroxidase (TPO). The hydrogen-peroxide-generating system is represented by hydrogen peroxide (H_2O_2). Organification occurs at or near this apical cell-colloid interface; the oxidized iodide is incorporated into tyrosine residues in peptide linkage in Tg. Two iodinated tyrosyl groups couple in ether linkage to form tetraiodothyronine (T4), which initially remains trapped in Tg. Hormone secretion first involves pinocytosis of colloid-containing iodinated Tg (large open circle) at the apical border of the follicular lumen and resolution into vesicles that fuse with lysosomes (LY, dark circle). Lysosome proteolysis (striated circle) then converts Tg to amino acids, T4,

triiodothyronine (T3), diiodotyrosine (DIT) and monoiodotyrosine (MIT). Iodotryosine dehalogenase regenerates iodide from MIT and DIT for reuse within the thyroid or release into the blood, accounting for the iodide leak in the chronic state of iodine excess in certain thyroid disorders. Type I iodothyronine deiodinase converts a fraction of the free T4 to T3. Both hormones (T4 and T3) are released into the blood circulation by a process that is not well understood. The thyroid also releases Tg, of which some is iodinated and some uniodinated as newly synthesized protein.

Although T4 is by far the major hormone secreted by the thyroid (typically at 8 to 10 times the rate of T3), T4 is considered a prohormone because about 33% of the T4 secreted undergoes 5'-deiodination to T3 in the peripheral tissues and T3 is about fourfold more potent than T4. Another 40% undergoes deiodination of the inner ring to yield the inactive material, reverse triiodothyronine (rT3), which recently has been postulated to play an inhibitory role on the conversion of T4 to T3. T3 is regarded as the active hormone because it is the form that appears to activate a response by nuclear DNA. Upon entering the circulation, both T4 and T3 are bound and transported in strong, but not covalent, association with plasma proteins.

The major plasma-protein carrier in humans is thyroxine-binding globulin, a glycoprotein with a very high affinity for T4 and a lower affinity for T3. In rats, the T4 and T3 are bound to prealbumin (PA) or albumin with a weaker attachment. Control of the circulating concentrations of these hormones is regulated primarily by a negative feedback involving three organs: (1) the thyroid, which produces thyroid hormone, and (2) the pituitary gland and (3) hypothalamus, which respond to and help maintain optimal T3 and T4 levels (Hill et al., 1998). Figure 3-2 shows the schematic for this hypothalamic-pituitary-axis and the feedback mechanisms.

The hypothalamus stimulates the pituitary gland through thyrotropin-releasing hormone (TRH) to produce TSH, which prompts the thyroid to produce T4 and T3. Once secreted into the blood, T4 and T3 are bound to plasma proteins (thyroid-binding globulin [TBG] in humans or prealbumin [PA] and albumin in rats). In addition to the aforementioned conversion of T4 to T3 in peripheral tissues, thyroid hormone also is metabolized irreversibly in the liver by uridine diphosphyl glucuronosyl transferases (UDPGTs) to either glucuronic (T4) or sulfate (mainly T3) conjugates that are excreted in bile. A portion of the conjugated material is hydrolyzed in the intestine, and the free hormones thus released are reabsorbed into the blood via enterohepatic circulation. The remaining portion of the conjugated material is excreted in the feces.

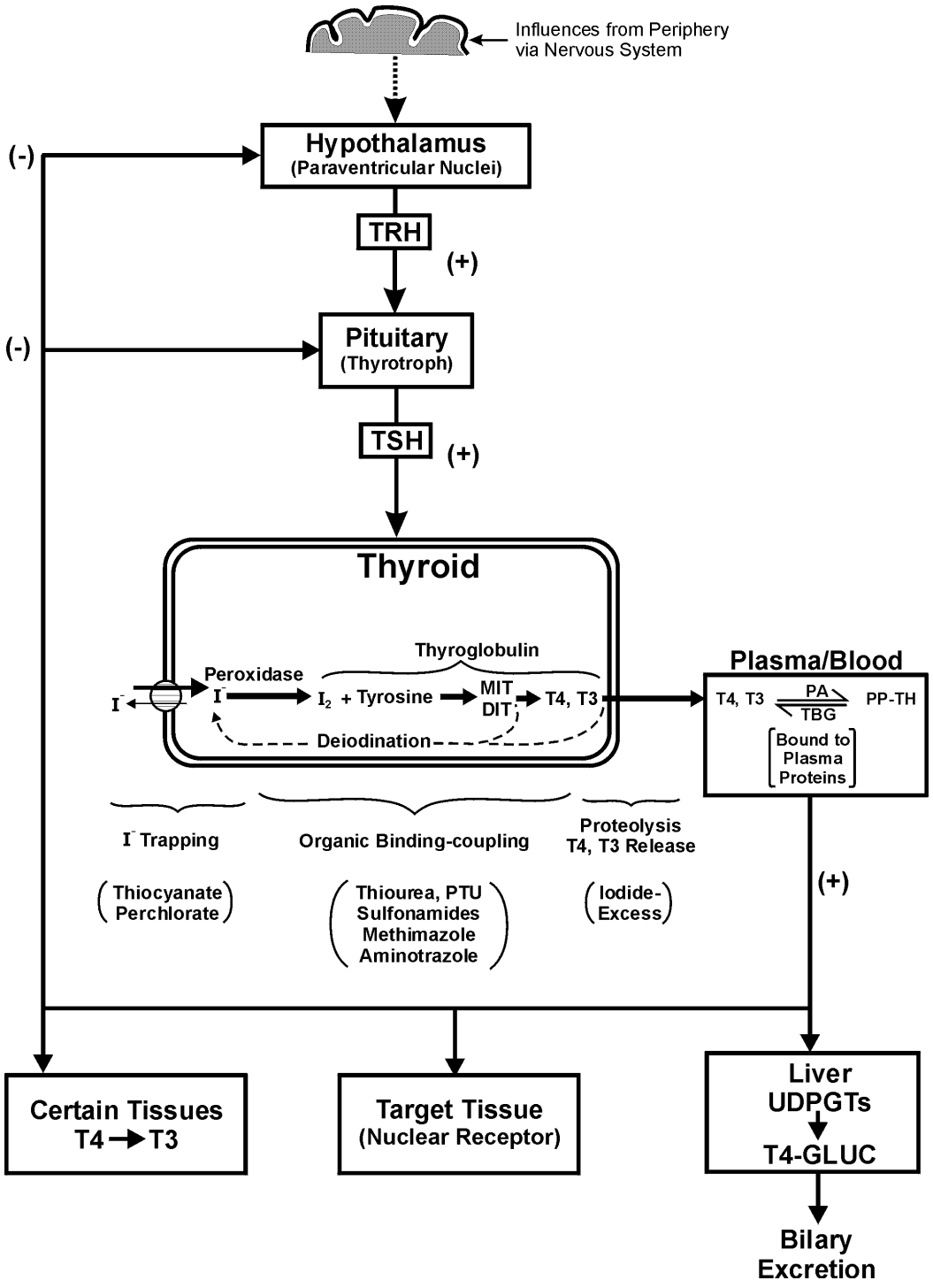

Figure 3-2. Schematic of the hypothalamic-pituitary-thyroid axis and feedback mechanisms (PP-TH = plasma protein-thyroid hormone, PTU = propylthiouracil, UDPGT = uridine diphosphyl glucuronosyl transferase, T4 GLUC = T4-glucuronide conjugate). (Modified from U.S. Environmental Protection Agency, 1998a; Hill et al., 1998; and Capen, 1997).

Cells in the hypothalamus and pituitary gland respond to levels of circulating T4 and T3 such that when thyroid production levels are high, there is a signal to reduce the output of (TRH) and TSH. Similarly, when thyroid hormone levels are low, the pituitary is prompted to deliver more TSH to the thyroid in order to increase the output of T4 and T3. This negative feedback loop helps the body respond to varying demands for thyroid hormone and to maintain hormone homeostasis. Thus circulating T4, T3, and TSH are monitored readily in experimental animals and humans and so may serve as biomarkers of exposure to and indicators of the effects from agents that disrupt the status of the hypothalamic-pituitary-thyroid axis (U.S. Environmental Protection Agency, 1998a).

In the absence of thyroid-binding globulin, as in the rat and mouse, a greater fraction of thyroid hormone is free of protein binding and subject to metabolism and removal from the body. As a consequence, the half-life of T4 in the rat is only about 1 to 24 hr, in contrast to the 6 to 7 day half-life in humans. Rats compensate for the increased turnover rate by secreting more TSH from the pituitary gland. Table 3-2 provides the interspecies and intraspecies differences in both thyroid hormone and gland structure between rats and humans. The consequences of disrupting the status of the hypothalamic-pituitary-axis will be discussed in Section 3.4.

3.3 TOXICOKINETICS OF PERCHLORATE

Because of the complex anatomy of the thyroid follicle, all of the locations where perchlorate inhibition is exerted remain to be established (Wolff, 1998). Perchlorate has been established as a competitive inhibitor of iodide uptake across the basolateral membrane (i.e., acts by the inhibition at NIS). Figure 3-3 shows a comparison of the molecular dimensions of perchlorate and iodide. The following potency series was constructed for monovalent anion-based inhibition of iodide transport in thyroid slices: $TcO_4^- \geq ClO_4^- > ReO_4^- > SCN^- > BF_4^- > I^- > NO_3^- > Br^- > Cl^-$ (Wolff, 1998). However, it is not clear whether this anion sequence, measured at very high concentrations, has any mechanistic relation to what occurs at low concentrations in the thyroid. It is important to determine which solution properties of the anions determine this sequence (e.g., crystal radius, hydrated radius, hydration enthalpy, charge density). Strong base anion-exchange resins (usually a large cation with a weak field) exhibit a marked preference for ClO_4^- (e.g., compared to Cl^-); thus, it seems likely that selectivity for iodide or perchlorate in the

TABLE 3-2. INTERSPECIES AND INTRASPECIES DIFFERENCES IN THYROID STRUCTURE AND T3, T4, AND TSH HORMONES (U.S. Environmental Protection Agency, 1998a)

Parameter	Human	Rat
Thyroxine-binding globulin	Present	Essentially absent
T_4 Half-life	5 to 6 Days	0.5 to 1 Day
T_3 Half-life	1 Day	0.25 Day
T_4 Production rate/kg body weight	1 ×	10 × that in humans
TSH	1 ×	6 to 60 × that in humans
Follicular cell morphology	Low cuboidal	Cuboidal
Sex differences		
Serum TSH	$M^a = F^a$	$M \leq 2 \times F$
Cancer sensitivity	$F = 2.5 \times M$	$M > F$

[a]M = male, F = female.

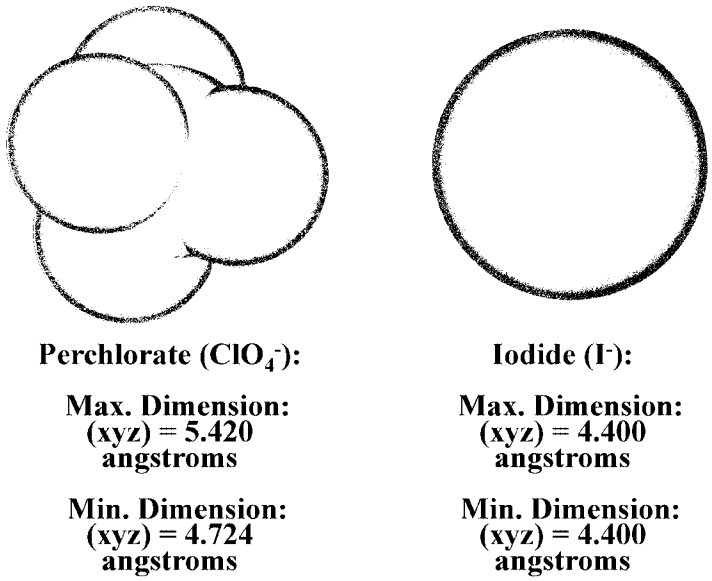

Figure 3-3. Comparison of the molecular dimensions for the perchlorate (left) and iodide (right) anions.

thyroid may be based on an anion-exchange mechanism using a large cation such as a quaternary amine (e.g., arginine) (Wolff, 1989).

Perchlorate also has been used to stimulate the efflux of iodide already stored in the follicular lumen of the gland (Atterwill et al., 1987). The exact nature of the mechanism for this effect has not been established, however. Transport of iodide out of the cell is downhill electrically, but this could be accounted for by the high concentration gradient that is established from follicular lumen (iodide stored in the colloid) to the basolateral and extracellular space. This may be the rate-limiting aspect for perchlorate efflux effect. Perchlorate added to the apical side of a polarized thyroid cell monolayer is substantially less effective than when added to the basolateral side (Wolff, 1998). Moreover, perchlorate rapidly increases the secretory response to TSH, and TSH increases iodide efflux before it increases iodide influx, suggesting that additional control points may exist.

Thus, perchlorate appears to have no effect on the iodination process itself but, rather, displaces iodide by competitive uptake at the NIS. Perchlorate is concentrated by thyroid tissue in a manner similar to iodide, but it is not significantly metabolized in the gland nor peripherally, as mentioned previously. It is not unequivocally established whether there are additional effects of perchlorate on iodide transport within the thyroid. Pharmacokinetic studies with perchlorate, both acute and particularly once steady state has been achieved, have provided some useful data with which to gain insight on this issue. The potential impacts as health endpoints of interest for human health risk assessment of this perturbation in the hypothalamic-pituitary-thyroid axis and hormone economy will be discussed in Section 3.4.

3.4 TOXICODYNAMICS OF THYROID HORMONE PERTURBATIONS

Given the established mode of action for perchlorate as the inhibition of iodide uptake at the NIS, it is important to distinguish the temporal aspects with respect to potential adverse tissue response.

3.4.1 Carcinogenic Effects

In higher organisms, when demands for more thyroid hormone are small, existing thyroid follicular cells can meet the demand. With increased need, as a result of certain chemical

exposures or iodine deficiency, the thyroid responds by increasing the size (hypertrophy) and number (hyperplasia) of thyroid follicular cells to enhance hormone output. With continued TSH stimulation, there is actual enlargement of the thyroid (goiter) and, at least in rodents, eventual neoplasia of the thyroid follicular cells. Because TSH-producing pituitary cells also are stimulated, they too sometimes undergo hyperplasia and neoplasia (U.S. Environmental Protection Agency, 1998a; Hill et al., 1998). The EPA Assessment of Thyroid Follicular Cell Tumors (U.S. Environmental Protection Agency, 1998a), as well as reviews recommended therein, provides details about thyroid follicular cell carcinogenesis. Figure 3-4 shows schematically the possible antithyroid effects that could influence carcinogenesis. Note that effects, not only in the thyroid but also in peripheral tissues and the liver, may cause demand on thyroid hormone production such that the TSH stimulation of the thyroid to produce more hormone is enlisted. Table 3-3 lists mechanisms of antithyroid-mediated neoplasia in rodents. The potential for an indirect effect of perchlorate has been established, but genotoxicity information was required to evaluate its potential for direct effects. As will be discussed in Section 3.5, a battery of genetoxicity assays was included in the testing strategy.

Long-term perturbations in the hypothalamic-pituitary-thyroid axis by the various influences listed in Table 3-3 are more likely to predispose the laboratory rat to a higher incidence of proliferative lesions (Capen, 1997). One factor that may play a role in this interspecies quantitative difference in sensitivity to thyroid stimulation is the influence of protein carriers of thyroid hormones in the blood (Table 3-2). Both humans and rodents have nonspecific, low-affinity protein carriers of thyroid hormones (e.g., albumin). However, in humans, other primates, and dogs, there is a high-affinity binding protein, thyroxine-binding globulin, which binds T4 (and T3 to a lesser degree). This protein is missing in rodents and lower vertebrates. As previously indicated, T4 is bound to proteins with lower affinity in the rodent and is more susceptible to removal from the blood, by metabolism, and through excretion than in dogs and primates.

In keeping with this finding, the serum half-life of T4 is much shorter in rats (less than 1 day) than it is in humans (5 to 9 days); this difference in T4 half-life results in a 10-fold greater requirement for exogenous T4 in the rat with a nonfunctioning thyroid than in the adult human. Serum T3 levels also show a species difference: the half-life in the rat is about 6 hr; whereas, it is about 24 hr in humans. High thyroid hormone synthetic activity is demonstrated in thyroid

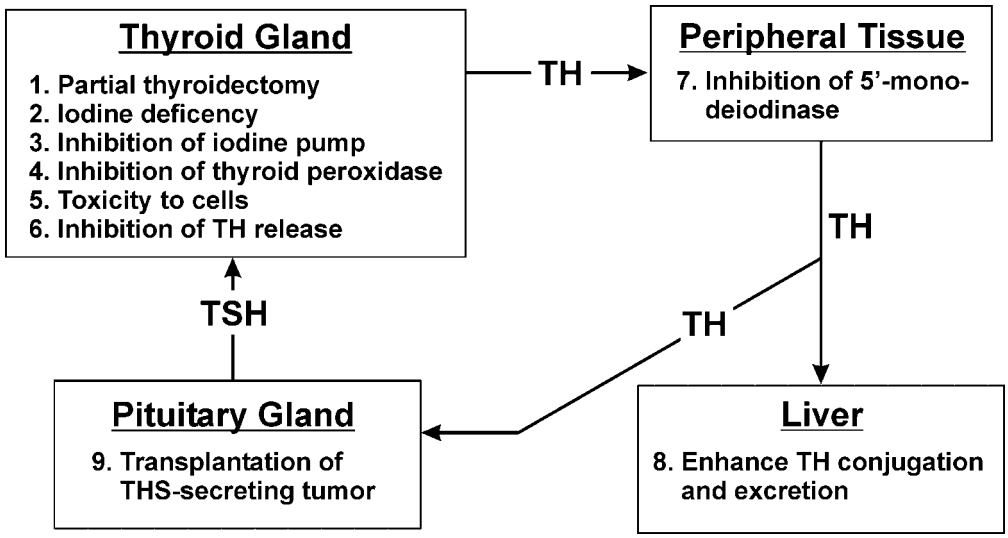

Figure 3-4. Schematic of antithyroid effects that influence thyroid carcinogenesis. (U. S. Environmental Protection Agency, 1998a; and Hill et al., 1998).

TABLE 3-3. MECHANISMS OF ANTITHYROID-MEDIATED NEOPLASIA IN RODENTS (U.S. Environmental Protection Agency, 1998a).

- **DNA Directed**
  - – X rays
  - – ^{131}I
  - – Genotoxic chemicals
- **Indirect**
  - – Partial thyroidectomy
  - – Transplantation of TSH-secreting pituitary tumors
  - – Iodide deficiency
  - – Chemicals inhibiting iodide uptake
  - – Chemicals inhibiting thyroid peroxidase
  - – Chemicals inhibiting TH
  - – Chemicals inhibiting conversion of T3 and T4
  - – Chemical inhibiting hepatic thyroid hormone metabolism and excretion

follicles in rodents, where the follicles are relatively small and are surrounded by cuboidal epithelium. Follicles in primates demonstrate less activity and are large with abundant colloid, and follicular cells are relatively flattened (low cuboidal) (McClain, 1992).

The accelerated production of thyroid hormones in the rat is driven by serum TSH levels that are probably about 6- to 60-fold higher than in humans. This assumes a basal TSH level in rats and humans of 200 ng/mL and 5 μU/mL, respectively, and a potency of human TSH of 1.5 to 15 IU/mg of hormone (U.S. Environmental Protection Agency, 1998a). Thus, it appears that the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased turnover of thyroid hormones. It follows that increases in TSH levels above basal levels in rats could more readily move the gland towards increased growth and potential neoplastic change than in humans. In addition to considerations about the influence of serum thyroid hormone carrier proteins, there are differences between humans and laboratory animals in size and life span and in the pharmacokinetics and pharmacodynamics of endogenous and exogenous chemicals. Any comparison of thyroid carcinogenic responses across species should be cognizant of all these factors.

A number of goitrogenic compounds, those that either interfere with thyroid hormone synthesis or secretion, have been demonstrated to result in thyroid follicular cell adenomas in rats. Excessive secretion of TSH alone has been reported to produce a high incidence of thyroid follicular cell adenomas. The pathogenic mechanism of thyroid follicular cell tumor development in rodents involves a sustained excessive stimulation of the thyroid by TSH. In the multistage model of this pathogenesis, the proliferative lesions often begin as hyperplasia, may proceed to the development of benign tumor (adenomas), and infrequently develop into malignant tumors (Figure 3-5).

The precise molecular steps in the carcinogenic process leading to thyroid follicular cell cancer have not been elucidated totally although significant insights into the problem have been described (Farid et al., 1994; Said et al., 1994). Normal cell division in the thyroid seems to be affected by an interplay among several mitogenic factors, namely TSH, insulin-like growth factor-1 (IGF-1), insulin, epidermal growth factor (EGF), and possibly fibroblast growth factor (FGF). Additionally, other factors, such as transforming growth factor β, certain interferons, and interleukin 1, may inhibit growth.

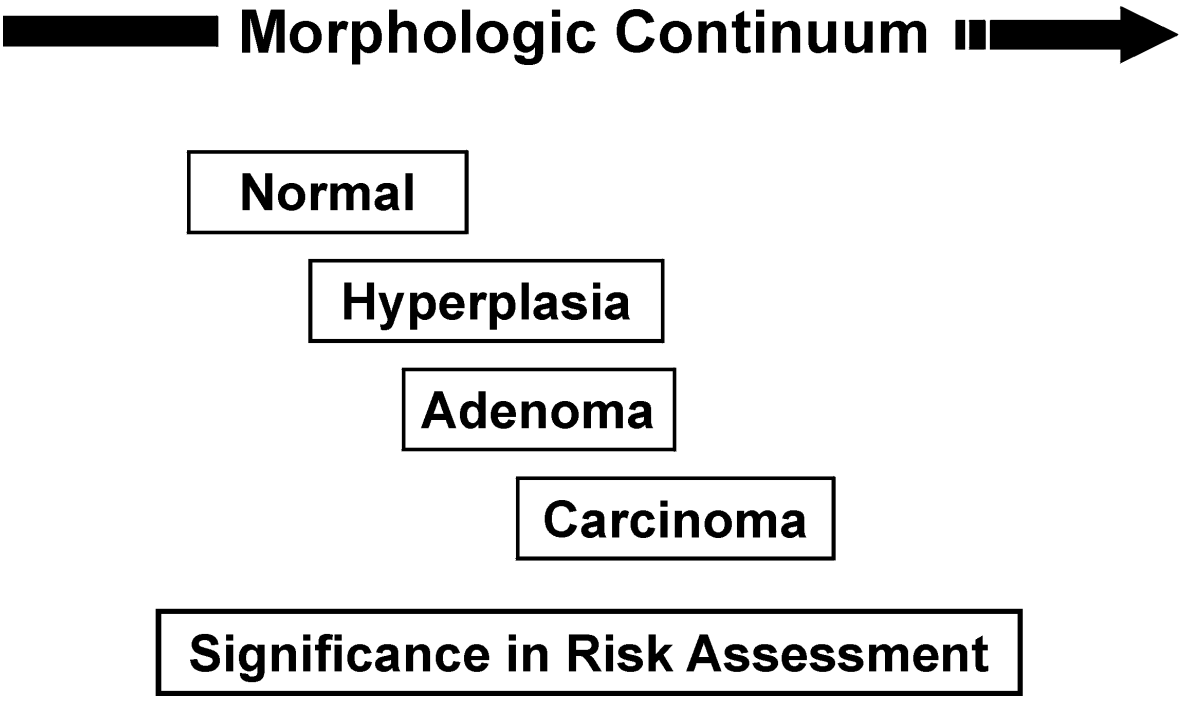

Figure 3-5. Proliferative changes involved in the multistage characterization of thyroid follicular cell neoplasia in rodents represent a morphologic continuum. Although these lesions typically are classified as discrete entities, the overlap in morphologic features should be emphasized because only imprecise criteria to separate borderline proliferative lesions exist. Thyroid neoplasia in rodents is considered relevant to human risk assessment (U.S. Environmental Protection Agency, 1998a) but thought to be protective (Capen, 1997).

Figure 3-6 shows the possible molecular events in human thyroid follicular carcinogenesis. In spite of the potential qualitative similarities, there is evidence that humans may not be as sensitive quantitatively to thyroid cancer development from thyroid-pituitary disruption as are rodents. Rodents readily respond to reduced iodide intake with the development of cancer; whereas, humans develop profound hyperplasia with "adenomatous" changes with only suggestive evidence of malignancy. Even with congenital goiters from inherited blocks in thyroid hormone production, only a few malignancies have been found in humans. Thus, despite a common physiology in regard to the thyroid-pituitary feedback system, the role of disruption of this axis in human cancer development is much less convincing. EPA has adopted the following science

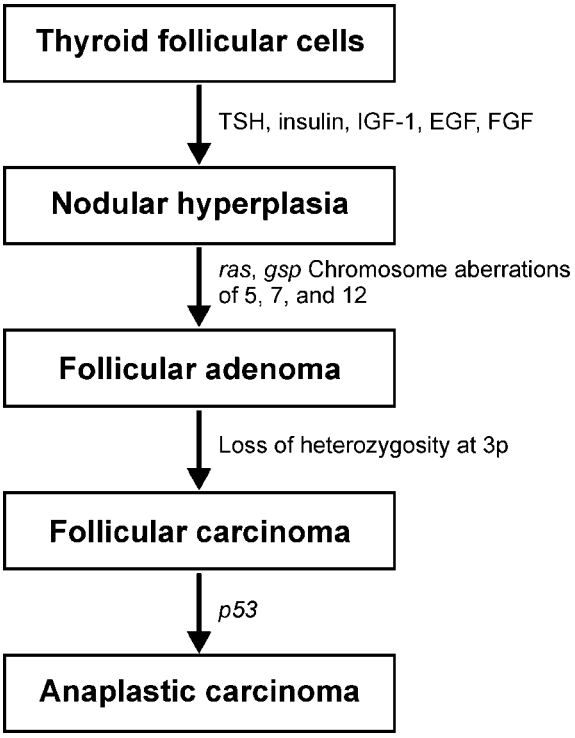

Figure 3-6. Possible molecular events in human thyroid follicular carcinogenesis (*ras* = *ras* protooncogene, *gsp* = GTP-binding protein mutation, *p*53 = *p*53 tumor suppressor gene) (U.S. Environmental Protection Agency, 1998a and Hill et al., 1998).

policy that recognizes the role of mode-of-action information regarding thyroid-pituitary disruption and mutagenesis to potential thyroid carcinogenesis (U.S. Environmental Protection Agency, 1998a).

- It is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid.
- In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer caused by thyroid-pituitary disruption. This is a conservative position when thyroid-pituitary disruption is the sole mode of action because rodents appear to be more sensitive to this carcinogenic mode-of-action than are humans. When the thyroid carcinogen is a mutagenic chemical, the possibility that children may be more sensitive than adults needs to be evaluated on a case-by-case basis.

- Adverse rodent noncancer thyroid effects (e.g., thyroid enlargements) following short- and long-term reductions in thyroid hormone levels are presumed to pose human noncancer health hazards.

The new data on the antithyroid activity of perchlorate that has resulted from the testing strategy will be evaluated in Chapter 7 according to criteria provided in the guidance (U.S. Environmental Protection Agency, 1998a) to determine the likelihood that the chemical would act indirectly, via disruption of the thyroid-pituitary axis, or directly on DNA.

3.4.2 Neurodevelopmental Deficits and Other Potential Adverse Effects Resulting from Thyroid Hormone Disruption

As expressed by the external review panel convened by Toxicology Excellence for Risk Assessment (TERA) in 1997, there was concern about other potential adverse effects of perchlorate-induced hypothyroidism. Humans respond as do experimental animals in regard to short- and mid-term disturbances in thyroid functioning from various anti-thyroid stimuli such as iodide deficiency, partial thyroidectomy (surgically or $^{131}I^-$ induced), and goitrogenic chemicals such as thionamides (U.S. Environmental Protection Agency, 1998a). For instance, thyroid hormone is critical to normal brain and physical development. This dependency begins in the uterus and extends to 3 years of age in humans. Thus, there was concern that hypothyroidism during pregnancy could result in neurodevelopmental effects.

The role of the placenta in thyroid hormone metabolism is shown in Figure 3-7. Although the fetus is initially dependent on maternal thyroid hormone levels, the potential for disruption of fetal hormone production remains once the fetal thyroid assumes this function because perchlorate can cross the placenta. Disruption of circulating thyroid hormones can have drastically different effects on fetuses and infants than on adults, depending on the developmental stage at exposure (Table 3-4). It is important to emphasize that even transient disruption may lead to permanent effects in the developing organism.

Chemical-induced alterations in thyroid hormone homeostasis are known to adversely affect the development of many organ systems, including the nervous and reproductive systems (Porterfield, 1994; Jannini et al., 1995). Severe developmental hypothyroidism caused by iodine deficiencies or a congenital condition has devastating effects on fetal and postnatal development, including mental deficiencies and hearing, speech, and motor deficits (Porterfield, 1994; Sher

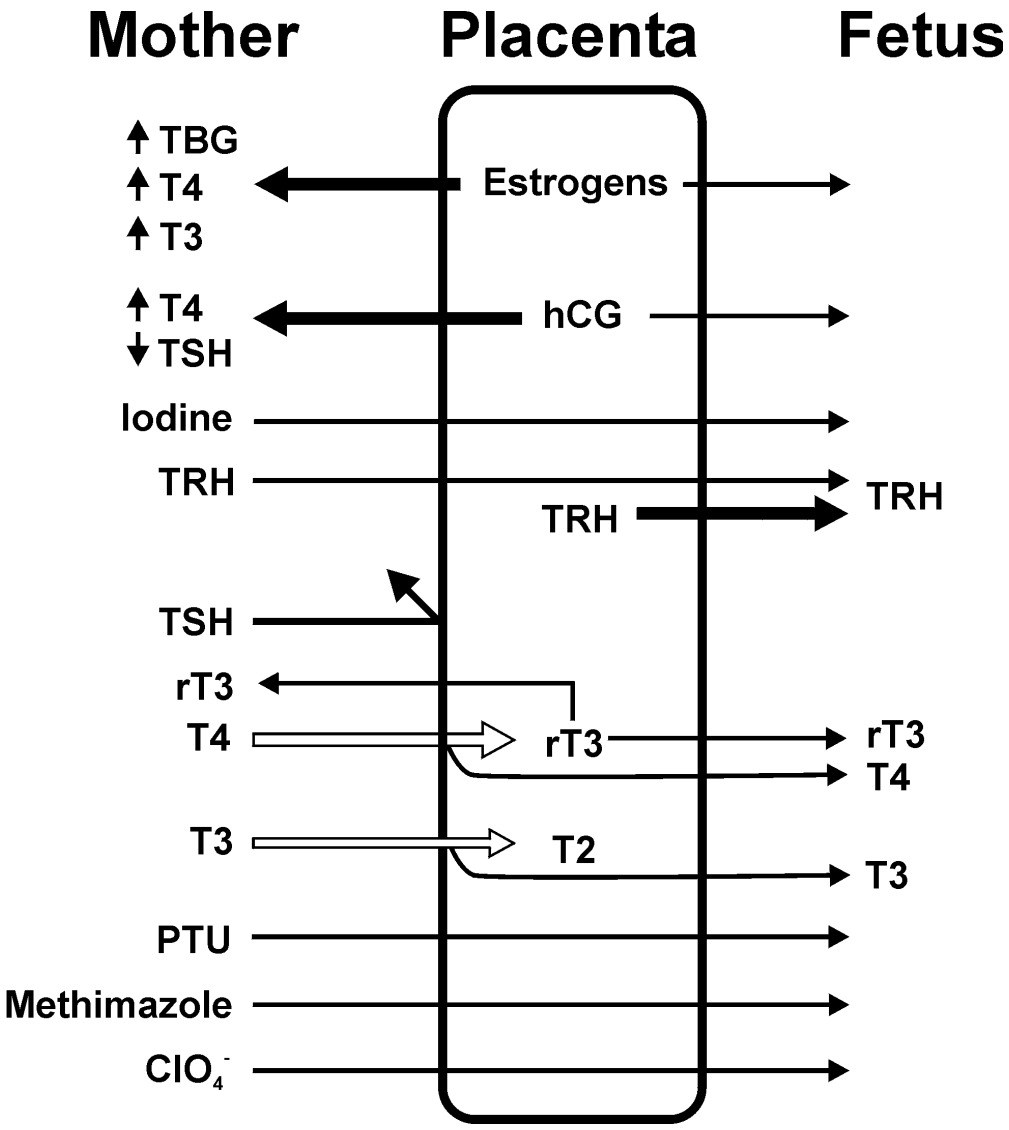

Figure 3-7. Schematic representation of the role of the placenta in thyroid hormone metabolism during human pregnancy. The placenta produces estrogens and hCG that increase maternal TBG levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal T4 and T3 concentrations and to inhibit maternal TSH secretion. Iodide and TRH readily cross the placenta, and the placenta itself synthesizes TRH. The placenta is impermeable to TSH and only partially permeable to T4 and T3. Placental Type III iodothyronine monodeiodinase enzymes degrade T4 to rT3 and T3 to 3,3'-diiodothyronine (T2). Propylthiouracil and methimazole readily cross the placenta. Given its physicochemical characteristics and similarity to iodide, perchlorate also is anticipated to cross readily. (Modified from Fisher, 1996 and Underwood, 1998).

TABLE 3-4. MAIN SYMPTOMS AND EFFECTS OF HYPOTHYROIDISM

Developmental *(Transient disruption leads to permanent effects.)*	**Adult** *(Transient disruption leads to transient effects.)*
• Delayed reflex ontogeny • Impaired fine motor skills • Deaf-mutism, spasticity • Gait disturbances • Mental retardation • Speech impairments	• Run down, slow, depressed • Sluggish, cold, tired • Dryness and brittleness of hair • Dry and itchy skin, constipation • Muscle cramps • Increased menstrual flow • Thyroid tumors in rodents

et al., 1998). It is important to emphasize that these effects are caused by a lack of thyroid hormones alone, rather than by tumor development or thyroid hypertrophy/hyperplasia due to increases in TSH. Thus, the important species comparison may be perchlorate's action of iodide uptake inhibition at the NIS. In fact, data discussed in Chapters 5 and 6 show that the sensitivity of the NIS is quite similar across species.

During development, thyroid hormones regulate cell proliferation, migration, and differentiation. Intracellularly, THs bind to thyroid hormone receptors that interact with thyroid response elements to alter expression of messenger ribonucleic acids (mRNAs) and subsequent protein synthesis. The pituitary-thyroid TSH feedback loop may or may not be activated during development, depending on the mechanism of action of the chemical. The adversity of congenital hypothyroidism, usually less severe than endemic cretinism, can be ameliorated via early postnatal thyroxine therapy. In contrast, the effects of developmental iodine deficiency can not be corrected with only postnatal therapy, indicating that iodine deficiency during pregnancy is the causative action (Cao et al., 1994). Clearly, xenobiotics that contribute to fetal or maternal hypothyroidism or hypothyroxenemia are of concern.

Since the previous external peer review, studies reported in the clinical and epidemiological literature have reinforced concerns for deficits in neuropsychological development related to maternal thyroid deficiency. Haddow et al. (1999) showed an effect on IQ scores in children (ages seven to nine) who had normal thyroid function at birth but were born to women with abnormal thyrotropin levels versus children born to a matched cohort of women with normal thyrotropin levels as controls. Haddow et al. (1999) concluded that even mild and probably

asymptomatic hypothyroidism in pregnant women can adversely affect their children's subsequent performance on neuropsychological tests.

Pop et al. (1995) noted an average impairment of 10.5 IQ points in the offspring of mothers with high thyroid peroxidase antibody (TPO-Ab) titers during pregnancy. In a later prospective study these same researchers evaluated developmental indices at 3 weeks, 10 months, 1 and 2 years of age and demonstrated that a maternal free T4 blood level that was less than the 10^{th} percentile of first trimester values (10.4 pmol/L in their study series) was associated with distinctly impaired psychomotor development whether or not TSH and TPO-Abs were elevated (Pop, et al., 1999). Smit et al. (2000) reported a similar relationship between free T4 and early neurodevelopment of children born from treated hypothyroid women.

Morreale de Escobar et al. (2000) evaluated epidemiological, clinical, and basic research data to ascertain if the principal factor leading to neurodevelopmental deficits in children was related to maternal hypothyroidism, whether clinical or subclinical (as defined by TSH higher than the 98^{th} percentile of the normal population); or if they were instead related to maternal hypothyroxinemia per se (decrement in T4 without concomitant increase in TSH). These researchers concluded that conditions resulting in hypothyroxinemia alone (a low for gestational age circulating maternal free T4 level whether or not TSH was increased) poses an increased risk for poor neuropsychological development of the fetus. T4 is the required substrate for the ontogenically-regulated generation of T3 in the amounts needed for optimal brain development, both temporally and spatially. Normal maternal T3 concentrations did not seem to prevent the potential damage of a low T4 supply (Morreale de Escobar et al., 2000). Hypothyroxinemia seems to be much more frequent in pregnant women than either clinical or subclinical hypothyroidism and autoimmune thyroid disease (AITD), especially in regions where the iodine intake of the pregnant woman is inadequate to meet her increased needs for T4 (Morreale de Escobar et al., 2000).

Figure 3-8 illustrates the windows of susceptibility for insults to the brain resulting from hypothyroxinemia. A similar map has been developed for rats, and time lines have begun to be compared and correlated (Rice and Barone, 2000), as shown in Figure 3-9. Morreale de Escobar et al. (2000) reported findings that altered early migration of cortical cells can be observed in rats with severe iodine deficiency. Porterfield (2000) has also discussed the potential for

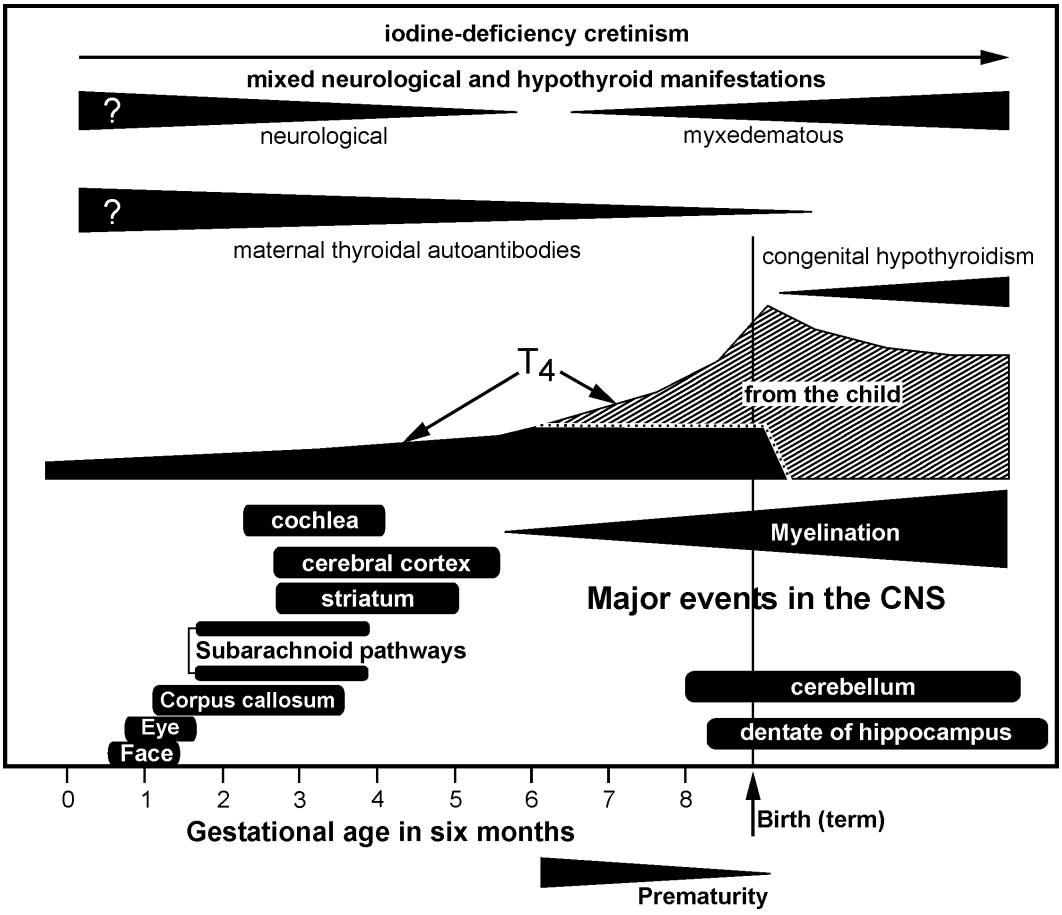

Figure 3-8. Approximate timing of major insults to the brain resulting from hypothyroxinemia, superimposed on major neurodevelopmental events in humans. Conditions resulting in early maternal hypothyroxinemia, combined to later impairment of the fetal thyroid, are the most damaging, with central nervous system (CNS) damage that is irreversible at birth. The most frequent cause is maternal iodine deficiency (ID) and the presence of maternal autoimmune thyroid disease (AITD). Unless ID is also present, the CNS damage in congenital hypothyroidism is preventable by early postnatal treatment because the normal maternal thyroxinemia has avoided damage to the brain until birth. If maternal hypothyroxinemia persists, normal maternal concentrations of T_3 do not protect the fetal brain because of its dependence on intracerebral regulation of local T_3 availability by deiodinating pathways using T_4 as a substrate. Interruption of the contribution of maternal T_4 in premature infants with an immature thyroid may also underlie their increased risk of neurodevelopmental problems, the more severe the earlier their birth. The *question mark* indicates that it is unknown whether very early CNS development, corresponding to a period when the general morphogenesis of the pros encephalon (neurolation and segmentation) is being determined, is thyroid hormone sensitive or not (Morreale de Escobar et al., 2000).

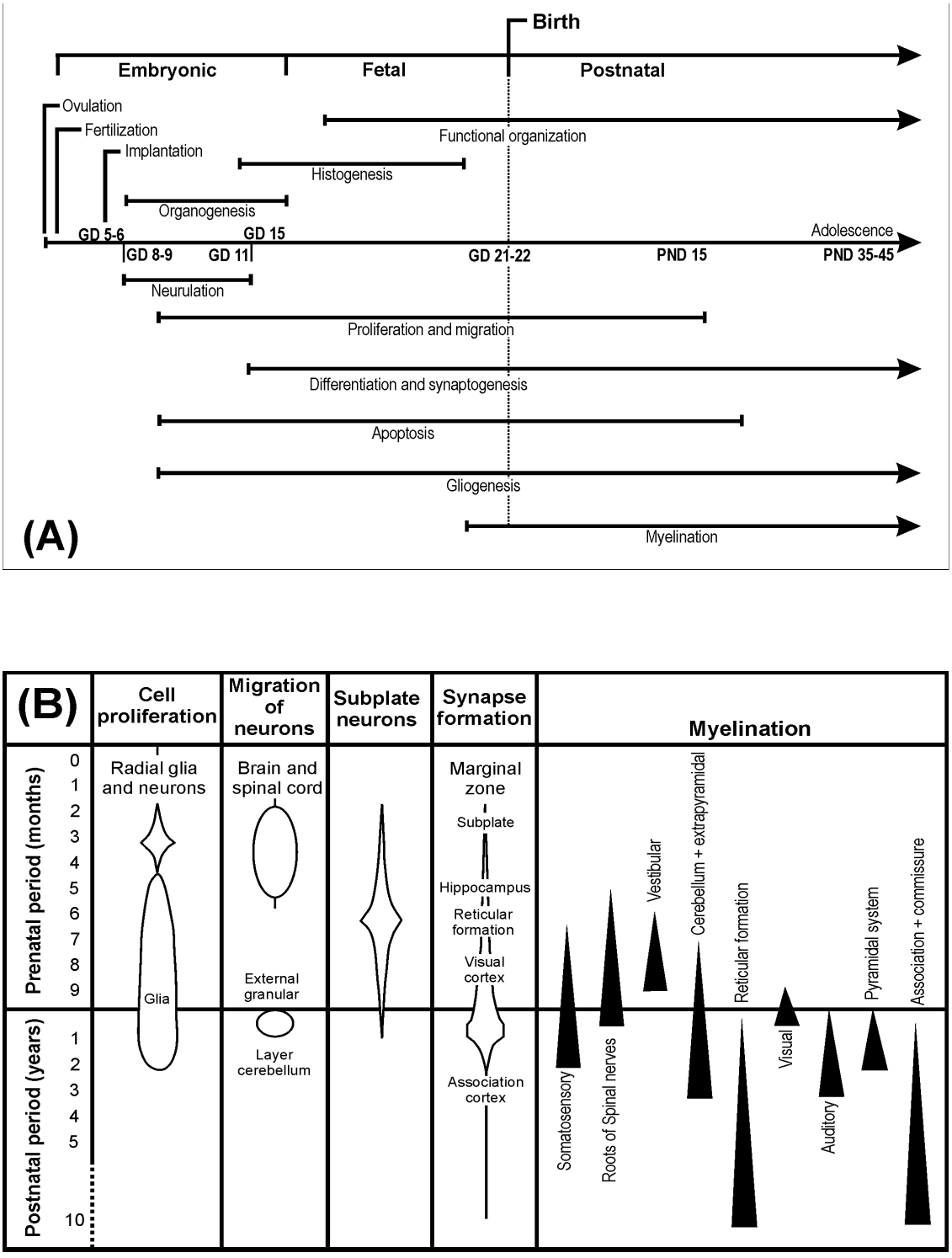

Figure 3-9. Timelines of developmental processes in the nervous system of rats (a) and humans (b). Rat timeline is compared to timing of fertilization, organogenesis, and histogenesis. Human perinatal period is scaled in months and the postnatal development is scaled in years (Rice and Barone, 2000).

neurotoxicity and altered brain development that may result from exposure to environmental chemicals that disrupt thyroid function even on a transient basis.

These concerns for the potential adverse effects of perchlorate on T4 and T3, especially during pregnancy, are compounded by the growing appreciation that women of childbearing age have relatively low iodide intake. A January 2001 report by the National Academy of Sciences (NAS) concerning the dietary reference intake of trace-mineral nutrients, including iodine, indicated that less than 25% of the total population was below the estimated average requirement for iodide and stressed a need to look at levels of adequacy for susceptible age groups and status during pregnancy and lactation. The higher requirements during this time indicate a potential susceptibility as shown in Table 3-5. The NAS also cautions against using urinary iodine as a biomarker for iodine status unless the data are from 24-hour collections or are normalized against creatinine**.** Other reports suggest that the level of iodide intake is less than a third of the range recommended for pregnant women by the World Health Organization (WHO) (Caron et al., 1997).

TABLE 3-5. DIETARY REFERENCE INTAKES (DRI) FOR IODIDE (National Academy of Sciences, 2001)

Age or Status	Adequate Intake (AI) μg/day	Estimated Average Requirement (EAR) μg/day	Recommended Dietary Allowance (RDA) μg/day
0-6 months	110		
6-12 months	130		
1-3 years		65	90
4-8 years		65	90
9-13 years		73	120
14-18 years		95	150
19-15 years		95	150
51 + years		95	150
Pregnancy		160	220
Lactation		209	290

The prevalence of abnormal thyroid function continues to be debated and this is confounded by the variable definitions of the disease state as well as the different measures of thyroid function (Canaris et al., 2000). Most reports are still defined by TSH levels rather than for hypothyroxinemia per se, but recent presentations suggest that TSH is a poor test to assess the severity of tissue hypothyroidism (Meier et al., 2001), and recommendations in the epidemiologic literature are proposing that screening of pregnant women should include the determination of free T4 (Morreale de Escobar et al., 2000). Age, sex and dietary iodine levels are confounding factors, although virtually all studies report higher prevalence rates for hypothyroidism (as defined by increased TSH) in women with age (Canaris et al., 2000). Rates as high as 24% among women older than 60 years have been reported. Suppressed TSH levels have been associated with decreased bone density, increased risk of atrial fibrillation, premature atrial beats, and effects on serum lipids notably elevated serum cholesterol levels.

Together these findings strongly suggest that a susceptible population of particular concern for perchlorate exposure is pregnant women with hypothyroxinemia and that the iodine deficiency represents an additional potential insult that could exacerbate the effects of perchlorate toxicity**.** The elderly, especially women, represent another potentially susceptible population, as well as people with cardiac dysfunction or risk factors such as elevated serum cholesterol.

As mentioned above, reproductive toxicity was also a concern as a potential effect of perchlorate's mode of action. In females, thyroid hormones appear to have a role in stimulating the onset of human chorionic gonadotropin (hCG) production by the placenta early in pregnancy. Human chorionic gonadotropin is essential for the maintenance of pregnancy. Therefore, a hypothyroid condition has potential to interfere with normal placental function and fetal survival, as well as the potential to interfere with lactation. Suppression of thyroid hormone secretion with radioactive iodine or goitrogens reduces milk yield in lactating animals. This effect may be caused by suppression of placental lactogen production. Thyroid-releasing hormone is known to play a role in prolactin release during the estrous cycle. Additionally, the thyroid is necessary for the transition to the anestrus state in seasonally breeding species. In summary, effects on thyroid hormone levels have roles in estrous cycle regulation, pregnancy maintenance, fetal growth, and lactation.

In males, the primary effects of hypothyroidism appear to occur during testicular development. The testis is responsive to thyroid hormones only during a limited time during the

perinatal and prepubertal periods. Thyroid hormone is a major regulator of seminiferous epithelium development by inducing the normal differentiation of Sertoli cells, gonocytes, and Leydig cells, and by limiting the proliferation of those cell types. In the hypothyroid condition, those cells proliferate beyond the norm, and the steroidogenic function of the Leydig cells, on a per-cell basis (but not necessarily in total), is impaired. Secretory activity of the Sertoli cells also appears to be impaired. In boys, untreated hypothyroidism is associated with marked and precocious testis enlargement, but low androgen activity. In a small study, hypothyroid men had complaints of reduced libido that was probably related to a defective leutenizing hormone response to gonadotropin-releasing hormone.

The inclusion of an immunological evaluation of mice exposed to perchlorate was warranted because of evidence from earlier clinical studies that indicated a link between the treatment of Graves' disease with perchlorates and serious hematological effects that may be linked to immune mechanisms. A small number of patients undergoing perchlorate therapy have been reported to develop aplastic anemia, agranulocytosis, lymphadenopathy, leukopenia, or skin rashes. The antithyroid drugs propylthiouracil and methimazoles are reported to exert their effects on the hematopoietic system through immune mechanisms. Because the use of these antithyroid drugs by a small number of patients also resulted in sequelae similar to that of some patients under perchlorate treatment, it has been postulated that perchlorate also may act via the immune system.

3.5 DEVELOPMENT OF A TOXICITY TESTING STRATEGY BASED ON MODE OF ACTION

Because the RfD is intended to be a lifetime dose-response estimate, the typical objective of a database to support such a quantitative assessment is to evaluate a comprehensive array of testing endpoints that represent various life stages during which potential effects could occur (e.g., the developing fetus through adult) and for effects on reproductive capability (shown schematically in Figure 3-10). As discussed in the previous sections, thyroid hormone deficiencies, such as those induced by perchlorate, can affect normal metabolism, growth, and development. No robust data existed prior to this time to evaluate other potential target tissues or effects. There were limited data on effects caused by long-term exposures and no data with

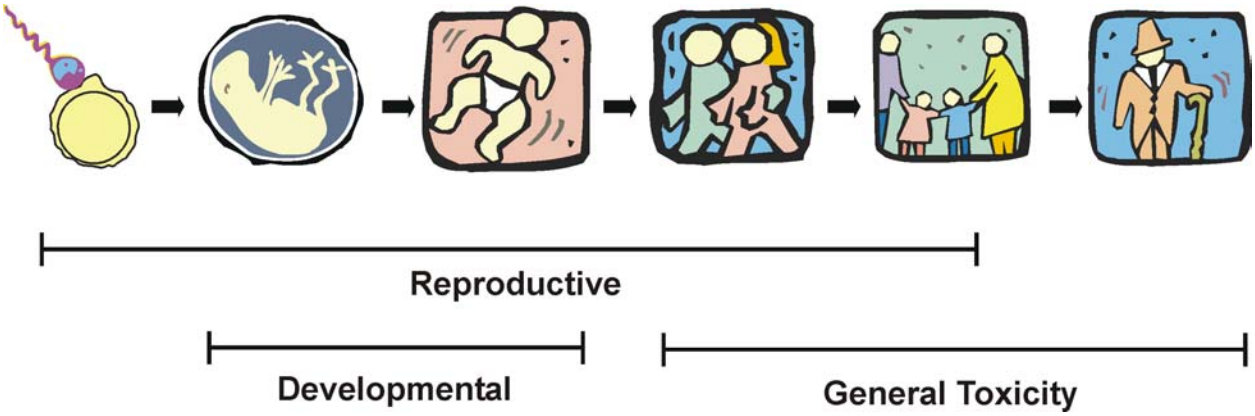

Figure 3-10. Schematic illustrating that a high confidence RfD is based on data that address all potentially critical stages over a lifetime.

which to evaluate the effects of perchlorate in potentially susceptible populations such as in developing fetuses, nor were there data on the effects of perchlorate on the reproductive capacity of male or female laboratory animals. Table 3-6 shows the minimum database for derivation of an RfD with low confidence (a 90-day bioassay) and the rationale for other tests typically included to bolster the confidence in the derivation–the same suite of tests that has been discussed for perchlorate. These data typically also reduce the uncertainty for which uncertainty factors are applied (see Table 3-7), either because the absence of data on a suspected endpoint (e.g., developmental toxicity) has been addressed or because mechanistic data provide insight on the relevance of the laboratory animal model, including the magnitude of interspecies and intrahuman variability in toxicokinetics and toxicodynamics. Any individual chemical database may fall in between this range of high and low certainty, depending on the quality of the individual studies and whether the dose response for suspected endpoints is characterized well.

The objective of the testing strategy was to provide a comprehensive database that described the mode-of-action-based pathogenesis in quantitative terms so that the resultant estimate could be more predictive and ultimately support the development of a robust RfD estimate that reduced the uncertainties inherent in the provisional, presumably protective values (see Figure 3-11).

TABLE 3-6. MINIMUM DATABASE FOR DERIVATION OF AN ORAL REFERENCE DOSE

Mammalian Database[a]	Confidence	Comments
Two chronic oral bioassays in different species One two-generation reproductive study Two developmental toxicity studies in different species	High[b]	Minimum database for high confidence
One subchronic oral bioassay	Low	Minimum database for estimation of an RfD

[a]Rationale is to use different species to evaluate variability in species sensitivity unless a particular laboratory animal model is more appropriate.
[b]Rationale is to address all potentially critical life stages.

TABLE 3-7. FACTORS FOR UNCERTAINTIES IN APPLIED EXTRAPOLATIONS USED TO DERIVE REFERENCE DOSES[a]

10_H	–	Human to sensitive human
10_A	–	Experimental animal to human
10_S	–	Subchronic to chronic duration
10_L	–	LOAEL(HEE)[a] to NOAEL(HEE)[a]
10_D	–	Incomplete to complete database
MF	–	Modifying factor. Professional assessment of scientific uncertainties of the study and database not explicitly addressed above. Default for the MF is 1.0 (e.g., applied for small sample size or poor exposure characterization).

[a]HEE = human equivalent exposure.

As illustrated in Figure 3-11, it is ultimately desirable to have a comprehensive biologically-based dose-response model that incorporates the mechanistic determinants of chemical disposition, toxicant-target interactions, and tissue responses integrated into an overall quantitative model of the pathogenesis (Jarabek, 1995a). Because the internal tissue dose of the chemical or its toxic moiety in a target tissue is not always proportional to the applied dose of a compound, emphasis has been placed on the need to distinguish clearly between the exposure concentration and the dose to critical target tissues. Consequently, the term "exposure-dose-response" has been recommended as more accurate and comprehensive (Andersen et al., 1992). This expression refers, not only to the determination of the quantitative relationship between exposure concentrations and target tissue dose, but also to the relationship between tissue dose and the observed or expected responses in laboratory animals and humans. The process of

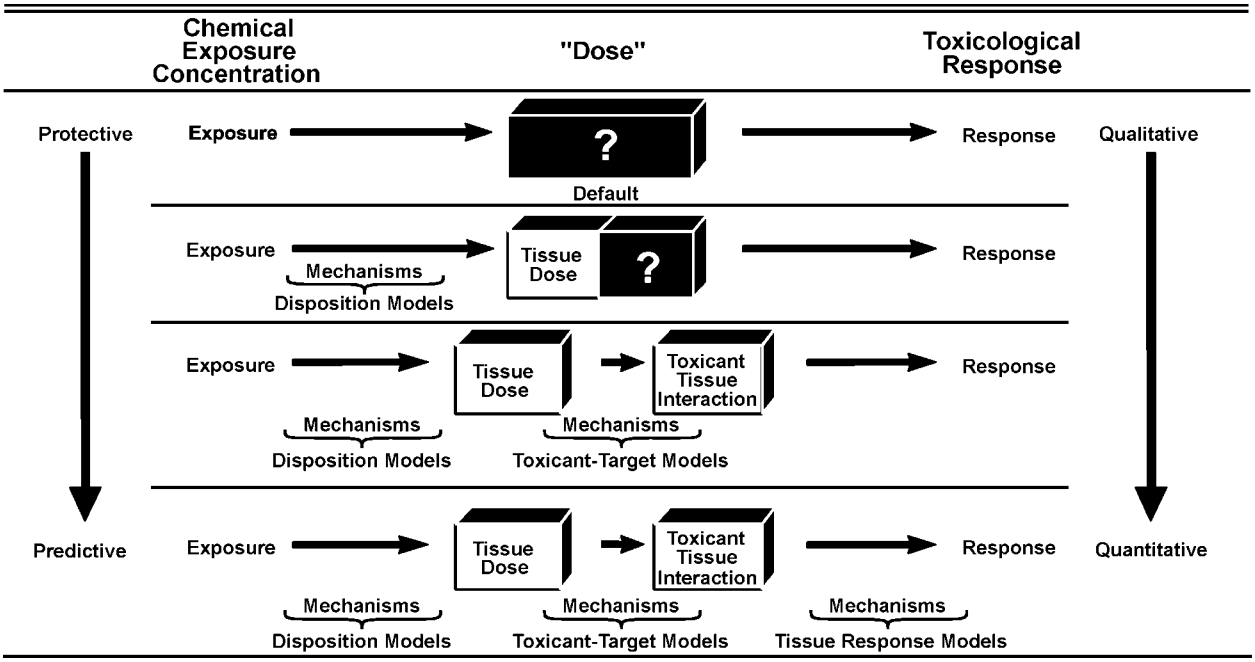

Figure 3-11. Schematic characterization of comprehensive exposure-dose-response continuum and the evolution of protective to predictive dose-response estimates (U.S. Environmental Protection Agency, 1994 and Jarabek 1995b).

determining the exposure-dose-response continuum is achieved by linking the mechanisms or critical biological factors that regulate the occurrence of a particular process and the nature of the interrelationships among these factors. This can be especially important for interspecies extrapolation and to understanding intrahuman variability.

Dose-response estimates based on characterization of the exposure-dose-response continuum at the rudimentary ("black box") level necessarily incorporate large uncertainty factors to ensure that the estimates are protective in the presence of substantial data gaps. With each progressive level, incorporation and integration of mechanistic determinants allow elucidation of the exposure-dose-response continuum and, depending on the knowledge of model parameters and fidelity to the biological system, a more accurate characterization of the pathogenesis process (Jarabek, 1995a). Because of the increase in accuracy of the characterization with each progressive level, dose-response estimates also progress from more protective to factually-based (predictive).

Eight new studies were recommended as part of the original testing strategy after the May 1997 external peer review to provide such a comprehensive array of endpoints. These studies are described below along with the role they were anticipated to play in informing the revised health risk assessment (see Table 3-8).

TABLE 3-8. PERCHLORATE PEER REVIEW RECOMMENDED STUDIES SUMMARY

Study	Description	Potential Use in Assessment
90-Day subchronic bioassay + TH[a] + reproductivity + genotoxicity + recovery	Tests for other target tissues; evaluates effect on TH in young adult rats; reproductive parameters added; mouse micronuclei and a recovery group	Minimum database for RfD dose-response for TH in young adult rats; additional information on others; may allow decrease in uncertainty factor (UF) for database deficiencies
Developmental neurotoxicity + TH	Evaluates nervous system in fetal and postnatal rats; TH in does (P0-generation) and pups (F1-generation)	Potentially critical effect; comparison of developmental versus adult effects on TH
Developmental study + TH	Evaluates birth defects in rabbits; TH in does at end of gestation	Potentially critical effect; data in second species for TH effects; may reduce UF for database deficiencies
Two-Generation reproductive toxicity + TH	Evaluates fertility of adult rats and toxicity in offspring over two generations; TH in parents (F0-generation) and offspring (F1- and F2-generations)	Potentially critical effect; may reduce UF for database deficiencies
ADME studies	Characterize absorption, distribution, metabolism, and elimination in rats and humans; iodine inhibition and perchlorate kinetices and hormone homeostasis	Interspecies extrapolation
Mechanistic studies	Evaluate mechanism of TH response and sensitivity in rats and humans	Interspecies extrapolation; determine susceptible subpopulation
Genotoxicity assays	Test for toxicity to DNA	Mode-of-action information for thyroid neoplasia; may reduce UF for database deficiencies
Immunotoxicity	Evaluates immune system structure and function	Potentially critical effect; may reduce UF for database deficiencies

[a]Thyroid hormones (T4 and T3); Thyroid stimulating hormone (TSH), a pituitary hormone, was also assayed in those studies.

(1) 90-Day Subchronic Oral Bioassay Study. This study was considered the minimum data requirement for derivation of an oral RfD. The study aimed to identify other target tissues, to test young adult rats, and to provide data on the effect of repeated exposure to perchlorate on thyroid hormone levels. The 30-day recovery phase, i.e., evaluation of the thyroid status 30 days after perchlorate was stopped, would provide data necessary to characterize its anti-thyroid effects with respect to carcinogenicity (U.S. Environmental Protection Agency, 1998a). These data were collected to allow reduction of the uncertainty factor applied for database deficiencies.

(2) Developmental Neurotoxicity Study. This study was designed to evaluate the potential for developmental neurotoxicity of perchlorate by assessing functional and morphological endpoints in offspring from the mother exposed during pregnancy and lactation. Neurotoxicity endpoints were likely to be a critical effect, and the developing organism a sensitive subpopulation. It was hoped that these data would allow reduction of the uncertainty factors applied for intrahuman variability and database deficiencies.

(3) Segment II Developmental Study. This study was conducted to evaluate the potential for perchlorate to cause birth defects in rabbits and to evaluate a potentially critical effect and subpopulation. This study also was conducted to provide data on the thyroid hormone effects in a second species (in addition to rats). These data might allow reduction of the uncertainty factor applied for database deficiencies.

(4) Two-Generation Reproductive Toxicity Study. This study was designed to evaluate the potential for perchlorate to cause deficits in reproductive performance in adult rats and for toxicity in the young offspring. The primary goal of this study was to identify a potentially critical effect and to allow for reduction of the uncertainty factor applied for database deficiencies.

(5) Absorption, Distribution, Metabolism, and Elimination Studies. These ADME studies aimed to understand the pharmacokinetics (i.e., how perchlorate is absorbed, distributed, metabolized, and excreted) of perchlorate in test animals and humans. These data were to

provide information to support construction of quantitative extrapolation of dose across species (e.g., rat to human).

(6) Perchlorate Mechanism Studies. These studies provided a link to the pharmacokinetic studies and were conducted via a comparison of existing literature and of new *in vitro* and *in vivo* data that evaluated the effects of perchlorate on the iodide uptake mechanism across species to aid in the quantitative extrapolation of dose.

(7) Genotoxicity Assays. These studies evaluated the potential for carcinogenicity by evaluating mutations and toxic effects on DNA. These data were useful to determining whether the benign thyroid tumors were likely to be a result of the proposed threshold pathogenesis process.

(8) Immunotoxicity Studies. These studies were planned to evaluate the potential for perchlorate to disrupt immune function and identify a potentially critical effect. These data would help to reduce the uncertainty factor applied for database deficiencies. Because concern was raised for these potential adverse effects based on the previous clinical experience with treatment of Graves' disease patients, these studies were considered necessary to a comprehensive database for perchlorate.

In the 1998 external review draft (U.S. Environmental Protection Agency, 1998d), a model based on mapping the events of the mode of action for perchlorate was proposed as shown in Figure 3-12. The key event was identified as the inhibition of iodide uptake at the NIS, followed by decreases in thyroid hormones and increases in TSH. Both the potential neurodevelopmental and neoplastic sequelae of this perturbation in thyroid hormone economy were proposed as downstream adverse health outcomes. The conceptual model was endorsed by the external peer review panel in 1999 (Research Triangle Institute, 1999), and additional studies were recommended to reevaluate indications of developmental and neurodevelopmental in rats for effects observed in the 1998 database. Delineating the continuum of histopathological changes in the thyroid was also recommended. The results of all the studies in the testing strategy (both

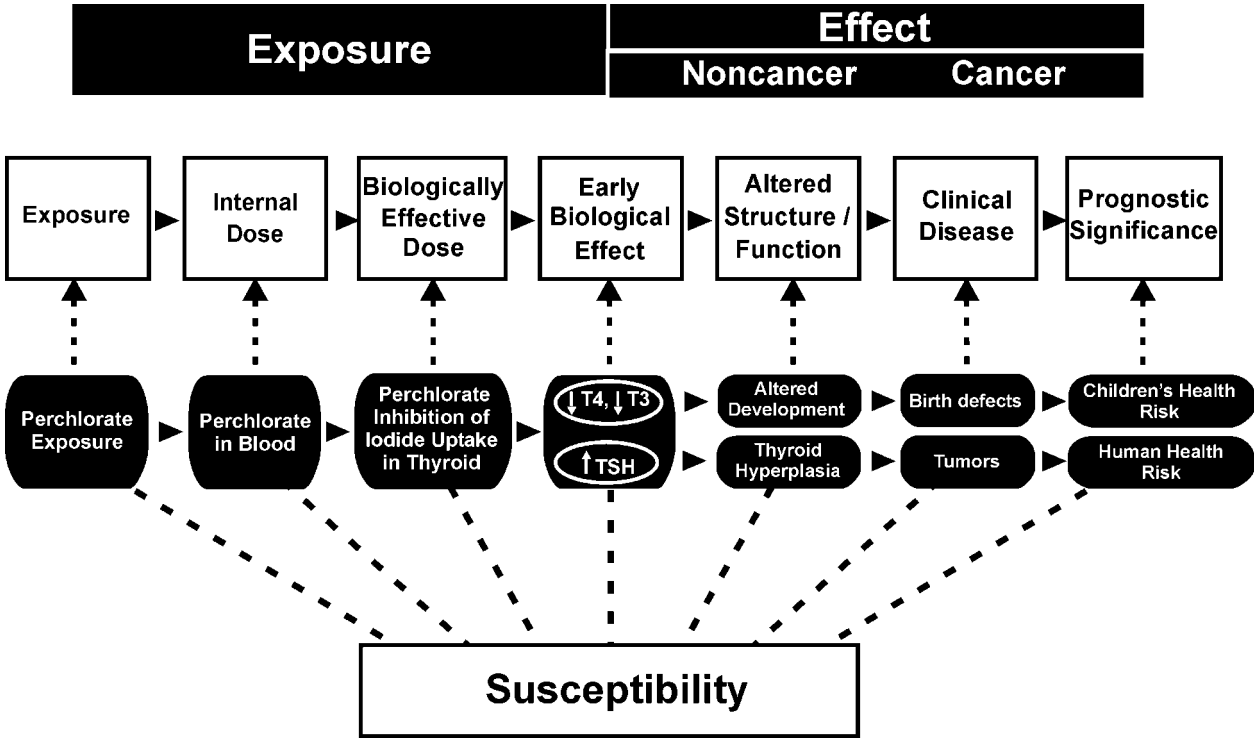

Figure 3-12. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U.S. Environmental Protection Agency, 1994; Schulte, 1989). The model maps the toxicity of perchlorate on this basis by establishing casual linkage or prognostic correlations of precursor lesions.

1 "old " 1998 and "new" 2001), as well as additional studies now available in the literature, will be
2 reported together with EPA's interpretation and evaluation in Chapter 5.

4. HUMAN HEALTH EFFECTS DATA

The available data on the human health effects of perchlorate exposures are limited. Until the emerging concern regarding environmental contamination, the majority of the studies were clinical reports on patients treated with potassium perchlorate for Graves' disease. The non-EPA, independent peer review held in March 1997 (Toxicology Excellence for Risk Assessment, 1998a) concluded that the experimental design limitations of the studies prior to that time precluded their use in quantitative dose-response assessment. The CA DHS also determined in 1997 that there were major limitations on the human studies. Nevertheless, the studies were useful in hazard identification and supported the conceptual model for the mode of action of perchlorate available at the time as described in Chapter 3.

Since the external peer review of the previous 1998 external review draft held in 1999 by the U.S. Environmental Protection Agency (Research Triangle Institute, 1999), some ecological studies have been performed that have addressed the limitations in the human data with some success. Two occupational populations with inhalation exposure to perchlorate were also studied, and some additional clinical studies in healthy adults performed. On December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. Environmental Protection Agency, 2001c). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Some of the clinical studies contained in this database fall in this category of studies not to be considered. However, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. Therefore, because of the scientific shortcomings of these studies, they will not be used as "principal studies" in the derivation of a RfD. The ethical issues surrounding the conduct of these studies or their use for

regulatory purposes in light of the Agency's interim policy will not be discussed in this document. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies which intentionally dose human subjects with toxicants to identify or quantify their effects.

4.1 EPIDEMIOLOGICAL DATA

To be informative to quantitative dose-response analysis for risk assessment applications, epidemiological studies must pose research questions that are based on appropriate physiological issues relevant to the mode of action for the chemical and its toxic effect. In some contexts, a sufficient specification may take relatively simple form. For example, with occupational cancer, the generally assumed underlying mechanisms lead to a simple test: does exposure to a substance or mixture specified as a dependent parameter, X, at time t_1 increase the incidence of specific cancers at time $t_2 > t_1 + a$, where $a > 0$ is some lag time. The relation of risk at t_2 to the history of prior exposure may be a complex one, but almost always, risk is an increasing function of exposure at various time intervals, $X(_{it})$. This test may require controlling for confounding factors, which is usually not difficult when relevant detailed information is available.

In contrast, determining the effect of an environmental exposure on a regulated system could be more of a challenge. Thus, cancers whose risk depends on endocrine status introduce increased complexity. Environmental perturbations of physiological systems that have inherent variability over time and are imbedded in control networks that function to minimize disruption make it a challenge to determine which endpoints to measure. Cross-sectional assessments during chronic exposures may capture variability in some regulated biological parameters while other parameters will tend to stabilize at "normal" levels despite substantial environmental impact on production and function. In such instances it can be difficult to distinguish alterations due to the xenobiotic from the variation that occurs in response to other environmental factors. Short-term fluctuations in exposure often have no effect independent of cumulative dose for chronic diseases such as lung cancer or other respiratory diseases but may be important for endocrine system functions that affect neurodevelopmental, hyperplastic, neoplastic, immune, or autoimmune events (Park, 2001).

The effect of the perchlorate anion on the hypothalamic-pituitary-thyroid feedback system is an example of a regulated system that is potentially difficult to characterize. Important effects may be evident as shifts in average levels of measurable factors, but more important effects may involve alterations in transient responses to demands on the regulated system (Park, 2001). Multiple covariates that may influence potential perchlorate health effects include iodine availability, age, gender, ethnicity, health status, diet, and possibly social class. For neonates, the birth process itself stimulates an endocrine cascade with the amplitudes of endpoint variation depending on birth weight, gestational age, age at sampling (in hours), and possibly environmental temperature. Post-partum developmental risk factors for the neonate and growing child include perchlorate exposure via lactation or consumption of contaminated water.

Individual perchlorate exposures are difficult to measure or even estimate in population-based studies. This makes their usefulness to quantitative dose-response analysis limited, particularly if confounding variables are not controlled and small population sizes are evaluated. The few population-based studies from geographic areas that have experienced perchlorate contamination offer little help beyond indicating that clinical thyroid disease is not greatly increased in populations with sustained drinking water contamination as high as 15 μg/L in the past. However, most of the studies have principally evaluated thyroid function or hormone status and have not evaluated neurodevelopmental or other deficits in children or adults resulting from perturbed thyroid function over sustained periods of exposure.

4.1.1 Ecological Studies

Rockette and Arena (1983) reviewed death certificates for workers known to have been exposed to perchloric acid, magnesium perchlorate, and other chemicals in a U.S. chemical plant. Because the workers had received multiple chemical exposures, the authors could not associate an elevated death rate for a particular time period or work area and a specific chemical.

The Environmental Health Investigations Branch within the CA DHS, under a cooperative agreement with ATSDR, conducted health assessment activities and consultations on the Aerojet-General Corporation Superfund site in Sacramento County, CA (California Department of Health Services, 1997; 1998a,b,c,d,e). A preliminary health review (California Department of Health Services, 1997) analyzed several statewide databases for possible perchlorate-related outcomes during the suspected years of contamination within the zip codes most likely exposed.

In California, thyroid hormone levels in newborns are measured and kept on file by the Genetic Disease Branch of the Centers for Disease Control and Prevention. Data for the period 1985 through 1996 from relevant zip codes was assessed for a total of 11,814 thyroid hormone screens. Although an extrapolation of the statewide rate predicted there would be 3.76 cases of hypothyroidism, four cases were observed. In the non-exposed areas, six cases of hypothyroidism were found although 6.41 cases were predicted. These data suggested no association between residence in the potentially-exposed zip codes and neonatal hypothyroidism. The TSH levels (ascertained only in neonates with initially low T4 levels) in the potentially-exposed areas were statistically significantly lower than those in the nonexposed areas. The database also was evaluated for diagnosis of goiter among the first five reported hospitalized individuals residing in the zip code of most likely contamination from the years 1991 to 1995. Because there are so many diseases or conditions that can produce a goiter other than perchlorate ingestion, and because the database can not differentiate this aspect, it was concluded that these data would not be useful in determining the prevalence of thyroid enlargement due to perchlorate in the affected water district. The same zip code also was evaluated for agranulocytosis or aplastic anemia as one of the top five diagnoses for the years 1991 to 1995. There were a total of 76 cases in 5 years, less than the statewide rate of 41.6 per year. The rate for aplastic anemia was 3.8 hospitalizations per 100,000 individuals per year, a rate higher than the statewide rate of 2.2. However, all but one of the hospitalizations also had an additional diagnosis of cancer with chemotherapy or radiation treatment; these treatments are likely explanations for this observation; acquired immunodeficiency syndrome (AIDS) may be another. The registry also was searched for cases of childhood leukemia (either acute lymphocytic leukemia or acute myelogenous leukemia). Again, the rate for the potentially exposed zip code was less than the corresponding rate for California.

The CA DHS concluded that the data on goiter, agranulocytosis, and aplastic anemia did not indicate an increase in incidence; however, these data do not provide definitive causitive information because other likely causes for these conditions existed. Increases in the incidence of decreased neonatal thyroid hormone levels, hypothyroidism, or childhood leukemia rates were not observed. The CA DHS noted that the major limitation on studies of this nature is that imposed by the absence of good exposure estimates and the absence of data on transport and transformation models which would provide dose reconstruction for the affected population. It is

unclear when the contaminated plume entered the drinking water supply; consequently, the time period analyzed may have been too broad. Improving this exposure information was one of the recommendations made in the report to Congress regarding perchlorate (U.S. Environmental Protection Agency, 1998e). Finally, that perchlorate is not specific for producing thyroid dysfunction or hematological abnormalities makes assessing these outcome surveys difficult. Table 4-1 shows the approximate prevalence of these disorders in the neonatal period (1:30,000 to 1:100,000), and suggests that studies with large numbers of subjects may be necessary to detect subtle effects.

Based on these results, the CA DHS investigated several other water service areas for exposure (California Department of Health Services, 1998a,b,c,d,e) and ascertained that complete exposure pathways to perchlorate contaminated water existed in several areas. These studies reinforced the need for this document which attempts to properly characterize the risk posed by perchlorate contamination by providing better exposure estimates and a revised health risk estimate.

Since the 1999 external peer review, eight new population studies have been performed. One of these studies has examined effects in the general population (Li et al., 2001), another in school-age children (Crump et al., 2000), and six have been devoted to evaluating neonatal endocrine status in areas with contaminated drinking water (Crump et al., 2000; Lamm et al., 1999; Li et al., 2000a,b; Brechner et al., 2000; Schwartz, 2001). In each study, the critical covariates were captured with varying degrees of success and only one study (Schwartz, 2001) offers a convincing description of neonatal perchlorate effects (Park, 2001).

In a study of the general population, Li et al. (2001) investigated physician-generated medical insurance claims for thyroid problems in a Medicaid insured population in Nevada, comparing all counties that were known not to have perchlorate contaminated drinking water with the one county that had contamination at approximately 10 μg/L. This was a study of period-prevalence, i.e., the proportion of the population that had claims for thyroid-related disorders anytime during a two-year period. Incident cases could not be identified within this database. Thyroid patients were defined as having one or more of the following diagnoses of thyroid disease according to the International Classification of Diseases, 9th Revision (ICD-9): (1) simple and unspecified goiter (ICD-9 Code 240); (2) non-toxic nodular goiter (ICD-9 Code 241); (3) thyrotoxicosis with or without goiter (ICD-9 Code 242); (4) congenital hypothyroidism

TABLE 4-1. THYROID DISORDERS AND THEIR APPROXIMATE PREVALENCES IN THE HUMAN NEONATAL PERIOD (Fisher, 1996).

Thyroid Dysgenesis	1:4000
Agenesis	
Hypogenesis	
Ectopia	
Thyroid Dyshormonogenesis	1:30,000
TSH unresponsiveness	
Iodide trapping defect	
Organification defect	
Defect in thyroglobulin	
Iodotyrosine deiodinase deficiency	
Hypothalamic-Pituitary Hypothyroidism	1:100,000
Hypothalamic-pituitary anomaly	
Panhypopituitarism	
Isolated TSH deficiency	
Thyroid hormone resistance	
Transient Hypothyroidism	1:40,000
Drug induced	
Maternal antibody induced	
Idiopathic	

(ICD-9 Code 243); (5) acquired hypothyroidism (ICD-9 Code 244); (6) thyroiditis (ICD-9 Code 245); (7) other disorders of the thyroid (ICD-9 Code 246) and (8) malignant neoplasms of the thyroid gland (ICD-9 Code 193). Two of these disorders have very low prevalence: congenital hypothyroidism (0.01%) and thyroid cancer (0.02%).

Comparisons were made between the exposed county, which includes Las Vegas, and (a) an unexposed county with a similar large city (Reno), and (b) all other counties (unexposed). There were no statistically significant period-prevalence rate differences between the exposed county and the two categories of comparison counties; however, the differences between the comparison county groups themselves were quite large, indicating that either important confounding risk factors were not controlled or estimates were unstable due to the small numbers of cases in the comparison counties. For acquired hypothyroidism, prevalences (%) in the two categories of unexposed counties were significantly different (Reno: 1.17 [95% CI = 1.05 to 1.30, using a normal approximation to the Poisson distribution for number of cases] and other

counties: 1.44 [95% CI = 1.29 to 1.59]). Age, gender, ethnicity, iodine intake, and other important risk factors were unavailable in this database and there could have been differential under- or over-diagnosis in this Medicaid population. Interestingly, when comparing the two counties with large urban centers and restricting focus to the 6 (out of 8) more prevalent outcomes (total n=3069), all 6 showed elevated (but not individually significant) rate ratios for the exposed county, ranging from 1.01 to 1.89. While these findings appear to rule out a large perchlorate-related excess (i.e., greater than two-fold) for some thyroid disorders such as acquired hypothyroidism (appearing as routine medical insurance claims), the study had a statistical power of less than 0.5 to detect a 50% excess for several specific thyroid disorders (i.e., the observed relative rises exceeded 1.50 but were not statistically significant). Unfortunately, owing to potentially overwhelming confounding (e.g., related to age, gender, ethnicity, or iodine intake) or because of small numbers of cases in the comparison counties, little else can be concluded from this study.

The Crump et al. (2000) study of school children (mean age 7.3 years) in three Chilean cities permitted comparisons on effects of drinking water with widely varying perchlorate content: 0, 5, and 100 ppb. A total of 162 school-age children were studied, 127 of whom had lifelong residence in their respective cities. Controlling for age, gender, and urinary iodine, a highly significant trend of increasing T4 levels—the opposite to the expected direction for effects on T4 from perchlorate—was observed with increasing perchlorate content in the water. The city with the highest concentrations (100 ppb) had a significant five-fold excess in family history of thyroid-related problems. Children in all three cities had elevated goiter prevalence, but it was highest in the city with intermediate concentrations (5 ppb) which was believed to also have iodine deficiencies. A variable introduction of iodized salt in earlier years may have affected these observations. It is not known what role boiling drinking water may have played or how the microbiological quality of drinking water varied across the cities studied. Ethnic and socioeconomic attributes were thought to be similar across the three groups of children but were not controlled for in the analysis. Whether ambient indoor and outdoor temperatures may have played a role in thyroid functional status was not investigated. It would appear that uncontrolled confounding effects, particularly from environmental or other factors, make it difficult to interpret the observed effects of drinking water contaminated with perchlorate at levels as low as 5 ppb on thyroid function in this study. Controlling for urinary iodine in the analyses would

better address whether iodine deficiency differences across the three cities studied may have distorted the association of T4 changes with perchlorate exposure. The paradoxical trend observed in this study remains unexplained.

Crump et al. (2000) also studied newborns screened for hypothyroidism by a heel-stick blood sample between February 1996 and January 1999 in the same three Chilean cities. A systematic laboratory error gamma counter contamination occurred between December 1, 1997 and June 30, 1998 which caused TSH to be reported very low (0.1 μU/mL) for a high proportion (29.1%) of the blood samples analyzed. The error was reported to be limited to this 7-month period and to have affected a similar proportion of samples from each of the three cities. All data obtained during the 7-month period in question were excluded, leaving 9,784 neonatal records for analysis. Analysis revealed a statistically significant decline in TSH (log-transformed) with increasing city-perchlorate levels, a trend opposite to that hypothesized. The analysis was adjusted for gender and age at screening as categorical variables in days but covariates lacking included iodine intake (known to be low in one city), ethnicity, and birth weight. The ages at screening differed across the three cities studied; the median ages were 3, 4, and 6 for the unexposed, low, and high perchlorate studies, respectively. Other important environmental factors may have played a role such as ambient temperatures, caloric intake, and social class. This paradoxical finding parallels the similar result in school age children in the same Chilean population discussed above, and remains unexplained.

Lamm et al. (1999) examined rates of congenital hypothyroidism in 7 counties of California and Nevada with perchlorate contaminated drinking water. This outcome is defined as a result of a mandatory screening program at birth that involves a preliminary T4 determination followed by a TSH assay in a prescribed subset with low T4. Age at screen is not considered in this procedure for selecting candidates for TSH determination and screening age distributions by county were not reported. County-specific levels of perchlorate contamination were unavailable. Rates for the California births were adjusted only for Hispanic ethnicity, observed to be a risk factor in this and other studies (Brechner et al., 2000; Schwartz, 2001). The county rate ratios for congenital hypothyroidism ranged from 0.6 to 1.1 relative to the statewide expected rates and were not statistically significant for all exposed counties combined, the rate ratio was 1.03 (95% CI = 0.90 to 1.16). Expected rates based on the non-exposed counties of the two states were not used. Had only non-exposed counties been used for comparison (given that the exposed counties

comprise a substantial fraction but assuming it is less than half of the state's population) the resulting rate ratios for the exposed counties would have been 1% or higher. Most critically lacking in the analysis was classification on age at time of blood sample for the screening test. Birth weight and further detail on ethnicity and other risk factors were also unavailable. Therefore, it is likely that uncontrolled confounding has played a role in this study, making it difficult to interpret and allows for some role of perchlorate in the almost two-fold observed variation in risk of neonatal hypothyroidism across counties.

Li et al. (2000a) compared the mean monthly T4 levels derived from mandatory screening of all newborns in Las Vegas (exposed) and Reno (unexposed), controlling for birth weight (within the restricted range 2.5-4.5 kg) and for age at sample (days 1, 2 or 3 versus 4), for the period April 1998 through June 1999. Statistical differences in the mean birth weight and mean age at time of sample were noted for the Las Vegas (n = 17,308) and Reno (n = 5,882) newborns. The exposure variable was based on monthly measurements made on Las Vegas finished water by the Southern Nevada Water Authority using IC with a detection limit of 4 ppb. The source of the Las Vegas water supply, Lake Mead, is known to have thermal stratification that causes seasonal variation in drinking water perchlorate content. The water supply in Reno comes from the mountains via Lake Tahoe, the Truckee River, and local wells. Tests of these water sources for Reno were reported to detect no perchlorate (data not shown nor was it specified if these measurements were made monthly). A highly significant period or seasonal effect was observed for both cities (perhaps suggesting an ambient temperature effect), but no difference was observed between cities during the period of exposure (7 out of the 15 months of observation when perchlorate content was high in Las Vegas drinking water). Highly significant age effects were observed, but the dependence of these age effects on exposure (i.e., an exposure interaction) was not examined. For reasons that are obscure, T4 levels reported in this study were considerably higher than those reported by others (17 versus 7-10 μg/dL). The restriction on birth weight would be inappropriate if birth weight were an intervening variable (i.e., itself affected by thyroid changes resulting from perchlorate exposure). Regressions on first trimester and 9-month cumulative exposures using monthly perchlorate levels and grouping birth outcomes by month in Las Vegas and Reno revealed no trends for T4 differences between the two cities although more powerful analyses could have been performed using individual observations. This study suggests that clinically significant individual neonatal T4 differences

have not resulted from current perchlorate exposures although the possibility of important variation with exposure conditional on neonatal age was not examined.

In a parallel study design, Li et al. (2000b) studied TSH levels in Las Vegas and Reno newborns over an eleven-month period from December 1998 to October 1999. Las Vegas water had measurable perchlorate levels in 8 of the 11 months. The perchlorate exposure measures and assumptions were the same as in Li et al. (2000a). TSH levels were determined on screening samples that were below the 10th percentile on T4 in each daily batch of samples collected throughout the state, selected without regard to age at screening. TSH levels from the two cities for birth weights restricted to 2.5-4.5 kg were compared adjusting for gender and age at screen (days 2-7 versus 8-30). Births whose screening sample was taken on the first day were excluded because those TSH levels were considered unstable. The study did not report whether the age at screen distribution differed between the two cities. Ethnicity and other risk factors were not available. Using a log-transformed TSH level to facilitate statistical testing, they found no difference in TSH levels between the two populations (a very small negative effect was estimated for TSH with exposure), however, the log transformation may have suppressed important differences at the high end of the TSH distribution and the analysis was not restricted to the 8 months when exposure differed between the two cities. Examination of an exposure with age interaction was not reported. Excluding births screened on the first day may have further obscured differences arising from perchlorate exposure, differences that pertain to thyroid responsiveness. This study suggests that TSH levels in newborns after the first day did not differ substantially between two cities with and without perchlorate contamination of drinking water as estimated by monthly measurements.

Brechner et al. (2000) studied TSH levels in Arizona newborns assayed over a three-year period between October 1994 and the end of December 1997 in the Arizona Newborn Screening Program. In this program, total T4 is assayed in daily batches of specimens received from all over the state. TSH is measured in selected samples, representing approximately 10% of the samples with the lowest T4 levels from each batch. TSH levels were compared between two cites, Flagstaff and Yuma, representing areas of nonexposure and exposure to perchlorate. Zip codes were used to determine that Yuma was the only area with essentially all of its drinking water supplied by the Colorado River below Lake Mead. Exposure data were not available for the period between 1994 and 1997. Measurements made by the U.S. Environmental Protection

Agency Region 9 laboratory in August 1999 reported perchlorate levels at 6 ppb in both raw and finished water for Yuma and not detectable in Flagstaff water. Because the water processing facilities have not changed in either city and perchlorate is known to persist for long periods, Brechner and colleagues presumed that comparable differences between the perchlorate levels in the two cities existed during the period of analysis. Controlling for age at screen (days 0, 1-4, 5+) and Hispanic ethnicity, these investigators found a statistically significant elevation in TSH for the exposed population in Yuma (crude TSH: 19.9 versus 13.4 mU/L; adjusted TSH effect not reported). However, the age-at-screening distributions differed considerably between these two cities presenting a possibility for some residual confounding on age. In Yuma (exposed) 5.9% of newborns were screened in the first 24 hours when TSH levels peak (mean TSH = 30 mU/L), compared with 2.4% of Flagstaff newborns (mean TSH = 23 mU/L). Thirty-one percent of Yuma births were screened at day 6 compared with 46% of Flagstaff births. Additionally, because of this negative association between age and exposure, the analysis of variance procedure employed had the potential for bias arising from colinearity. The age and exposure effect estimates would be jointly affected: overestimating exposure and underestimating age effects, or visa versa. Other factors not controlled included gender and birth weight. This study offers positive support for an association of increased neonatal TSH with perchlorate exposures; however, similar to other studies on this question, it has some unresolved methodological issues, most notably the strong association between age at screen and perchlorate exposure.

There is a subtle form of bias in the Brechner and other studies where TSH was determined on a low - T4 percentile subset of all births that mixes on a daily basis ages at screen for samples from all over the state. Bloods with low T4 are selected, but the T4 distribution depends on age. Births with screen ages that usually have higher T4 (typically after 24 hr) are less likely to be selected for TSH determination; conversely, at ages under 24 hr, births are more likely to be selected. Both summary and age-specific TSH comparisons would be unbiased with respect to exposure effects only if the same age at screen distributions were obtained in both the exposed and unexposed populations. The effect of this bias on estimation of overall perchlorate exposure effects is difficult to predict, depending in part on how perchlorate exposure affects T4 as well as on its effects on TSH, and on how sampling age varied with exposure status. It is conceivable that this bias could explain some of the elevated TSH in perchlorate-exposed neonates of the

Brechner et al. (2000) study, but the same sampling bias was potentially present in the Li et al. (2000b) study that found no effect. The latter study, however, excluded neonatal blood samples taken during the first 24 hours. That is the period when the strongest perchlorate-related differences were observed in the Brechner et al. (2000) study.

Schwartz (2001) analyzed both T4 and TSH levels for all California newborns screened in 1996, making use of detailed covariate information on age, birth weight, ethnicity and birth multiplicity. Perchlorate exposure was assigned using the mothers' postal zip codes that were linked to state water testing data on all drinking water sources. These estimates of perchlorate levels were ultimately collapsed into four exposure categories: 0, 1-2, 3-12, 13+ ppb. This level of exposure detail far exceeded that of any other studies, very likely resulting in the least exposure misclassifications.

An analysis of covariance (ANCOVA) model was used in this analysis. The ANCOVA model is a multiple linear regression model that can simultaneously estimate effects for levels categorical variables like gender as well as for continuous variables like age or birth weight. Controlling for age at screening (6-hour increments up to 48 hours), gender, single versus multiple birth, birth weight (in 5 levels), and ethnicity (20 categories), a highly statistically significant declining trend was observed for T4 with the four perchlorate exposure levels (0, −9.7, −11.2, −18.2). T4 levels in this model declined with age (relative to its final level after 48 hours) until about 18 hrs (-50 mg/dL below final level) and then increased over the next 30 hours (to 36 mg/dL above final level) before assuming its final level after 48 hours. For TSH (log-transformed), there was a significant increasing trend with perchlorate exposure (0, 0.029, 0.03, 0.128), and the TSH level followed a more rapid time course increasing immediately after birth, then declining to a final level by 24 hours. Substantial birth weight, gender, ethnicity and birth multiplicity effects were observed for T4, and smaller effects were observed for TSH.

The models specified in this study tested for uniform additive exposure effects for T4 and TSH across all covariate categories, including baseline shifts. Another issue of considerable physiological interest would have been whether the amplitudes of the T4 and TSH surges depended on perchlorate exposure with baseline levels relatively unaffected, which could be tested by evaluating an interaction between age and exposure. An examination of interaction was not reported. The bias in TSH measurements introduced by the T4-triggered sample selection described above for other effects studies would also affect the Schwartz study. This bias would

not affect inferences on exposure effects if the age at screen distribution were similar across the four exposure levels. These distributions were not reported in the Schwartz study.

The Schwartz study also modeled the effect of two screening performance criteria on the same set of predictors: (a) "presumptive positive criterion" and (b) a positive finding of congenital hypothyroidism. Not surprisingly, these models did not predict the standard screening outcomes well because the screening algorithm does not take into account the several very important predictors identified in this study. Rather, finding a presumptive positive is based entirely on T4 without regard to age at screen, birth weight, etc. Similarly, identifying a case of congenital hypothyroidism is based only on T4-triggered sample selection and subsequent TSH determination (>25 μU/ml).

The Schwartz study is by far the most convincing of the neonatal studies, being based on the most elaborate exposure assignment and the most detailed collection of covariate information pertaining to neonatal thyroid function. It is unlikely that bias arising from the TSH sampling could produce such a consistent TSH exposure response and would play no role in the stronger (based on narrower confidence intervals for the parameter estimates) exposure response observed for T4.

4.1.2 Occupational Studies

There are two publications investigating workers in ammonium perchlorate production (Gibbs et al., 1998; Lamm et al., 1999). The route of exposure for each was by inhalation to perchlorate dust, introducing a considerable uncertainty in dose-response analysis especially due to poor characterization of particle size distribution. Both studies were also cross-sectional in design and, therefore, subject to survivor bias in that workers experiencing adverse effects could have left employment. This issue was not addressed in either study. It would have been particularly noteworthy had any former employee no longer in the cohort experienced thyroid disorders, aplastic anemia, or related hematological disorders, each of which have been reported in settings where perchlorate is used for short periods at higher doses in the treatment of disease (Lawrence et al., 1999). The airborne exposures that were characterized corresponded to daily doses on the order of 20 to 50 mg and possibly higher as the air-sampling methods excluded large particulate (> 50 μm) that could add considerable mass to the daily inhaled or ingested

dose. In the study that investigated this (Lamm et al., 1999), the daily absorbed dose based on urinary perchlorate actually exceeded the inhaled dose.

There was no clear evidence for any perchlorate effect on thyroid function, as defined by the investigators, in these two cross-sectional occupational studies. However, historical exposure classification was limited in one study and absent in the other. Former employees were lost to follow-up, and neither study controlled for potential confounding arising from body mass, environmental temperatures, or socioeconomic status. There was no measurement of thyroid iodine status or of any index thyroid dynamic responsiveness that conceivably could be altered even though steady-state TSH and T4 levels appear to be in the normal range. Because of the cross-sectional design and measured endpoints, the studies did not evaluate the dynamics of hypothalamic-pituitary-thyroid feedback that are likely important in target populations such as hypothyroxinemic pregnant women and their fetuses.

Gibbs et al. (1998) performed a case control occupational epidemiology study to evaluate thyroid function and standard clinical blood test parameters of liver, kidney, and bone marrow function in employees exposed to ammonium perchlorate airborne dust at a production facility and an associated cross-blending facility. Exposure estimates were based on multiple samples (average sample number = 17) for eight homogenous exposure groups defined by similar job activities: control, maintenance/foreman, and six discrete operator job categories. Personal breathing zone samples (n = 119) were used for the work categories and full-shift area samples were used for the control group (n = 19). The control exposure was not zero but was several orders of magnitude below any exposure category. In their 1997 analyses, when ammonium was quantified using National Institute for Occupational Safety and Health Method 6016 which had a minimum reporting limit of 0.017 mg/m^3, concentrations in a large number of the samples were reported as undetectable. The 1998 analyses were performed using the modified EPA 300.0 methodology that determines perchlorate using ion chromatography and has a reporting limit of approximately 0.00004 mg/m^3.

Effects were examined in either a single-shift design (pre- and post-shift parameter measurements) or working lifetime design based on medical surveillance data that included thyroid examination since 1996 (blood tests, physical exam, and history since 1994). Dose was reconstructed based on personnel records for job type and area samples.

Despite the lack of particle size distribution data, an inhaled "dose" was calculated for a single shift as (Gibbs et al., 1998):

$$\begin{pmatrix}\text{respiratory}\\ \text{rate}\end{pmatrix} \times \begin{pmatrix}\text{inhalation}\\ \text{concentration}\end{pmatrix} \times \begin{pmatrix}\text{exposure}\\ \text{duration}\end{pmatrix} \times \begin{pmatrix}\text{fraction}\\ \text{absorbed}\end{pmatrix}. \qquad (4\text{-}1)$$

Working lifetime exposure estimates were calculated as:

$$\sum (\text{mean group exposure}) \times (\text{years in exposure group}) \times 2{,}000, \qquad (4\text{-}2)$$

where the 2,000 was an average of the number of hours worked yearly based on typical overtime rates at the facilities.

Daily respiratory rates of 0.0165 m^3/kg-hr and 0.0068 m^3/kg-hr were estimated for "active" and "sedentary" workers, respectively, based on Beals et al. (1996). These estimates are slightly lower than the default EPA respiratory rates and are moderately lower than those recommended by the International Commission on Radiological Protection in its recent human respiratory tract model (International Commission on Radiological Protection, 1994). Average body weights of the workers were larger than the typical default body weights. Because current practice usually scales ventilation rate based on body weight, higher ventilation rates were expected.

The absence of particle size diameter and distribution data is a significant limitation of the study because this data is required to assess the potential inhalability of the ammonium perchlorate aerosol. Data from another production facility indicate the majority of particles are 200 μm (Hancock, 1998). Particles larger than 30 μm are typically not inhalable by humans (U.S. Environmental Protection Agency, 1996b). Further, there was no mention of face volume performance of the personal samplers using 5-μm filters although this is an important consideration in dusty environments when the particles under investigation have a large diameter. This consideration is especially important here because the filter cassettes were changed when respirators were used. Even if a 5-μm particle diameter could be assumed, the inhaled "dose" calculation should have included an adjustment for inhalability and deposition efficiency to

calculate the deposition fraction, approximately 0.3 at 5 μm (U.S. Environmental Protection Agency, 1996b).

The assumption about the solubility of the inhaled particles is also problematic because this parameter is particle-diameter dependent. The particle diameter dictates the location (extrathoracic, tracheobronchial, pulmonary) where the particle deposits and the local milieu and clearance vary with location also influence solubility (U.S. Environmental Protection Agency, 1996b; Snipes et al., 1997). The solubility of cesium chloride (CsCl) in beagles was used to estimate a fraction absorbed. Although CsCl and NH_4ClO_4 may have similar solubilities, additional uncertainty is introduced because the CsCl particle diameter or inhalability function for the beagles was not taken into account; and the hydroscopicity, which influences the initial deposition site, may not be the same. The assumptions about dose could have been validated with a mass balance approach. For example, perchlorate could have been measured in the blood when samples were taken for thyroid hormone analyses. Additionally, urine samples could have been monitored for perchlorate because it is excreted in the urine. These additional measurements would have afforded some confidence that the inhaled dose estimates were reasonable.

Standard clinical thyroid profiles included a total serum T4, triiodothyronine resin uptake, and TSH. Bone marrow function was evaluated during medical surveillance examinations with standard tests from the complete blood count which included hemoglobin, hematocrit, red blood cell count, mean corpuscular volume, white blood cell count, and platelet count. Standard serum chemistries were used to assess kidney (serum creatinine level and blood urea nitrogen) and liver (serum glutamyl pyruvic transaminase [SGPT], serum glutamyl oxaloacetic transaminase [SGOT], g-glutamyl transpeptidase [GGTP], and alkaline phosphatase) functions.

Dependent variables for the single-shift study were the cross-shift change in measures of thyroid function. Explanatory variables included race, gender, age, hours awake prior to the pre-shift test, number of hours slept during the most recent period prior to the test, time of day, and shift length. Dependent variables for the working lifetime included measures of thyroid, bone marrow, liver, and kidney functions. For the thyroid tests, an additional explanatory variable was used to indicate whether the measurement was from a routine physical in 1996 or from a pre-shift or a post-shift examination in 1997 or 1998. The dose variables were group (control, low dose, or high dose) and estimated cumulative exposure. The dose group

designation was an arbitrary stratification of <8 mg/kg-day and >8 mg/kg-day. Multiple regression was used to analyze the relationship between effect measures and explanatory variables. A sequential approach was used to determine whether a dependent variable should be log-transformed and whether any outliers (defined as a value corresponding to a residual larger in absolute value than three standard deviations) should be eliminated from an analysis.

Estimated doses for the single shift-study ranged from 0.0002 to 0.436 mg/kg-day with a mean of 0.036 mg/kg-day and median of 0.013 mg/kg-day. The dose estimate was not a significant predictor of thyroid function parameters measured in 83 control (65 male, 18 female) or 18 exposed (15 male, 3 female) individuals. Working lifetime exposure estimates ranged from 0.5 to 7.0 (mean 3.5) mg/kg for the low-dose group and from 8.0 to 88.0 (mean 38.0) mg/kg for the high-dose group. The duration of exposure ranged from 1 to 27 years (mean 8.3).
No significant correlations were detected in any measures of thyroid, bone marrow, liver, or kidney function; however, significant gender and race differences were apparent in the clinical tests of bone marrow, liver, and kidney functions. Females were slightly lower in hemoglobin, hematocrit, SGPT, GGTP, and creatinine than males; black workers were slightly lower than whites in hemoglobin and hematocrit and slightly higher in creatinine.

The only significant finding ($p = 0.01$) was that cross-shift TSH changes were greater for those who worked a 12-h shift than for those who worked 8-h shifts, accounting for a 0.45 urinary IU/mL increase across the shift. This was attributed to the influence of circadian changes in serum TSH. However, the TSH increase (10%) across a single work shift in an exposed group (n = 18) compared to an unexposed group (n=83) was observed in groups that together comprised less than half of employees eligible for study. Comparison of workers in three groups (unexposed, low and high cumulative exposure) resulted in consistent patterns for all thyroid parameters in which the unexposed group had values intermediate between those of the low and the high cumulative dose groups. This suggests that important confounding was present (i.e., that the comparison group, which apparently included office workers, differed from the exposed groups on other important risk factors) as well. For thyroid (TSH) and liver outcomes (SGOT, GGPT, SGPT), there were subtle indications of exposure effects: the standard deviation increased substantially in the high dose group, as did the average values but not the percentiles up to the 75^{th}, suggesting that a small subgroup had undergone a considerable upward

excursion. Statistical tests (regression analysis) of these effects were severely limited by the apparent confounding that affected baseline levels.

In the second study of ammonium perchlorate workers, Lamm et al. (1999) assembled a comparison group at the same facility from an unrelated process thought to have low exposure to inhaled perchlorate. Workers were classified using presumptive exposure based on visible dust generated. Pre- and post-shift urine samples were collected to measure urinary perchlorate, iodine and creatinine levels. Post-shift blood samples were analyzed for complete blood count (CBC), hemoglobin, hematocrit and additional red cell parameters (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular concentration). A clinical chemistry panel was also run on post-shift serum samples. Thyroid parameters included TSH, free T4, T4, T3, thyroid hormone binding ratio, thyroid peroxidase antibodies, and clinical examination. Urinary perchlorate measurements were used to calculate a post-shift level of perchlorate (mg) per g of creatinine as an excretion dose, D:

$$D = k[Ei - 0.354\ E0]/0.646. \qquad (4\text{-}3)$$

The right hand term in brackets is the post-shift adjusted level in mg perchlorate per gram of creatinine. Perchlorate absorption was calculated as a time-weighted average exposure using an assumption that the percent absorbed which is excreted is 95%. The human adult creatinine excretion rate was then used to link perchlorate excretion rate in terms of creatinine to rates in terms of time, so that the exposure dose was then calculated as:

12 hours x 60 minutes / hour x 0.001 g/mg x 1 mg creatinine/min x [post-shift]/0.646. (4-4)

While particle size distribution data were collected, this information was not utilized in the analyses. Inhalation exposure was instead categorized into either “total” or “respirable”. While these categories correlated with each other to a good degree (r = 0.82), perchlorate absorption (mg/shift) did not correlate as well to respirable (r = 0.45) as it did to total particles (r = 0.54). The comparison group had current absorbed doses equal to 20% of the low perchlorate-exposed group and 3% of the high exposed group even though the inhaled dose of the comparison group was 4% of that of the low dose and 0.02% of the high dose group. This suggests that there was

considerable exposure misclassification, arising perhaps from general environmental contamination at the work site or in clothing. In one subject, urinary perchlorate increased over a 12 hr period during which there was thought to be no exposure. No significant associations were observed between perchlorate exposure and thyroid parameters; however, measures of cumulative exposure were not considered. Suggestions of increasing trends for T3, T4, and maximum-T3 were not statistically significant but were based on small numbers (numbers of workers in exposure groups: 21 for the unexposed versus 14, 8, and 15 in the low, medium, and high exposure groups).

4.2 CLINICAL STUDIES

The historical clinical data on perchlorate have been predominantly case reports of patients whose results would be confounded either with thyroid disease or other pharmaceutical agents. A few more recent studies have begun to evaluate thyroid function in healthy volunteers. This section will discuss the available data on thyroid function from several clinical studies. A more formal development of the pharmacokinetic data in humans is presented in Chapter 6.

4.2.1 Studies in Healthy Human Subjects

Few data are available to demonstrate the effects of perchlorate on healthy individuals and issues of ethics are likely to preclude clinical evaluation in sensitive populations such as pregnant women. Exposure duration to perchlorate is typically short, from a few days to 4 weeks.

Burgi et al. (1974) examined the effects of perchlorate on the secretion of endogenous iodine by the normal human thyroid gland. Five healthy volunteers (3 males, 2 females; ages 24 to 27 years) received tracers of ^{125}I-iodide and ^{131}I-thyroxine for 17 days, followed by 600 mg/day perchlorate (9.7 mg/kg-day, based on actual reported average body weight of 61.8 kg) for 8 days. Urine and serum were analyzed for ^{125}I and ^{131}I to determine if perchlorate can cause the discharge of endogenous, as well as exogenous iodide, from the thyroid. Results show that this dose of perchlorate also was sufficient to completely block iodide uptake by the thyroid. In addition, perchlorate caused a 65% increase in excretion of nonthyroxine iodide over background. The authors attributed this increase to additional secretion of endogenous iodide

from the thyroid. Treatment with carbimazole plus perchlorate caused an additional increase in the secretion of nonthyroxine iodide, suggesting that perchlorate causes only a partial release of endogenous iodide. This study suggests a Lowest-Observed-Adverse-Effect-Level (LOAEL) of 9.7 mg/kg-day for thyroid effects in healthy patients.

Brabant et al. (1992) administered potassium perchlorate to five healthy male volunteers (age 25 to 28 years) to study changes in TSH concentration and release in response to a decrease in iodine supply to the thyroid. During the first 4 weeks of the study, the volunteers were given 200 μg/day iodine. After iodine supplementation was discontinued, the volunteers were administered 900 mg/day of potassium perchlorate orally for 4 weeks to induce a state of iodine depletion. At the end of the 4-week perchlorate treatment, levels of thyroid hormones were measured. Although perchlorate treatment had no effect on thyroid volume or levels of triiodothyronine (T3) and thyroxine (T4), intrathyroidal iodide concentration and serum levels of TSH were decreased significantly, and serum levels of thyroglobulin were nearly doubled. The authors speculate that the decrease of TSH, which is the opposite of the expected response, may be an early adaptive mechanism to the iodine deficiency induced by perchlorate. They suggest that, early in iodide deficiency, the thyroid becomes more sensitive to TSH creating a feedback mechanism that decreases TSH levels. Only as iodide deficiency becomes more prolonged do TSH levels increase. This study defined a LOAEL of 13 mg/kg-day for thyroid effects. In a follow-up study, Brabant (1994) repeated the earlier studies with perchlorate treatment lasting longer than 4 weeks. As a result of the longer treatment, thyroid volumes increased in all subjects although TSH levels did not increase.

Lawrence et al. (2000) performed a 14-day clinical study with nine euthyroid volunteers (ages 22 to 30 years). Each subject was enrolled after a normal complete physical exam that included a thyroid exam. Blood was obtained for baseline measurement of thyroid function tests, TPO antibodies, CBC, and routine blood chemistries. A spot urine was obtained for routine urinalysis. All baseline tests were normal.

Ten mg of perchlorate as potassium perchlorate was dissolved in 1-L bottles of spring water. Each subject was instructed to consume the 1-L bottle intermittently during waking hours. Assuming a body weight of 70 kg, this dosage is equivalent to 0.14 mg/kg-day. Blood specimens were drawn between 8:00 and 9:00 a.m and 24-hour urine samples were obtained on days 7 and 14 during exposure and then again after another 14 days after perchlorate was discontinued.

Thyroid function was assessed by assays for TSH, free thyroxine index (FTI), total T3, (TT3) and T4. Blood chemistries and CBC were also measured. Baseline thyroid radioactive iodine uptake (RAIU) was measured using ^{123}I at 4, 8, and 24 hours after the ingestion of 150 μCi ^{123}I.

As reported by the authors, statistical analysis for the thyroid RAIU was carried out by analysis of variance (ANOVA) with post hoc pairwise comparisons using Tukey's method. The outcome measure variable was log-transformed to achieve greater homoscedasticity and a more Gaussian distribution. Serial analyses were done: a three-factor ANOVA with factors as patient, treatment, and time and a set of two-factor ANOVAs, one for each of the three times. The analogous mixed-model ANOVAs were also run with subject as a random effect to confirm that repeated measures among the subjects did not affect the results. Statistical analyses of the thyroid function tests and urine and serum perchlorate and iodine values were carried out by ANOVA and Student Newman Keuls (SNK).

Urine and serum perchlorate levels at baseline and during and after ingestion of the daily 10 mg perchlorate dose are presented in Table 4-2. Perchlorate levels returned to baseline after the two week recovery period. There was also no significant changes in urinary iodine excretion during, or 2 weeks after stopping the perchlorate administration as shown in Table 4-3. The authors note that the iodide ingestion of the volunteers was not controlled in the diet and were variable. It may also be worthwhile to note that the urinary iodine values are relatively high (see Chapter 3), indicating a potential protective status in these subjects for the inhibition of the NIS by perchlorate.

TABLE 4-2. URINE AND SERUM PERCHLORATE (ClO_4^-) VALUES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg OF ClO_4^- DAILY FOR 14 DAYS (Lawrence et al., 2000)

Time	Urine Perchlorate[a] (mg/24 hr)	Serum Perchlorate[a] (μg/mL)
Baseline	< 0.5	0
7 Days ClO_4^-	7.7 ± 0.8[a]	0.61 ± 0.02
14 Days ClO_4^-	7.5 ± 1.0	0.59 ± 0.02
14 Days After ClO_4^-	< 0.5	0

[a]Mean ± SE.

TABLE 4-3. URINE AND SERUM IODINE VALUES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg OF ClO_4^- DAILY FOR 14 DAYS (Lawrence et al., 2000)

Time	Urine Iodine[a] (μg/24 hr)	Serum Iodine[a] (μg/dL)
Baseline	254 ± 69	6.5 ± 0.42[a]
7 Days ClO_4^-	233 ± 49	6.2 ± 0.34
14 Days ClO_4^-	385 ± 123	6.4 ± 0.37
14 Days After ClO_4^-	208 ± 42	6.3 ± 0.57

[a]Mean ± SE.

A highly significant decrease in the ^{123}I thyroid RAIU with respect to baseline measurements at all three time points was noted (Table 4-4), 34%, 39%, and 41% at 4, 8, and 24 hours. The decrease averaged over all three time points was 38%. Two weeks after perchlorate was discontinued, the thyroid RAIU values were significantly higher at all three time points (average increase of 25%), indicating a rebound that may represent upregulation of the NIS. The time course of the iodine inhibition could not be calculated since the subjects drank the dose ad libitum over the day and there was evidence that the full 10 mg/day dose was not achieved for at least some subjects because the average daily urinary excretion of perchlorate was 7.6 for the 2-week course of perchlorate administrations. There was a corresponding increase in urinary iodide excretion during dosing followed by a drop below baseline during rebound. T3 levels were observed to rise throughout the 28-day trial (trend not tested).

In a subsequent study reported as a letter to the editor by these same investigators, Lawrence et al. (2001) used nine healthy male volunteers and a dose of 3 mg/day (.04 mg/kg-day assuming 70 kg body weight) and again observed decreased RAIU. The mean 8-hour decrease from baseline was reported to be at 10% and at 24-hours to be 10.3%. Neither were significant based on Tukey paired t-test (data not shown). The RAIU after stopping the perchlorate ingestion for 14 days rebounded as in the first study and was reported to be an increase of 22% at 8 hours and 18% at 24 hours ($p < 0.02$). It is worthwhile to note when evaluating these results that these data (Lawrence et al., 2000; 2001) were evaluated for use in the physiologially-based pharmacokinetic (PBPK) models described in Chapter 6, but the data were excluded due to the

TABLE 4-4. THYROID ^{123}I UPTAKES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg ClO_4^- DAILY FOR 14 DAYS (Lawrence et al., 2000)

	Thyroid ^{123}I Uptake[a] (% Dose)		
Time	Baseline	14 days on ClO_4^-	14 days after ClO_4^-
4 Hours	12.5 ± 1.3	8.2 ± 0.7[b]	16.6 ± 2.4[c]
8 Hours	17.3 ± 1.9	10.6 ± 1.0[b]	21.9 ± 2.8[c]
24 Hours	23.6 ± 2.6	14.0 ± 1.6[b]	27.1 ± 3.3[d]

[a]mean ± S.E.
[b]$p < 0.01$ vs. baseline and after ClO_4^-.
[c]$p < 0.01$ vs. baseline.
[d]$p < 0.05$ vs. baseline.

lack of availability of all records to the QA/QC process and unresolved issues regarding sample sequences. Variability of serum and urine perchlorate results, potentially due to the unstructured drinking water regimen (Merrill, 2001a,b) was noted. Serum levels from the 0.04 mg/kg-day dose group ranged from non-detect to 495 mg/L on days when the subjects were supposed to have consumed perchlorate. Given this variability and the unknown consequence of a 10% change in thyroid RAIU of a small sample of healthy euthyroid individuals to potentially hypothyroid or hypothyroxinemic pregnant women, it would be difficult to designate this effect as a No-Observed-Adverse-Effect-Level (NOAEL) with any confidence.

Greer et al. (2000) described a third study of RAIU in healthy euthyroid subjects in an abstract. Perchlorate was dissolved in 400 ml of drinking water at one of three doses to twenty-four euthyroid volunteers (4 males and 4 non-pregnant females per dose; 18 to 57 years old). The subjects were instructed to drink 100 ml at 4 set times throughout the day for 14 days. Measurement of 8- and 24-hour RAIU was performed prior to perchlorate ingestion (baseline), on exposures days 2 and 14, and on post-exposure Day 15. Expressed as a percentage of baseline (mean ± S.E.), the abstract reports 24-hour RAIU values for the 0.02, 0.1 and 0.5 mg/kg-day dose groups as: 83 ± 5.6, 59 ± 3.5 and 31 ± 2.6 on exposure day 2; 85 ± 5.6, 57 ± 4.7, and 34 ± 4.5 on exposure day 14; and 111 ± 5.1, 96 ± 12, and 108 ± 12 on post-exposure Day 15. These correspond to RAIU inhibition values expressed as % of baseline (where "–" indicates inhibition relative to baseline) for the 0.02, 0.1 and 0.5 mg/kg-day dose groups of –17, –41, and –69 on

exposure Day 2; −15, −43, and −66 on exposure Day 14; and +11, −4, and +8 on post-exposure Day 15. The authors report no difference between males and females and that a linear log-dose relationship was observed with the regression slopes indistinguishable between the 8- and 24-hour measurements (data not shown).

In other unpublished data provided in Merrill (2001a; Attachment #7) these same investigators tested seven euthyroid subjects (six non-pregnant females and one male) at a dose of 0.007 mg/kg-day. Expressed as a percent of baseline, the average 8- and 24-hour RAIU inhibition values measured on exposure Day 14 were −6.2 and −1.8%. The inhibition values ranged from −38.6% to +27.9% of baseline at the 8-hour time point and −26.7 to +39% of baseline at the 24-hour time point. The range for the post-exposure Day 14 RAIU inhibition values was −19.3 to +45% of baseline. No measurements were made on Day 2 when the RAIU inhibition would have been greater. There was no RAIU inhibition measured on post-exposure Day 15. In the Greer et al. (2000) abstract, the authors estimate the no effect level at 0.007 mg/kg-day.

In order to evaluate whether the 0.007 mg/kg-day dose had a sufficient sample size to detect a difference of the observed magnitude as in the other doses tested, the EPA calculated the power of the usual t-test for the 14-day exposure data. A log transform of the ratio of the individual values at Day 14 to their baseline values was based on the non-central t distribution. The power at the 0.007 mg/kg-day dose was low (0.1) compared to the other doses: 0.95, 0.998, and 0.999 at 0.02, 0.1, and 0.5 mg/kg-day.

The EPA has also been made aware of another human clinical study being performed at Loma Linda and funded by Lockheed Martin (Beck, 2001). The study is not yet completed because the objective sample size for each dose group has not yet been attained. Human euthyroid volunteers (male and non-pregnant females) have been dosed with perchlorate in gel caps at 0.007, 0.014, and 09.04 mg/kg-day. Measurements were made at baseline, 3-months, 6-months, and after recovery from exposure for RAIU, T3, T4, and TSH levels. These dosages are the same as already tested so the added value to the human database, especially with respect to the now prominent concern for neurodevelopmental effects secondary to hypothyroxinemia or even transient decrements in T4, is not readily apparent. The additional data may potentially reduce the variability and low power due to the small sample sizes of the previous studies if sufficiently comparable in design.

4.2.2 Studies in Patients with Graves' Disease

Potassium perchlorate had been used to treat Graves' disease in humans; consequently, most of the prior data on perchlorate effects on humans are in patients with this disease. Graves' disease is an autoimmune disorder which causes patients to carry immunoglobulins in their blood that bind to TSH receptors on thyroid cells and act like TSH to stimulate DNA synthesis and cell divisions, leading to a hyperthyroid state. Symptoms of the disease include increased synthesis and secretion of iodide-containing hormones into the blood by the thyroid gland, thyroid gland enlargement, increased basal metabolism, and weight loss. Perchlorate inhibits the excessive synthesis and secretion of thyroid hormones by inhibiting the uptake of iodide into the thyroid and causes an efflux (discharge) of accumulated iodide in the gland.

Stanbury and Wyngaarden (1952) evaluated therapeutic perchlorate use in patients (n = 8, although reporting of exact numbers for various aspects [e.g., different dose levels] of the study is sketchy) with Graves' disease and found that perchlorate caused the discharge of iodide accumulated in the thyroid and blocked the uptake of iodide into the thyroid. Within 30 min of administration, a single dose of 100 mg potassium perchlorate caused the nearly complete release (≈80%) of ^{131}I from the thyroids of Graves' disease patients previously treated with tracer amounts of ^{131}I and 1-methyl-2-mercaptoimidazole (MMIA). MMIA was given to cause accumulation of ^{131}I in the thyroid because MMIA prevents the oxidation of iodide ion to iodine and its attachment to tyrosyl groups (see Chapter 3). A single dose of 10 mg perchlorate appeared to cause a ~50% release of accumulated iodine. The authors reported that perchlorate doses as low as 3 mg caused detectable, but incomplete, release of iodide from the thyroid (although quantitative data for doses less than 10 mg were not presented). In addition, Stanbury and Wyngaarden (1952) reported that the uptake of tracer levels of ^{131}I into the thyroid glands of two patients with Graves' disease was markedly inhibited for as long as 6 hr when 100 mg of potassium perchlorate was given orally 1 h prior to administration of the tracer. Beyond 6 h, uptake of ^{131}I recommenced. Inhibition of iodide uptake also occurred in three patients without MMIA treatment. The authors stated that no toxic effects were encountered in any patients who were given, in more than three doses, a total not exceeding 600 mg potassium perchlorate. This

study was used to identify a LOAEL of 1.4 mg/kg-day[1] for complete release of iodine from the thyroid for the RfD reviewed in March 1997 (Toxicology Excellence for Risk Assessment, 1997). Because it was not clear what degree of iodide efflux constitutes an adverse effect, a NOAEL was not designated for this study. An expert peer review panel later determined this study was inadequate for RfD derivation (Toxicology Excellence for Risk Assessment, 1998b).

Godley and Standbury (1954) report using potassium perchlorate to treat 24 patients with Graves' disease. Patients were treated with 600 to 1,200 mg/day (typically 200 mg every 8 h) for at least 11 weeks with a few patients treated as long as 45 to 52 weeks. A decrease in iodide uptake was observed. Five patients became euthyroid after continuous administration for 28 weeks. Two patients developed gastrointestinal problems that were assumed to result from perchlorate treatment. In one of these patients, these effects occurred at 600 mg/day, but the dose that the other patient received is not specified. Other side effects of antithyroid agents, such hematological changes, liver damage, and skin rash, were not observed. This study suggested a LOAEL of 9 mg/kg-day in humans for short-term exposures.

Crooks and Wayne (1960) observed one case of skin rash and three cases of nausea (12%) among 35 patients treated with 600 mg/day (9 mg/kg-day) and 165 patients given 1,000 mg/day (14 mg/kg-day). All patients had diffuse goiters and exophthalmos, classic signs of Graves' disease. In another group of 10 patients given 1,500 mg/day (21 mg/kg-day) and 40 patients given 2,000 mg/day (29 mg/kg-day), five cases of skin rash, two cases of nausea, and one case of agranulocytosis occurred (16%). Leukocyte counts returned to normal in the patient with the agranulocytosis when perchlorate treatment was stopped. The length of treatment was unclear but generally appears to have been less than 8 weeks although it appears that one patient was monitored for 22 weeks. The authors report that the "time to cure" Graves' disease using perchlorate is approximately 9 weeks. The authors also report that 1 of 12 infants born of mothers given 600 to 1,000 mg/day was born with a very slightly enlarged thyroid that returned to normal size in 6 weeks; no other abnormalities were noted. This study suggested a LOAEL between 9 and 14 mg/kg-day.

[1]Unless otherwise indicated, for human studies in which the actual body weight of the subjects was not reported, the dose in milligrams per kilogram per day was calculated assuming a body weight of 70 kg. Thus, a dose of 100 mg/day ÷ 70 kg is 1.4 mg/kg-day.

Morgans and Trotter (1960) reported that 3% of 180 patients treated with 400 to 1,000 mg/day (6 to 14 mg/kg-day) potassium perchlorate and 18% of 67 patients treated with 1,200 to 2,000 mg/day (17 to 29 mg/kg-day) displayed a variety of adverse reactions that included skin rash, sore throat, gastrointestinal irritation, and lymphadenopathy. Reactions occurred within 2 to 3 weeks of drug administration. This study suggested a LOAEL between 6 and 14 mg/kg-day.

Connell (1981) reported a case study of a single 72-year-old female Graves' disease patient who was treated with 200 mg/day (3 mg/kg-day) potassium perchlorate for 22 years without any indication of adverse side effects. Thyrotoxicosis recurred 4 weeks after stopping potassium perchlorate administration, suggesting that this dose level provided sufficient clinical control of the hyperthyroidism. The study also suggested that the adverse reactions seen at higher doses may not occur at lower doses, even after long-term treatment.

4.2.2.1 Hematological Effects

Between 1961 and 1966, the occurrence of severe hematological side effects in patients receiving long-term potassium perchlorate treatment for Graves' disease led to a decreased use of potassium perchlorate as a therapeutic agent. Several authors (Hobson, 1961; Johnson and Moore, 1961; Fawcett and Clarke, 1961; Krevans et al., 1962; Gjemdal, 1963) report case studies in which a single patient suffered fatal aplastic anemia after treatment doses ranging from 6 to 14 mg/kg-day. The duration of treatment ranged from 3 mo (Johnson and Moore, 1961) to 8 mo (Hobson, 1961). In all cases, patients were started at the high end of the treatment range for a period of time and then were reduced to the lower end of the treatment range after the appearance of side effects. In two cases (Hobson, 1961; Gjemdal, 1963), patients had co-exposures to other drugs. Other case reports are available that report nonfatal agranulocytosis in patients treated with 14mg/kg-day for 12 days (Southwell and Randall, 1960) or 3 mo (Sunar, 1963). Barzilai and Sheinfeld (1966) report that 11% of 76 patients developed leukopenia or other unspecified side effects after treatment with 1,000 mg/day (14 mg/kg-day) for a little as 2 mo. Within this group, there was one case of fatal aplastic anemia and one case of fatal agranulocytosis.

These studies suggest that doses in the range of 6 to 14 mg/kg-day may represent a frank effect level in patients with Graves' disease although there were questions as to whether these effects were caused by the disease itself, whether there was some contamination, or whether the

effects occurred only at high doses. A review by Wenzel and Lente (1984) concluded that the "severe adverse reactions, such as agranulocytosis, were likely to occur only when large doses of more than 1,000 mg potassium perchlorate were administered." There is no information to suggest that humans without Graves' disease would have a similar reaction to perchlorate.

Antithyroid drugs appear to exert their effects on the hematopoietic system through an immune mechanism. Wing and Fantus (1987) reviewed the adverse effects of two antithyroid drugs, propylthiouracil and methimazole, and concluded that most reactions were related to the immunologic effects of these drugs. They noted that skin rash and granulocytopenia were among the most commonly reported adverse effects of these drugs. Less commonly reported effects include aplastic anemia, leukopenia, and antibodies to insulin and glucagon. In fact, Wing and Fantus (1987) recommend that patients be instructed to report skin rash immediately, as this may be an early sign of adverse immune reaction caused by the antithyroid drugs. Although these authors did not include perchlorate in their investigation, the similarity of the effects seen after perchlorate treatment—including rash, leukopenia, agranulocytosis, and aplastic anemia—suggest that perchlorate also may act in a similar fashion to induce an immune effect.

There is a tight functional connectivity between the immune and endocrine systems which is mediated, at least in part, by shared receptors and mediators among the systems (Kammuller, 1995). Thus, although the mechanism of perchlorate action on the hematopoietic system is not known, it is likely to be an immune reaction. Although it is possible that perchlorate may cause hematological effects in healthy humans, it appears that Graves' disease patients are likely to be more sensitive to this type of immune-induced adverse effect than are healthy people. The increased sensitivity to immunologic function in Graves' disease patients arises because of the underlying abnormal immunologic function in Graves' disease. Immunoreactivity to antithyroid drugs is another expression of the compromised immune system in these patients (Wall et al., 1984; Wing and Fantus, 1987).

4.3 SUMMARY OF CONCLUSIONS REGARDING HUMAN HEALTH EFFECTS STUDIES

The recent human studies support the established effect of perchlorate at the NIS. Using these data as the basis for quantitative dose-response assessment is more difficult. Of the five

population studies investigating the effects of perchlorate exposures on TSH levels in newborns (Lamm et al., 1999; Li et al. 2000b; Crump et al., 2000; Brechner et al., 2000; Schwartz, 2001), the Brechner et al. (2000) study had a somewhat better exposure classification owing to a more narrow, but still ecological, geographical focus (two small cities) and Schwartz had a relatively detailed exposure classification down to the level of zip codes. Only these two studies had positive findings in newborns. The restriction of birth weight in Li et al. (2000b) could have reduced study sensitivity if thyroid endpoints in non-normal birth weights are especially effected by perchlorate. The strong dependence of thyroid endpoints on birth weight observed in several studies raises the possibility that birth weight itself could be an intervening variable in perchlorate effects. That is, perchlorate exposure may affect birth weight. This would be a testable hypothesis in several of the studies. If birth weight were an intervening variable, birth weight restriction in the Li et al. (2000a,b) studies or controlling for birth weight as a confounder in the Li et al. (2000a,b), Brechner et al. (2000) and Schwartz (20001) studies may have resulted in an underestimation of perchlorate exposure effects.

In the one study that reported age-specific perchlorate exposure effects on TSH (Brechner et al., 2000), the largest effect was in the first 24 hours after birth. This observed exposure-age interaction was not statistically evaluated. The study with the strongest findings (Schwartz, 2001) actually focused only on the first 2 days after birth. Therefore, excluding day-one screened births as in the Li et al. (2000b) study may severely reduce or eliminate the ability to detect a perchlorate effect.

The well-known TSH surge at birth is thought to represent a response to temperature change (Schwartz, 2001). This suggests that ambient temperatures – prenatal and perinatal – might be important determinants of thyroid endpoints. The strong period/seasonal effect observed in the Li et al. (2000b) study supports this temperature conjecture and the unexpected trends across Chilean cities in the Crump et al. (2000) and variations across U.S. counties in the Lamm et al. (1999) and Schwartz (2001) investigations could also be related to temperature.

It should also be noted that all of the studies in this review examined endpoints that may be insensitive to the consequences of altered thyroid function. No detailed models of thyroid dynamic response were postulated with subsequent analysis of relevant endpoints that would reliably detect the specific perchlorate- or environmentally-induced defects. Nonetheless, one study examining neonatal thyroid status in the first five days found a perchlorate effect that was

greatest in the first 24 hours and that rapidly declined over the next two days, suggesting alteration of thyroid response to the birth event. The issue of iodine depletion in exposed populations was not directly evaluated although experimental evidence of short-term depletion in adults at high doses was observed.

All of the observational field studies utilized "ecological" exposure rather than individual-specific dose measurements; the relative specificity of the dose metric varied widely from "exposed/not exposed", to an average concentration in drinking water for a given zip code. The occupational studies used air sampling to estimate homogeneous exposure groups. Nevertheless, there was evidence of perchlorate effects on neonatal thyroid status, with the studies by Brechner et al. (2000) and Schwartz (2001) contributing the most compelling observations, and iodine depletion was observed experimentally. The presence of exposure misclassification and potentially serious confounding in many of the studies makes interpretation difficult and allows for the possibility of missed effects even at the level of current thyroid function (e.g., steady state levels of TSH or T4). The full implications of these findings are unclear; however, they should be taken seriously, especially in populations already at risk for thyroid deficiency. These considerations are summarized in Table 4-5.

The present review differs from a recent summary co-authored by two major participants in industry-funded perchlorate research (Soldin et al., 2001). That review argues that there is now sufficient evidence to recommend safe levels for regulatory purposes. The authors see no immediate need for refinement of the physiological issues underlying the existing epidemiologic study designs or for new initiatives in evaluating such issues in human populations. Potentially important aspects of the mode-of-action for perchlorate not well addressed in the available human studies include: (1) short-term effects of variable exposure during pregnancy, for example, on critical neurodevelopmental effects; (2) the effects of iodine depletion on the T4 or TSH surge response at birth, i.e., whether the effect of perchlorate on fetal thyroid status depends additionally on prior cumulative exposure; (3) the equilibration of this regulated system under chronic exposure and the masking of potential deficiency states such that steady-state T4 or TSH levels appear normal despite substantial impact on production and function; and (4) the special situation of populations or individuals with inadequate iodine intake where thyroid responsiveness may be compromised.

The recent clinical data (Lawrence et al., 2000; 2001; Greer et al., 2000) may be more useful in helping to characterize the potential effects on thyroid function if the mode of action framework is superimposed on the interpretation of the data (i.e., that prevention of significant iodide inhibition would preclude adverse neurodevelopmental and neoplastic sequelae). However, given the current controversy in evaluating thyroid status, particularly in pregnant women, it is difficult to ascertain the degree of iodide inhibition to designate as adverse. Further, there is considerable uncertainty associated with using small sample sizes of euthyroid individuals as the basis of such a determination, so that the use of a factor to account for this in a risk derivation would be warranted, particularly when the variability as noted is considered and the range of inhibition of iodide uptake at levels suggested to be "No-Observed-Adverse-Effect-Levels" include values as great as 38.6% below baseline. A discussion considering these human clinical data in comparison to the laboratory animal toxicological study results can be found in Section 7.1.5.1.

TABLE 4-5. SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
1	Gibbs JP, Ahmad R, Crump KS, et al JOEM 1998; 40:1072-1082. *Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function.*	Kerr-McGee workers in voluntary medical surveillance 1994-98; 170 out of 254 did survey; 130 did single shift evaluation	Airborne exposure to AP in 8 homogenous exposure groups: 0.04-627 $\mu m/m^3$ using closed face cassettes	1 day 1-27 yr.	T3U, T4, FTI, TSH, liver, kidney and hematol fcn T4: 7.5 μg/dL TSH: 2.0 μIU/ml	Indication of increase in TSH over work shift: 2.2 -> 2.5. In workforce, T4 declines and TSH increases from low to high exposure but also from low exposure to unexposed; see inconsistent TSH trends using two lab groups; for both thy and liv outc, SDs increased in high dose group: for thy and liv fcn, averages for low vs high AP very different but %iles up to 75th are not. Implies big excursion at high exposure end.	Possibly half of eligibles did not participate in shift study; possibly confounded by shift duration. Did not evaluate ITR. Suggestion of inappropriate unexposed comparison group. In this steady state and cross-sectional population, difficult to assess thyroid regulatory status. SDs suggest heterogeneity of effect. **Indications of chronic effects.**
2	Lamm SH, Braverman LE, Li FX, et al. JOEM 1999; 41:248-260. *Thyroid health status of ammonium perchlorate workers: a Cross-sectional occupational health study.*	American Pacific workers: 37 AP and 21 azide workers: full feasible participation; all from same site with same other work attributes	Airborne exposure in 3 AP groups based on visible dust level; total and respirable AP by individual closed-face samplers 10-11 hrs on subset from each exp group; levels: total dust (mg/day): .01, .34, 6.57, 59.4; resp fraction (mg/day): .02, .09, .60, 8.6	1 day n=58; 6 days n=2	Urine AP, T3, T4, FTI, TSH, THBR, and hematologic fcn T4: 7.0 pg/dL TSH: 2.6 JLrU/InI	18% of total airborne Mb is respirable (range *8-25);* urinary excretion of P shows much higher absorbed dose in unexposed workers than expected from air samples: (mg): .88. 4.0, 10.9, 33.6 (assuming 8 hr halflife). Thy, hematol by current exp group: no association (T3, T4]; absorb dose greatly exceeds resp total inhaled dose [F51. See aberrant clearance in 1 of 2 6-day subjects fF2]. Authors conclude no AP health effects.	Some misclassification apparent among exposure groups based on absorbed dose; non-inhalable contribution may constitute important deficit in air sampling results. Steady-state, cross-sectional population difficult to interpret. Thy, hematol results based on current, non-cumulative AP exposure are uninterpretable for chronic effects. Possible increasing trend for max(T3) with exposure group.
3	Lawrence JE, Lamm SH, Braverman LE. J Endocrinol Invest 1999; 22:405-407. *The use of perchlorate for the prevention of thyrotoxicosis in patients given iodine rich contrast agents.*	Radiocontrast patient series	Therapeutic high oral doses (1000 mg) in day prior to contrast agent	1 day	Misc. thyroid parameters —	Recommend in high risk patients (low iodide areas and elderly) a combination of perchlorate and contrast agent.	Not relevant to and uninformative on chronic exposure effects in adults and acute effects in infants.

TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
4	Li FX, Squartsoff L, Lamm SH. JOEM 2001; 43:630-634, *Prevalence of thyroid diseases in Nevada counties with respect to perchlorate in drinking water.*	Medicaid population at risk for thyroid disease in Nevada in 1997-98.	Perchlorate in drinking water in one county (P= 8.9-11.6 μg/L) versus all others	Lifetime	ICD 240-246; ICD 193: thy cancer	Exposed county (Clark) with Las Vegas compared to another county with a city (Reno/Washoe) as well as with all other counties. No significant excesses found for exposed county for the 8 outcomes studied. Actually, the comparison counties (one with a city, and all others) for all important outcomes differed more between them than with the exposed county. For the 6 more prevalent outcomes (n=3069) the exposed county had higher rates than the unexposed (Washoe) county.	Based on period-prevalence rates. Two outcomes with small numbers are not informative: congenital hypothyroidism (n=22) and thyroid cancer (n=44). **The difference in the comparison counties suggests that uncontrolled confounders or uncertain estimates are affecting this analysis and that the study is uninterpretable for all but large effects.** Confounders might include age, gender, body mass, diet, iodine intake, ethnicity, occupational exposures.
5	Crump C, Michaud P, Tellez R et al. and Crump KS, Gibbs JP. JOEM 2000; 42:603-612. *Does perchlorate in drinking water affect thyroid function in newborns or school-age children?*	School children from 1 or 2 schools in three cities in Chile (n=53,49,60 in 0, low and high P cities); all newborns 2/96-1/99 in same cities (n=8888,468,428)	Geological Na-P in drinking water (0, 5.5, 111.6 μg/L)	Recent and lifetime for 6-8 yr-olds; gestation	T3, T4, free T4, FTI, TSH, hematol, liver, kidney, prev:goiter, prev:family H_x thy disease T4:10.0 μg/dL TSH: 3.0 μIU/mL	Did comparisons across cities. Urinary I/creatine low in city-2 lifetime residents: (1,092, 862, 963); goiter high in city-2 recent residents: (17.7, 26.5, 23.3%) and high in city-3 lifetime residents: (22.2, 19.5, 26.0 based on 8, 8, 13 cases); family H_x of the disease high in city-3: OR=4.9 (11.1, 9.8, 30.0); highly significant increase in T4 with increased P (1.25, 1.34, 1.50). Highly significant decrease in log (TSH+1) in newborns in city-3–high P (.91, .91, .66) [T9], which is in the unexpected direction. There was a diverse age-at-screen distribution across cities.	Dietary, ethnic, birthwt, SES confounders of thy fcn uncontrolled; observe trends in unexpected directions; suggesting confounding. Unknown if some Chileans boil drinking water. **Significant paradoxical effects indicate uncontrolled confounding and inappropriate thy fcn model in relation to P in this population**. Possible role of ambient temperatures.
6	Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE. Thyroid 2000; 10:659-663. *The effect of short-term low-dose perchlorate on various aspects of thyroid function.*	9 healthy, male volunteers K-perchlorate – 10mg/day	Potassium perchlorate 10 mg/day	14 days	T3, T4, FTI, TSH, THBR, RAIU, liver, hematology T4: 7.0 μg/dL TSH: 1.0 μIU/mL	Assumed identical P doses. Upward trend for T3 at BL, 7-, 14-, and 28-days (136, 140, 151, 157; trend not tested). See depressed I-uptake at 14 days (40%) with rebound at 28 days; non-24 hour urinary- and serum-I was unchanged throughout. Authors conclude: no thyroid impact because of large I-storage.	Hematol, liver test results clinically "normal" but no data presented. **Inappropriate assessment: clinical rather than epidemiological**. T3 effect not addressed; dietary I not controlled or reported. **Suggests long term iodine depletion.**

TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
7	Lawrence JE, Lamm S, Braverman LE. Thyroid 2001. 11:295 (letter) *Low dose perchlorate (3 mg daily) and thyroid function.*	8 healthy volunteers	Potassium perchlorate 3 mg/day	14 days	T3, T4, FTI, TSH, THBR, RAIU, liver, hematol	No signif changes (data not presented) except for depressed I-uptake at 14 days (10%) with significant rebound (22%) at 28 days;	**Implies some I depletion over 2 weeks at 3 mg/day** (seen by other investigators at 1.4 mg/day).
8	Lamm SH, Doemland M. JOEM 1999; 41:409-411. *Has perchlorate in drinking water increased the rate of congenital hypothyroidism?*	Newborns in CA and NV in 1996-97 in 7 counties	Perchlorate in drinking water: 4-16 μg/L	Gestation	Congenital hypothyroid-ism based on neonatal screen (expected= $35/10^5$) —	Compared counties. Hispanic-adjusted prevalence ratios by county: 0.6 (n=8) to 1.1 (n=136); none statistically significant.	No county-specific levels of P; no individual consumption. Should have used other CA and NV counties for expected rates. **Identification of cases is limited by screening procedure that dues not consider age at screen, ethnicity and birthweight.** Unable to address transient developmental sequelae.
9	Li Z, Li FX, Byrd D, et al. and Lamm. JOEM 2000; 42:200-205. *Neonatal thyroxine level and perchlorate in drinking water.*	Newborns in Reno (n=5,882)and Las Vegas (n=17,308) NV 4/98 – 6/99 with birthwt 2.5-4.5kg and age at screen < 5 days and non ICU	Perchlorate in drinking water of Las Vegas: 0 up to 15 μg/L, measured monthly	Gestation	T4 T4:17.0 μg/dL	Compared cities. Significant period effect (seasonal) (ΔT4=.60) when adj for birthweight (.85/kg), age at screen (day 1,2,3 vs. 4: –1.275, .408,. 758) and gender (.727). No city * period interaction implies no P effect. Age * exposure interaction not investigated. Did regressions on monthly means (T4, cum.P); also, used 10 percentile T4 as an outcome–no effect. See jump in T4 at newborn return visits in days 2-4.	**These T4 levels are much higher than in other neonate studies (7-10).** Birthweight may be intervening variable: P causing reduced birthwt via impaired thy fcn. Loss of power in regressions using monthly means instead of individual obs. Early return visits have selection bias: reason for early return.
10	Li FX, Byrd DM, Deyhle GM et al. and Lamm. Teratology 2000; 62:429-431. *Neonatal thyroid-stimulating hormone level and perchlorate in drinking water.*	Newborns in Reno and Las Vegas NV 12/98 – 10/99 with birthwt 2.5-4.5 kg	Perchlorate in drinking water of Las Vegas: 0 up to 15 μg/L, measured monthly	Gestation	TSH TSH: 10.0 μIU/mL	Compared cities. TSH levels, adjusted for gender and age at screen (2-7 vs. 8-30): no difference for LV vs. Reno.	TSH log transformation for variance stabilization could suppress TSH differences in the high range; inadequate control for age at screen (LV vs Reno), ethnicity and birthwt (2.5-4.5 kg); birthwt may be intervening variable. TSH levels may not be relevant vs T4. **Insensitive to developmental issues and short-term time variability of P exposure.**

TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
11	Brechner RJ, Parkhurst GD, Humble WO et al. JOEM 2000; 42:777-782. *Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona.*	Newborns 10/94-12/97 in two Arizona cities whose T4 screen was below state-wide daily 10%ile	Perchlorate in drinking water <16 μg/L	Gestation	TSH TSH: 13.4 μIU/mL	Compared cities. TSH higher in newborns from exposed city (median: 19.9 vs 13.4); age at screen distribution very different between two cities: exposed screened sooner. Stratifying on age at screen (0, 1-4, 5+ days) and Hispanicity, see signif increase (p=.017); adj effect not reported.	TSH levels (13-20) higher than reported for other newborns (7-10).] Selection on T4 level is problematic due to strong age dependence of T4 surge at birth thus causing variable percentile discrimination with age (8-40% were screened depending on age). This effect could increase TSH of the exposed city relative to unexposed city but the effect of the bias is difficult to predict. Uncontrolled other confounding e.g., birthwt, gest. age, iodine intake, SES.
12	Schwartz J.Dissertation, UC Berkeley, 2001. *Gestational exposure to perchlorate is associated with measures of decreased thyroid function in a population of California neonates.*	99% of California newborns screened for thy disease in 1996	Perchlorate in drinking water classified in 3 levels and assigned by zip code: 1-2,3-12, 13+ μg/L	Gestation	T4, TSH, presumptive positive; congenital hypothyroidm T4: 160 mg/dL TSH: 7.6 μIU/mL	Compared across four levels of estimated exposure. Has detailed covariates: birthweight, age at screen in hours, ethnicity in 20 groups; birth multiplicity; ANCOVA model with extensive control of most confounders finds highly significant decrease in T4 (mean=166) with P level (0, −9.7, −11.2, −18.2) and large effects for birthweight (−72 for birthweight 1500-2500), age (−50 for hours 7-18) and ethnic groups (−10 to −30); see initial T4 fall followed by surge by 12 hours and stays elevated until 36 hours; initial onset of TSH surge unresolvable in time; stays elevated till 18 hours. Significant P effect on TSH (0, .029, .03, .128) but birthweight effects models (−.09 for <1.5 kg). Model for presumptive positives shows strong age at screen and ethnicity effects; for congenital hypothyroidism, insignificant effect.	[T4 is reported at levels 10,000-fold higher than in other studies.] presumptive positive criterion not clear (all at or below 9 mg/dl plus lowest 5% immediately above 9 mg/dl?). NO P-ITR reported, e.g., P * age (especially on surge amplitude), P * birthweight; possible selection bias in identification of TSH subjects. Age at screen was not included in logistic regression model of congenital hypothyroidemia. **This study presents strong evidence of perchlorate health effects in neonates from drinking water contamination with perchlorate.**

TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
13	Soldin OP, Braverman LE, Lamm SH. Therapeutic Drug Monitoring 2001; 23:316-331. *Perchlorate clinical pharmacology and human health: a review.*	Review of animal and human evidence				This review, co-authored by two major participants in industry funded perchlorate research, argues that there is now sufficient evidence to recommend safe levels for regulatory purposes, i.e., at this time there is no need for further refinement of the physiological issues underlying the existing epidemiologic study designs or for new initiatives in evaluating such issues in human populations.	Not considered in this review are issues such as (1) short term effects of variable exposure during pregnancy, (2) the effects of maternal iodine depletion on T4 or TSH surge response at birth, (3) the equilibration of this system under chronic exposure and the masking of potential deficiency states, and (4) the special situation of populations with inadequate iodine intake.

I = iodine; P = perchlorate; AP = ammonium perchlorate; exp = exposure; thy = thyroid; liv = liver; hematol = hematologic; ITR = interaction; outc = outcomes; SD = standard deviation; H_x = history; [Tn] = table in paper; [Fn] = figure in paper.

5. TOXICOLOGICAL EFFECTS IN LABORATORY ANIMAL STUDIES

This chapter provides a review of the relevant laboratory animal toxicity data for quantitative dose-response analysis of the toxic effects of perchlorate exposure. Evidence that both the neoplastic and non-neoplastic effects of perchlorate derive from its anti-thyroid effects at the sodium (Na^+)-iodine (I^-) symporter (NIS) should be appreciated. Studies completed before the initiation of the perchlorate testing strategy described in Chapter 3 are included here, but the major emphasis is on these newer studies given their contemporary design and integrated approach to evaluating perchlorate's mode of action. This introduction provides a brief review of the status of issues after the previous external peer review and a summary of studies recommended and performed since that time. In response to the 1999 external peer review, the EPA committed to a second external peer review to address these recommendations and to evaluate the data from new analyses and studies (Noonan, 1999).

At the external peer review in February 1999, it was noted by the EPA that the thyroid histopathology that had made a significant contribution to the risk assessment had never undergone an independent peer review by a second pathologist in any of the studies. In addition, these studies had been performed at several different laboratories with several different study pathologists using different lesion grading systems. The external peer review panel agreed that these inconsistencies between study reports made it difficult to compare studies and could contribute to variability in the resultant dose-response estimate (Research Triangle Institute, 1999).

In response, the National Center for Environmental Assessment (NCEA) committed to a Pathology Working Group (PWG) process in collaboration with the NIEHS. The purpose of the independent peer review and PWG was to decrease variability in response across the studies by providing a common nomenclature for lesions and a consistent pathology review. Determination of No-Observed-Adverse-Effect-Levels (NOAELs) or designation of adversity was not the objective of this review. NCEA asked Dr. Douglas C. Wolf in the EPA's National Health and Environmental Effects Research Laboratory (NHEERL) to conduct the requisite independent

peer review (second pathology review) using one consistent lesion grading system on the materials. Dr. Wolf was chosen because he had not been involved in any of the work performed with ammonium perchlorate and because he had developed a thyroid grading scheme (Hooth et al., 2001) to analyze a similar thyroid response in rodents exposed to sodium chlorate that would be useful to the perchlorate review.

After the initial pathology review of 100% of the thyroid slides by Dr. Wolf, Dr. Peter Mann of Experimental Pathology Laboratories, Inc. (EPL), reviewed 100% of the slides for quality assurance/quality control (QA/QC) and consistency. Subsequent to this QA/QC review of the independent peer review, a NIEHS-sponsored PWG of 5 experienced veterinary pathologists was conducted on a subset of the slides. Recommendations of that PWG (Experimental Pathology Laboratories, 2000) were then incorporated into the final report on the independent review of 100% of the slides conducted and reported by Dr. Wolf (Wolf, 2000). Both of these reports were made available almost immediately to the public on the NCEA website. During subsequent analyses it was appreciated that the slides provided for the two-generation study (Argus Research Laboratories, Inc. 1999) were from animals not on test and some of the mean severity scores were miscalculated. These minor changes are provided in Wolf (2001).

The thyroid slides that underwent the PWG review included materials from the following studies: Argus Research Laboratories, Inc. (1998a,b,c); Caldwell, et al. (1995); Keil et al. (1998); and Springborn Laboratories, Inc. (1998). It should be noted that the two-generation reproduction study performed by Argus Research Laboratories (1999) was completed at the time of the PWG, and the review included all final thyroid tissue slides despite its listing in the PWG and Wolf (2000; 2001) reports as 1998c. The newest study, that of Argus Research Laboratories, Inc. (2001) described below in Section 5.3.3, was also performed with the new nomenclature and grading system. The study pathologist had been a member of the PWG; therefore, the pathology results can be considered consistent with the results of Wolf (2000, 2001). However, a second independent review of the pathology in that study has not been performed.

All analyses performed on thyroid histopathology in this revised risk assessment rely on either the PWG data (Wolf, 2000; 2001) or the new 2001 study (Argus Research Laboratories, Inc., 2001). The revised benchmark dose (BMD) analyses for thyroid colloid depletion, hypertrophy, and hyperplasia diagnosed in the studies reviewed by the PWG are presented in

Table 5-1 (Geller, 2001a). Figures 5-1 and 5-2 present these estimates and their distributions graphically in comparison to the previous 1998 assessment values. It is worthwhile to note that while hyperplasia occurs at slightly higher concentrations in the analysis of the overall data array, there is considerable overlap with the distributions of the other two thyroid histopathology indices (colloid depletion and hypertrophy). This overlap is especially evident when evaluating BMD or benchmark dose lower confidence level (BMDL) values within individual studies.

The potential for variability due to inconsistent handling of the radioimmunoassay (RIA) kits used for serum thyroid and pituitary hormone levels was also noted at the external peer review (Research Triangle Institute, 1999). In response, the Air Force Research Laboratory (AFRL) conducted a study to compare serum thyroid hormone and TSH data obtained by RAI procedures for three different research laboratories that participated in perchlorate toxicity studies involving hormone analysis (Narayanan, 2000). The purpose was to statistically investigate the reproducibility (i.e., variability across laboratories) and the repeatability (i.e., variability within a laboratory) of the hormone measurements expressed as counts per minute (CPM). RIA kits from the same batch number and with the same expiration date were used for all the hormone measurements for all the standard and unknown samples. For unknown samples, six rat serum samples plus six samples obtained from different species (dog, guinea pig, rabbit and mouse) were used. Assays were performed using the RIA kits according to the manufacturers' recommended procedures and each laboratories' standard operating procedures.

Reproducibility limits (RL) for each sample and for each hormone were determined. The RL was defined as approximately 95% of all pairs of means from the same hormone and same sample; different laboratories should differ in absolute value by less than the RL. The difference in means between any two laboratories is a normally distributed random variable with a mean of zero. The range ± RL is then the middle 95% for this distribution (i.e., 2.5% in each tail). The reproducibility varied for each hormone with T3 showing the best reproducibility and TSH the least. Three replicates ensured a more reproducible sample even when repeatability was not as consistent. The results suggest that the variability in the RIA determination should be considered when determining effect levels.

It was also recommended at the external peer review, by the biostatistician Dr. Joseph Haseman, that different approaches to the thyroid and pituitary hormone analyses be explored (Research Triangle Institute, 1999). EPA complied with this request and developed two new

TABLE 5-1. BENCHMARK DOSE (BMD)[a] AND BENCHMARK DOSE LOWER CONFIDENCE LIMIT (BMDL)[a] ESTIMATES CALCULATED FROM THE WOLF (2000, 2001) THYROID HISTOPATHOLOGY DATA (Geller, 2001a)

Study Name, Time Point Wolf (2000; 2001) Table Number	Ammonium perchlorate dose levels test (mg/kg-day)	Colloid Depletion				Hypertrophy				Hyperplasia			
		BMD	BMDL	χ^2 [b]	Exp[c]	BMD	BMDL	χ^2 [b]	Exp[c]	BMD	BMDL	χ^2 [b]	Exp[c]
1. Caldwell Tbls. 1 and 2	0, 1.25, 5, 12.5, 25, 50, 125, 250	13.29	0.72	0.97	4.37	Not done[d]				35.29	0.78	0.20	0.88
2. Subchonic, 14-day Tbls. 3 and 6	0, 0.01, 0.05, 0.2, 1.0, 10.0	2.55	0.28	0.20	0.74	0.75	0.017	0.54	0.78	NOE[e]			
3. Subchronic, 90-day Tbls. 4 and 7	0, 0.01, 0.05, 0.2, 1.0, 10.0	0.13	0.03	0.70	0.50	0.21	0.008	0.74	0.55	8.36	2.09	1.00	7.87
4. Subchronic, 120-day Tbls. 5 and 8	0, 0.05, 1.0, 10.0	NOE				NOE				NOE			
5. Neurobehav., F0 Fem Tbl. 9	0, 0.1, 1, 3, 10	NOE				NOE				NOE			
6. Neurobehav., PND5 Tbls. 10 and 11	0, 0.1, 1, 3, 10	0.45 0.53	0.009 0.33	0.46 0.67[f]	0.94 1.0	0.92 1.27	0.24 0.88	.024 0.26[f]	0.81 1.0	15.18 11.02	1.86 3.62	0.70 0.32[f]	0.36 1.0
7. Neurobehav., adult Tbls. 12 and 13	0, 0.1, 1, 3, 10	0.72	0.029	0.23	0.89	3.48	NC	0.72	0.29	NOE			
8. 2-gen., P1 Tbls. 14 and 15	0, 0.3, 3, 30	1.97	0.11	0.68	3.84	Poor fit[g]				7.89	2.44	0.41	0.72
9. 2-gen., P2 Tbls. 16 and 17	0, 0.3, 3, 30	2.16	0.90	0.06	1.16	0.99	0.15	0.67	0.70	4.62	0.0004	0.14	0.31
10. 2-gen., F1-weanling Tbls. 18 and 19	0, 0.3, 3, 30	2.51	0.80	0.17	1.2	0.21	0.057	0.40	0.79	2.74	0.66	0.85	0.52
11. 2-gen., F2-weanling Tbls. 20 and 21	0, 0.3, 3, 30	Poor fit				1.19	0.32	0.25	0.52	NOE			
BMDL Range: Rat Studies		**0.009 - 0.90**				**0.008 - 0.74**				**0.0004 - 3.62**			
12. Dev tox., rabbit dams Tbl. 22	0, 0.1, 1, 10, 30, 100	0.12	0.008	0.19	0.36	Poor fit				1.53	0.42	0.13	0.61
13. Immunotox. Mice, combined studies Tbl. 23	0, 0.1, 1, 3, 30	26.07	5.15	1.00	7.88	1.62	0.97	0.58	0.84	24.92	4.48	1.00	7.86

[a] Units of mg/kg-day.
[b] χ^2 p-value.
[c] Exponent in Weibull model fit not restrained to ≥ 1.0 unless indicated.
[d] Not done: Because of non-routine staining, cytological characteristics were not adequate to make determination of hypertrophy on these samples (Wolf, personal communication).
[e] No observed effect (NOE): Either no incidence of endpoint noted in animals tested or no notable difference between dosed and controls.
[f] Exponent in Weibull model fit restrained to ≥ 1.
[g] Poor fit: $p < 0.05$ for χ^2 test.

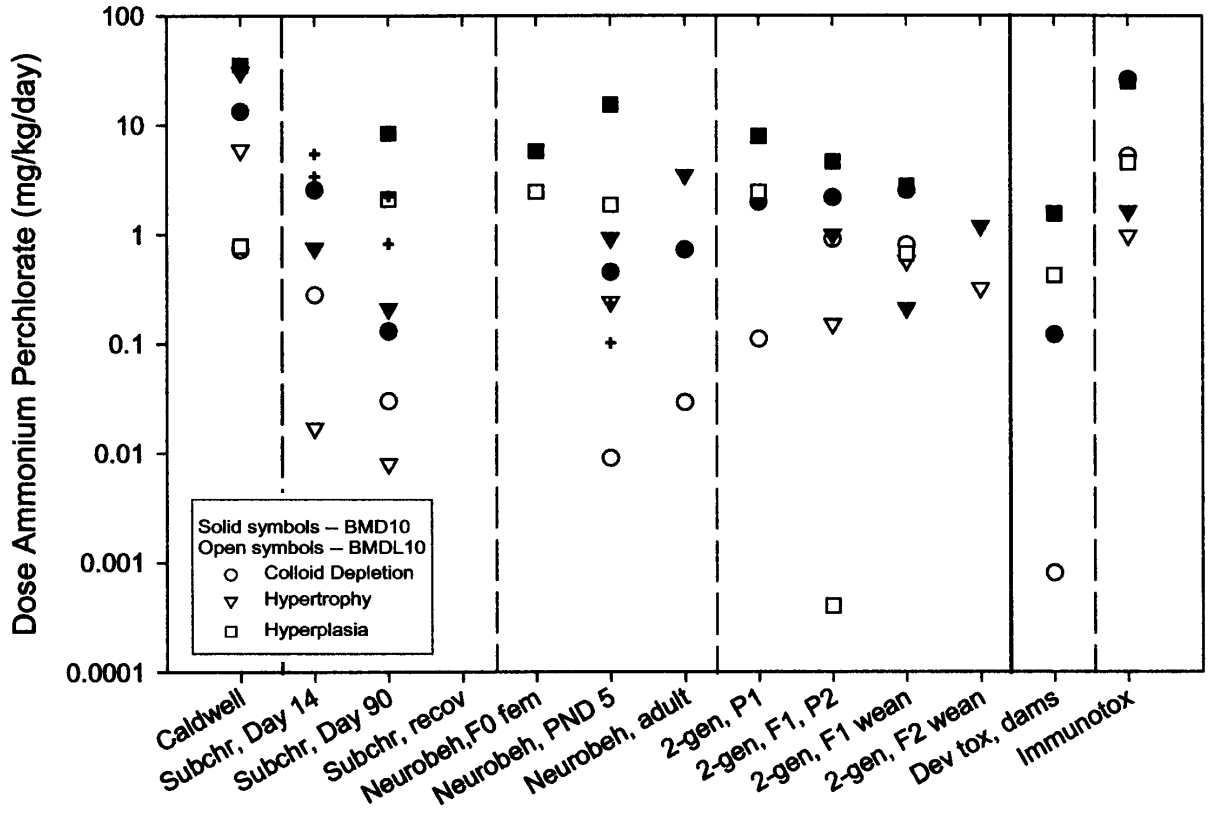

Figure 5-1. Benchmark dose (BMD) and benchmark dose lower limit (BMDL) estimates recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Data on incidence of colloid depletion, thyroid hypertrophy and thyroid hyperplasia were submitted to the EPA for the perchlorate risk characterization. Values used are presented in Table 5-1 (Geller, 2001a). Greater value represents the BMD and lesser value represents the BMDL. The + denotes BMD and BMDL from previous EPA risk characterization (U.S. Environmental Protection Agency, 1998d; Geller, 1998a). Values to the left of the vertical solid line are from the rat studies. Values to the right are from the developmental study in rabbits (Argus Research Laboratories, 1998c) and the mouse immunotoxicity studies (Keil et al., 1998). Study denoted by "Caldwell" refers to Caldwell et al. (1995); "Subchronic" to Springborn Laboratories, Inc. (1998); "Neurobeh" to the 1998 developmental neurobehavioral study (Argus Research Laboratories, 1998a); and "2-gen" to the completed 2-generation reproductive toxicity study in rats (Argus Research Laboratories, 1999).

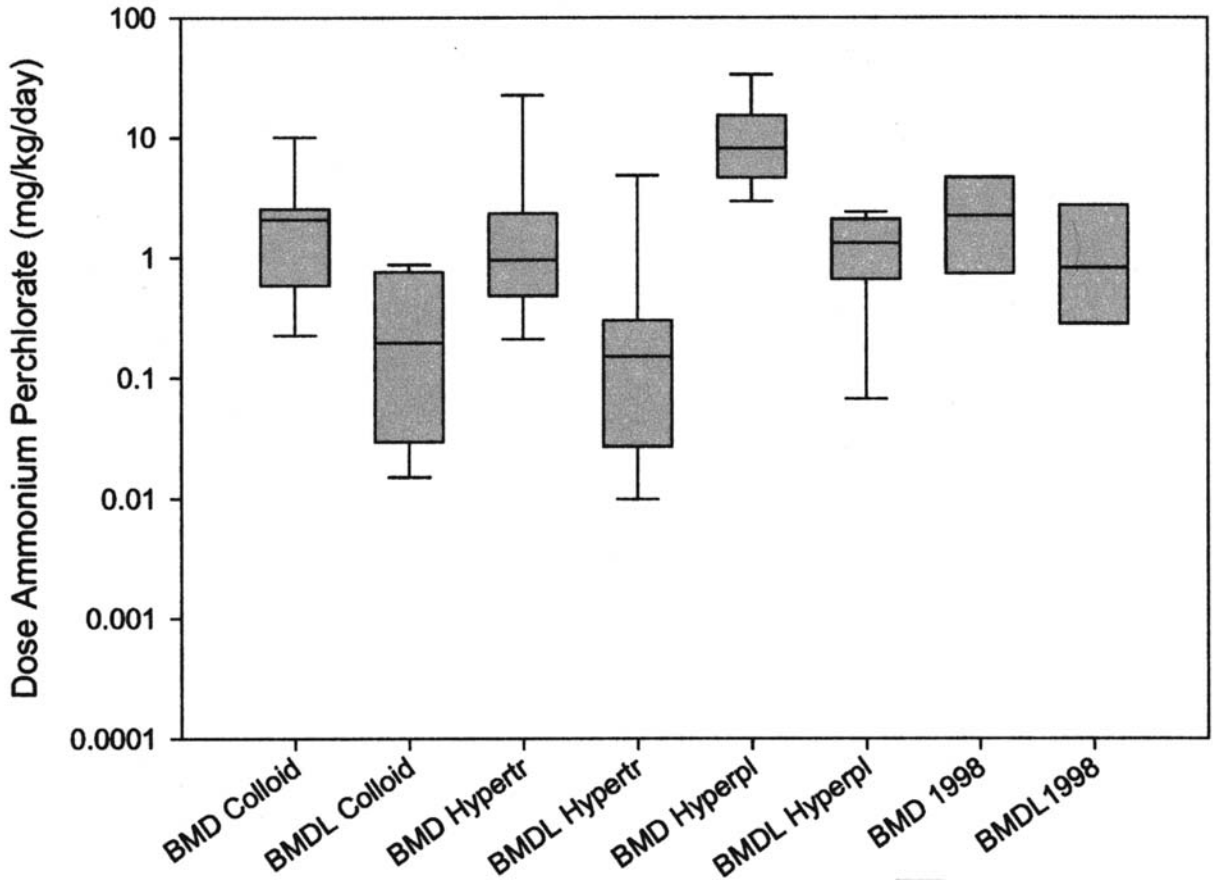

Figure 5-2. Distribution of BMD and BMDL estimates shown by "box and whisker" plots of colloid depletion (colloid), hypertrophy (hyptry), and hyperplasia (hyppls) from rat studies recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Values are presented in Table 5-1. Study #4 was excluded since it was a 30-day recovery experiment and #5 was excluded due to lack of monotonicity. The boundary of the box closest to zero indicates the 25th percentile, a line within the box denotes the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. The two rightmost boxes plot values from the combined rat studies from the 1998 EPA risk characterization (U.S. Environmental Protection Agency, 1998d; Geller, 1998a).

approaches to the analyses that address these comments (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a). All thyroid and pituitary hormone analyses presented will utilize these new approaches. The reanalyses of the hormone data for the previous set of studies can be found in Table 5-2.

Finally, a number of additional new toxicology studies were recommended by the EPA and the external review panel in 1999. These included a study of the developmental effects of perchlorate (Section 5.4.3); a re-evaluation of the effects of perchlorate on neurodevelopmental motor activity (Section 5.3.2); refinement of the evaluation of immunotoxicity concerns with a repeat of the sheep red blood cell (SRBC) response using the established plaque-forming cell (PFC) assay for humoral effects and an additional test for contact hypersensitivity (Section 5.6); and what has become known as the "Effects Study" (Section 5.3.3). The objective of the "Effects Study" (Argus Research Laboratories, Inc., 2001) was to reevaluate brain morphometry effects and to evaluate thyroid histopathology and thyroid and pituitary hormones at various stages of development, including during gestation and post-natal days 5, 10 and 22.

5.1 CHRONIC STUDIES AND GENOTOXICITY ASSAYS

This section discusses the data establishing perchlorate as a carcinogen. A few long-term studies at comparatively high doses performed before the 1997 perchlorate testing strategy showed that perchlorate causes thyroid tumors. These studies are discussed in Section 5.1.1. In order to invoke the conceptual mode-of-action framework for the anti-thyroid effects of perchlorate causing thyroid neoplasia via a non-genotoxic mechanism, the testing strategy had to determine whether or not perchlorate acts directly with DNA. This evidence is discussed in Section 5.2.2. The completed genotoxicity data were presented at the 1999 external peer review as Attachment A to the February 1, 1999 submission provided by NCEA to the peer review panel (Zeiger, 1999a,b; Dellarco, 1999; BioReliance, 1999; Moore, 1999). Dr. David Brusick, the genetic toxicologist on the previous external peer review panel, agreed with the EPA conclusions (Research Triangle Institute, 1999) that perchlorate's ability to cause thyroid tumors was not likely to be via a directly genotoxic mechanism.

It should be noted that perchlorate exposure also caused a statistically-significant increase in tumors at the 30 mg/kg-day dose in the F1-generation pups of the two-generation rat

TABLE 5-2. A COMPARISON OF NOAELs AND LOAELs FROM THE ORIGINAL 1998 ANALYSES AND THE 2001 RE-ANALYSES FOR HORMONE AND MORPHOMETRY ON THYROID FOLLICULAR LUMEN SIZE (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)

Species/Study	Time Point, Age *(Doses, mg/kg-day)*	Endpoint	Sex	Original Analyses NOAEL	Original Analyses LOAEL	Re-Analyses[a,b] NOAEL	Re-Analyses[a,b] LOAEL
Rat 14-Day (Caldwell et al., 1995)	14-Day *(males - 0.0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, 22.16) (females - 0.0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, 24.86)*	T3	M	0.11	0.44	0.11	0.44
			F	—	0.11	—	0.12
		T4	M	—	0.11	—	0.11
			F		0.12		0.12
		TSH	M	0.44	1.11	0.44	1.11
			F	0.12	0.47	**—**	**0.12**
		hTg	M	—	0.11	—	0.11
			F	—	0.12	—	0.12
		rT3	M	0.44	1.11	**0.11**	**0.44**
			F	0.47	1.23	**0.12**	**0.47**
Rat Subchronic Study (Springborn, 1998)	14-Day *(0, 0.01, 0.05, 0.2, 1.0, 10.0)*	T3	M	—	0.01	—	0.01
			F	10.0	—	10.0	—
		T4	M	1.0	10.0	**—**	**0.05**
			F				
		TSH	M	0.05	0.2	**0.01**	**0.05**
			F	0.01	0.05	**—**	**0.01**

TABLE 5-2 (cont'd). A COMPARISON OF NOAELs AND LOAELs FROM THE ORIGINAL 1998 ANALYSES AND THE 2001 RE-ANALYSES FOR HORMONE AND MORPHOMETRY ON THYROID FOLLICULAR LUMEN SIZE (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)

Species/Study	Time Point, Age *(Doses, mg/kg-day)*	Endpoint	Sex	Original Analyses		Re-Analyses[a,b]	
				NOAEL	LOAEL	NOAEL	LOAEL
Rat Subchronic Study (Springborn, 1998) (cont'd)	90-Day *(0, 0.01, 0.05, 0.2, 1.0, 10.0)*	T3	M	—	0.01	—	0.01
			F	—	0.01	—	0.01
		T4	M	—	0.01	—	0.01
			F			—	0.01
		TSH	M	0.05	0.2	0.05	0.2
			F				
Rat Subchronic Study (Springborn, 1998)	120-Day *(0, 0.05,1.0, 10.0)*	T3	M	1.0	10.0	1.0	10.0
			F				
		T4	M	—	0.05	—	**0.05**
			F			**1.0**	**10.0**
		TSH	M	10.0	—	—	**0.05**
			F				
Rat Developmental Neurotoxicity Study (Argus, 1998a)	PND5 *(0, 0.1, 1.0, 3.0, 10.0)*	Lumen size	M	1	3	**0.3**	**3**
			F				
	PND90 *(0, 0.1, 1.0, 3.0, 10.0)*	Lumen size	M	Data not available for original analyses		**10**	—
			F			**10**	—
	PND5 *(0, 0.1, 1.0, 3.0, 10.0)*	T4		1.0	3.0	**0.1**	**1.0**
		T3		0.1	1.0	0.1	1.0
		TSH		3.0	10.0	3.0	10.0
	PND90	T4, T3, and TSH		No data available			

TABLE 5-2 (cont'd). A COMPARISON OF NOAELs AND LOAELs FROM THE ORIGINAL 1998 ANALYSES AND THE 2001 RE-ANALYSES FOR HORMONE AND MORPHOMETRY ON THYROID FOLLICULAR LUMEN SIZE (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)

Species/Study	Time Point, Age *(Doses, mg/kg-day)*	Endpoint	Sex	Original Analyses		Re-Analyses[a,b]	
				NOAEL	LOAEL	NOAEL	LOAEL
Mouse Hormone and Immunotoxicity (Keil et al., 1998)	14-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	3.0	30.0	—	**0.1**
		T3	M	Data not available at time of 1998 analysis		—	**0.1**[c]
		TSH	M	No data			
	90-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	0.1	3.0	—	0.1[c]
		T3	M	Data not available at time of 1998 analysis		—	**0.1**[d]
		TSH	M	30.0	—	—	**0.1**[d]
	120-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	30.0	—	30.0	—
		T3	M	Data not available at time of 1998 analysis		30.0	—
		TSH	M	30.0	—	—	—
Rabbit Developmental Toxicity (Argus, 1998b)	Gestation Day 28 *(0.0, 0.1, 1.0, 10.0, 30.0, 100.0)*	T4	F	0.1	1.0	0.1	1.0
		T3	F	100	—	100	—
		TSH	F	100	—	100	—

[a]Bold indicates where 2001 analyses is different than 1998 analyses.
[b]Results from the liberal and conservative statistical approaches were the same.
[c]No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.
[d]No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.

reproductive study (Argus Research Laboratories, 1999). These pups were used as the parents of the second generation (F2) pups in the study. When these F1 animals were sacrificed after only 19-weeks, tumors were observed (Wolf, 2000). The type was the expected benign thyroid adenoma consistent with the anti-thyroid effect at the NIS (iodine uptake inhibition) with thyroid hormone disruption followed by TSH upregulation. The early onset at 19 weeks is remarkable and suggests the potential for in utero imprinting, a phenomenon beginning to be appreciated with other endocrine-disrupting compounds (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997). These tumor results will be discussed in Section 5.5.

5.1.1 Cancer Studies

Kessler and Krüskemper (1966) provided potassium perchlorate in drinking water at a concentration of 0 or 1% to male Wistar rats for 2 years. Body weights and thyroid weights were reported for groups of 6 to 8 rats sacrificed after 0, 40, 120, 220, and 730 days of treatment, and thyroid glands from the animals were examined histologically. Using body weight data provided in the report to calculate a time-weighted average body weight of 0.336 kg and using an estimated water consumption of 0.045 L/day (calculated with the allometric equation recommended by U.S. Environmental Protection Agency [1987]), a dose of 1,339 mg/kg-day can be derived. Body weights of control and treated animals were comparable throughout the experiment. In contrast, thyroid weights, both relative and absolute, were increased markedly in treated rats compared to controls at each examination interval. Histological examination of thyroids from treated rats at 40 days revealed follicular cell hyperplasia. The authors characterized these changes as typical for a thyroid gland stimulated by TSH during a relatively short period of time. After 200 days of perchlorate treatment, diffusely degenerative changes with fibrosis and increased colloid were observed. The authors commented that the course of the histological changes in the thyroid was similar to that produced by long-term administration of thiouracil, another antithyroid agent. The authors further reported that 4 of 11 rats treated with potassium perchlorate for 2 years developed benign tumors of the thyroid gland and that 20 untreated Wistar control rats displayed no thyroid gland tumors. The 1,339 mg/kg-day dose suggested a free-standing LOAEL because no other doses were tested.

Pajer and Kališnik (1991) administered 0 or 1.2% sodium perchlorate in drinking water to groups of 36 female BALB/c mice (12/group) for up to 46 weeks. Eight or 12 weeks after the

beginning of the experiment, one group of treated and control mice were totally irradiated with 0.8 Gy on 5 consecutive days at a dose rate of 1.45 Gy/min so that each mouse received a total of 4 Gy. Assuming a body weight of 0.0353 kg and a water consumption rate of 0.0063 L/day (U.S. Environmental Protection Agency, 1987), a dose of 2,147 mg/kg-day can be calculated. Thirty animals died during the experimental period; however, details about the cause of death were not provided. Forty-two animals were sacrificed at 46 weeks for histological examination of the thyroid and pituitary gland. No other tissues were examined. Obvious treatment-related histological changes were observed in the thyroid and pituitary gland, including thyroid follicular cell carcinoma. Immunoperoxidase staining of pituitary thyrotropic cells and antihuman TSH serum provided qualitative evidence of increased TSH production in the pituitary gland. Perchlorate treatment was associated with an increased total volume of the thyroid and of the distal parts of the anterior pituitary gland (adenohypophysis). In addition, increased average volume and numbers of epithelial, thyrotropic, and parafollicular cells were observed. Irradiation appeared to enhance the effects of perchlorate treatment. This study suggested a free-standing LOAEL of 2,147 mg/kg-day for thyroid effects.

5.1.2 Genotoxicity Assays

ManTech Environmental Technology, Inc. (1998) performed a battery of three genotoxicity assays (*Salmonella typhimurium*/microsome mutagenesis assay [Ames assay], the mouse lymphoma cell mutagenesis assay [L5178Y-TK test], and the *in vivo* mouse bone marrow micronucleus induction assay) with ammonium perchlorate to help determine its potential for various interactions with DNA and to gain insight into its possible carcinogenicity. To confirm the findings of ManTech Environmental Technology, Inc., the EPA requested that the National Toxicology Program (NTP) also evaluate ammonium perchlorate in the Ames assay and the mouse bone marrow micronucleus test (Zeiger, 1999a). The sponsor (PSG) also had the mouse lymphoma assay repeated (BioReliance, 1999).

Ammonium perchlorate was evaluated in the Ames assay (*Salmonella typhimurium*/ microsome mutagenesis assay), which is a well-defined assay for detection of mutagens. It measures the reversion from a histidine-independent state (his^-) induced by chemicals that cause base-pair changes or frameshift mutations in the genome of the organism (i.e., it measures for point mutations [e.g., substitution, addition, or deletion of one or a few DNA base pairs

within a gene]). In this assay, bacteria are exposed to the test chemical with and without a metabolic activation system (Arochlor 1254-induced rat liver S9 with co-factors). The mutagenicity is evaluated by the increase in the number of revertant colonies. The L5178Y mouse-lymphoma assay is another short-term *in vitro* assay to detect both point mutations and structural chromosomal changes. The *in vivo* mammalian micronucleus test detects the damage of chromosomes or of the mitotic apparatus caused by a clastogenic chemical in bone marrow cells (polychromatic erythrocyte [PCE] stem cells) of treated animals. Micronuclei are believed to be formed from chromosomes or chromosome fragments left behind during anaphase of mitosis. The induction of micronuclei indicates changes in either chromosome structure or number in bone marrow cells. ManTech Environmental Technology, Inc. (1998) performed this assay in Swiss-CD-1 mice and the NTP used B6C3F1 mice (Zieger, 1999a). The micronucleus assay also was performed as part of the 90-day bioassay in Spraque-Dawley rats (Springborn Laboratories, Inc., 1998). This is considered an adequate series of tests to determine the mutagenic and clastogenic (chromosomal breaking) potential of an agent. It should be noted that perchlorate is not likely to be mutagenic, given its physical and chemical properties (i.e., it is simply an anion). Although perchlorate is an oxidizing agent, it is not expected to produce oxidative DNA damage because of the kinetic considerations discussed in Chapter 2.

5.1.2.1 *In Vitro* Assays

Ammonium perchlorate was not found to be mutagenic in the *Salmonella typhimurium* (Ames assay) with and without Arochlor 1254-induced rat liver S9 activation by two separate laboratories (ManTech Environmental Technology, Inc., 1998; Zeiger, 1999a). In the ManTech study, ammonium perchlorate was dissolved in distilled water and tested at five concentrations (5,000, 2,500, 1,250, 625, and 312.5 μg/plate) in tester strains TA98, TA100, TA1535, and TA1537, with and without Arochlor 1254-induced rat liver S9 using the plate incorporation assay. Although this study was regarded as adequate, the EPA requested that the Ames assay be repeated by the NTP to confirm the negative findings and to include additional tester strains (i.e., TA102, and TA104) that are able to detect a variety of oxidative mutagens. Therefore, the NTP evaluated ammonium perchlorate in the Salmonella/Ames assay in tester strains TA98, TA100, TA1535, TA97, TA102, and TA104 (Zeiger, 1999b). Ammonium perchlorate was dissolved in distilled water and tested using the preincubation procedure at doses of 10,000, 3,333, 1,000,

333, and 100 μg/plate, with and without metabolic activation from Arochlor-induced rat and hamster livers. Ammonium perchlorate was neither toxic nor mutagenic under the conditions of the NTP assay.

The L5178Y/$tk^{+/-}$ mouse lymphoma assay also was used to evaluate the mutagenic and chromosomal breaking potential of ammonium perchlorate in vitro. Ammonium perchlorate was reported to be negative both in the absence and presence of rat Arochlor-induced S9 liver activation (ManTech Environmental Technology, Inc., 1998). Ammonium perchlorate was evaluated at 5.0, 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL without S9 activation, and at 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL with S9 activation. Although a small increase in mutation frequency was found in the absence of S9 activation at 2.5 mg/mL, which appeared to be statistically significant ($p < 0.05$) by the two-tail Student's t-test, a repeat assay found no increase in mutation frequency at this concentration compared with controls. Therefore, ammonium perchlorate is considered to be negative in the absence of S9 activation. Confidence in the negative findings without S9 activation is reinforced by the wide range of ammonium perchlorate concentrations evaluated. Although ammonium perchlorate also was reported as negative in the presence of S9 activation, the response of the positive control, 3-methyl cholanthrene (MCA), in the actual experiment was too low (182.6×10^{-6}) to be acceptable. The highest dose of ammonium perchlorate produced a mutation frequency of 194×10^{-6}. The MCA at 2.5 μg/mL should induce a mutation frequency of 300 to 350×10^{-6} or higher. Such a low positive control response weakens the confidence for the negative finding with S9 activation. In addition, the cloning efficiencies for the S9 test appear to be too high (143%), further reducing the confidence in a negative finding. Therefore, only the assays on ammonium perchlorate without S9 are considered unequivocally to be negative. Although perchlorate is not expected to be metabolized to a mutagenic intermediate, these S9 data were not of sufficient quality to support a negative-response conclusion.

Because of the problems described above, the sponsor (PSG) had the mouse lymphoma assay repeated. In this recent mouse lymphoma assay, ammonium perchlorate was evaluated at concentrations of 1000, 2000, 3000, 4000, and 5000 μm/ml without and with Arochlor 1254-induced rat liver S9 activation (BioReliance, 1999). No increase in mutant frequencies were found after treatment with perchlorate. The data were judged to be of sufficient quality to determine perchlorate to be nonmutagenic both with and without S9 activation. Although the

background mutant frequency was low, particularly in the S9 experiment, the data set still is considered to be very good overall, as well as internally consistent. The problems that were observed in the data generated by the first laboratory (ManTech Environmental Technology, Inc., 1998) were not present in the data form the BioReliance (1999) study.

5.1.2.2 *In Vivo* Assays

The potential for ammonium perchlorate to induce micronuclei was evaluated in mice and rats. Ammonium perchlorate was administered by drinking water gavage for 3 consecutive days to Swiss CD-1 mice (5 females and 5 males per dose group) at 1,000, 500, 250, 125, and 62.5 mg/kg-day (ManTech Environmental Technology, Inc., 1998). Twenty-four hours after the last dose, the mice were sacrificed, and the frequency of micronucleated cells were evaluated by counting 1,000 PCEs per animal. The assay was conducted in accordance with existing EPA Federal Insecticide, Fungicide, and Rodenticide Act/Toxic Substances Control Act (FIFRA/TSCA) testing guidelines. No increase in the frequency of micronuclei were found for any dose group. There is some uncertainty whether a maximum tolerated dose (MTD) was reached in this study. The study authors reported that at 2,000 mg/kg, 4 out of 6 animals died after one dosing of ammonium perchlorate. Typically, the assay is performed at 85% of the MTD, and the 1,000 mg/kg-day represents approximately 50% of the LD_{50}. There was no indication of toxicity to the bone marrow cells because the polychromatic erythrocyte to normochromatic erythrocyte (PCE/NCE) ratio was not different from negative controls. Furthermore, the study authors did not report any indication of clinical signs of toxicity in the highest dose group. Despite a rebuttal submitted by Dourson (1998) on behalf of the sponsor (PSG), EPA remained concerned because of the importance of this test in the overall determination of the approach to be taken for the carcinogenicity assessment (i.e., to rule out direct genotoxicity).

The NTP agreed to expedite and repeat this test in response to an EPA request. The assay was performed by ip injection to ensure the greatest delivery to the bone marrow. Male B6C3F1 mice were treated with 125, 250, 500, 1,000, 1,500, and 2,000 mg/kg ammonium perchlorate in buffered saline, plus solvent and positive (cyclophosphamide) controls. Note that this study uses two dose groups higher than those used in the previous study (i.e., 1,500 and 2,000 mg/kg). Furthermore, the use of ip injection as the route of administration would result in a direct

delivery of the compound to the bone marrow cells versus delivery from drinking water gavage. Five mice per group were injected daily for 3 consecutive days and were sacrificed 24 h after the last injection; 2,000 PCEs were scored per animal for micronuclei. All animals in the 1,500- and 2,000-mg/kg groups died after the first ip injection, and 4/5 animals died in the 1,000-mg/kg group after the second ip injection. No increases in percent PCE were observed in any of the remaining test groups (125, 250, and 500 mg/kg). No bone marrow toxicity was seen as indicated by the percent of PCE (Zeiger, 1999a,b). These results are interpreted to be consistent with those of the ManTech Environmental Technology, Inc. (1998) study that used gavage drinking water administration, and confirm that perchlorate does not induce micronuclei in rodents.

The 90-day subchronic bioassay using Spraque-Dawley rats also evaluated micronuclei induction (Springborn Laboratories, Inc., 1998). The frequency of micronuclei induction was examined in both the males and females after the 90-day sacrifice in the 10-mg/kg-day dose group of ammonium perchlorate administered by drinking water. Although there was no induction of micronuclei at this dose, 10 mg/kg-day does not appear to reach a MTD because there were no overt signs of toxicity. However, the definition of MTD may be somewhat moot, given the changes in thyroid hormone economy and histopathology seen in the thyroids at that dose. There was significant reduction in the PCE/NCE ratio (i.e, an indicator of toxicity to the bone marrow cells).

5.1.2.3 Summary of Genotoxicity Battery Results

Negative results were reported in all genotoxicity assays conducted on ammonium perchlorate when evaluated by two independent laboratories. Ammonium perchlorate was not mutagenic in the Ames assay (with or without S9 activation). Negative results were also found in the mouse lymphoma gene mutation assay without and with S9 activation. Ammonium perchlorate did not induce chromosomal anomalies when evaluated for micronuclei induction in the bone marrow of mice when administered via drinking water gavage or ip injection.
No increases in micronuclei were found in Spraque-Dawley rats when evaluated from the 90-day study at the highest dose, which produced both thyroid hormone perturbations and follicular cell hyperplasia.

In conclusion, ammonium perchlorate does not have the potential to be mutagenic or clastogenic. The *in vitro* and *in vivo* studies discussed above provide support for that conclusion. Therefore, mutagenicity is not considered a possible mode of carcinogenic action for this chemical.

5.2 GENERAL TOXICITY: SHORT-TERM AND SUBCHRONIC TESTING

The majority of the data on perchlorate toxicity available from previous studies or as a result of the current perchlorate testing strategy involved either short-term or subchronic exposures and are presented in this section. As discussed in Chapter 3, the testing strategy included targeted studies to evaluate different endpoints, e.g., developmental neurotoxicity (Section 5.3), developmental studies (Section 5.4) reproductive studies (Section 5.5) and immunotoxicity assays (Section 5.6). The rationale behind the 90-day study (Section 5.2) with satellite examination of thyroid and pituitary hormones and a 30-day recovery period was to evaluate anti-thyroid effects as possible precursor lesions. If a NOAEL could be established for these precursor lesions, it was thought that a two-year bioassay would not be required. This assumption is now more tenuous due to the tumors observed in the F1-generation at 19 weeks. The integration of these results with the available human data to arrive at a risk assessment will be discussed in Chapter 7.

5.2.1 Historical Data

Mannisto et al. (1979) measured serum levels of TSH, T3, and T4 by RIA in groups of 5 to 6 male Sprague-Dawley rats weighing 180 to 220 g that were exposed to potassium perchlorate in their drinking water at concentrations of 0, 10, 50, 100, or 500 mg/L for 4 days. Potassium perchlorate doses of 0, 1.5, 7.6, 15.3, or 76.3 mg/kg-day, respectively, were calculated assuming a body weight of 0.2 kg and a water consumption rate of 0.0305 L/day (U.S. Environmental Protection Agency, 1987). Perchlorate produced statistically significant increases in serum TSH levels and decreases in serum T3 and T4 levels. Significant changes in all three parameters were measured in the 100 and 500 mg/L (15.3 and 76.3 mg/kg-day, respectively) dose groups. In the 50 mg/L (7.6 mg/kg-day) dose group, levels of T3 and T4 were decreased significantly; TSH

levels were increased slightly, but the increase was not significant. At the low dose, T3, T4, and TSH levels were unchanged from controls. This study suggested a NOAEL of 1.5 mg/kg-day and a LOAEL of 7.6 mg/kg-day for short-term exposures to potassium perchlorate.

Shigan (1963) administered 190 mg/kg-day of potassium perchlorate in water to rabbits and white rats (number, sex, and strain not identified) for 3 mo. The author did not indicate whether the compound was administered in drinking water or by gavage with water. The animals were examined for cardiac function; liver function, based on changes in serum proteins; immune function, based on leukocyte phagocytosis; and adrenal function. Perchlorate at the dose tested caused a change in the electrocardiogram and a decrease in serum proteins, indicating a disruption of the glycogen-forming function of the liver. Shigan (1963) did not indicate whether these changes were observed in both rabbits and rats. Perchlorate had no effect in the remaining tests. This study suggested a LOAEL of 190 mg/kg-day although the study translation is reported incompletely, limiting its usefulness for risk assessment.

In a second set of experiments, Shigan (1963) also treated rabbits and white rats (number, sex, and strain not identified) with 0, 0.25, 2.0, and 40 mg/kg-day of potassium perchlorate for 9 mo. The medium for dosing was not reported. The animals were examined for cardiac and liver function, for conditioned reflexes, and for uptake and discharge of iodide by the thyroid. In the two highest dose groups, there was a statistically significant increase in the amount of iodide excreted from the thyroid; this increase was not observed in the 0.25-mg/kg-day dose group. The study does not indicate if the effect was seen in one or both species tested. This study suggested a NOAEL of 0.25 mg/kg-day and a LOAEL of 2 mg/kg-day for thyroid effects.

Hiasa et al. (1987) measured serum levels of T3, T4, and TSH by radioimmunassay in groups of 20 male Wistar rats administered 0 or 1,000 ppm potassium perchlorate in the diet for 20 weeks. Assuming a body weight of 0.34 kg (the average final body weight of rats treated with perchlorate) and a food consumption rate of 27.4 g/day (U.S. Environmental Protection Agency, 1987), an estimated dose of 80.7 mg/kg-day was calculated. Absolute and relative thyroid weights were significantly increased compared to controls in perchlorate-treated rats. No effects were seen on liver weights. The T4 levels decreased slightly, but the decrease was not statistically significant. The T3 levels were unchanged compared to controls. The TSH levels were increased statistically significantly compared to controls. Histological examination

of the thyroid revealed diffused small follicles in perchlorate-treated rats and one case of follicular hyperplasia. Thus, the 80.7-mg/kg-day dose could be considered a LOAEL.

Gauss (1972) fed female NMRI mice a diet containing 0 or 1% potassium perchlorate for up to 160 days. Mice were between 50 and 60 days old at the beginning of treatment and weighed between 19 and 28 g (average, 23.23 g). During the first 2 mo of treatment, body weights increased about 12%; body weight data for longer treatment periods were not reported. Assuming a body weight of 23 g and a food consumption value of 4.625 g/day (U.S. Environmental Protection Agency, 1987), a dose of 2,011 mg/kg-day was calculated. Thyroid glands were examined histologically at 10- to 20-day intervals throughout the 160-day study period. Thyroid and nuclei volumes and height of epithelial follicles were increased in treated mice throughout the treatment period compared to controls. The histological examinations showed a progressive change in the histological appearance of the thyroid of treated mice, beginning with colloid loss, nuclei volume expansion, and rising epithelium height, followed by the appearance of hypertrophy and hyperplasia of the thyroid parenchyma. At later stages of the treatment period, hyperplastic follicles, areas of adenomatic tissue, adenoma complexes, and secreting cystadenomas were observed; however, no progression to malignancy was apparent. The 2,011 mg/kg-day dose suggested a free-standing LOAEL because no other doses were tested.

5.2.2 Caldwell et al. (1995) 14-Day Study

Caldwell et al. (1995) administered ammonium perchlorate in drinking water at concentrations of 0, 1.25, 5.0, 12.5, 25, 50, 125, or 250 mg/L to Sprague-Dawley rats (6/sex/group) for 14 days. The actual dose administered to each animal was calculated by multiplying the concentration of ammonium perchlorate administered in the drinking water by each rat's average water consumption over the 14-day period and dividing this number by each animal's average body weight for the same period, resulting in doses (male/female) of 0, 0.11/0.12, 0.44/0.47, 1.11/1.23, 2.26/3.06, 4.32/4.91, 11.44/11.47, and 22.16/24.86 mg/kg-day, respectively (Caldwell et al., 1995). Caution must be used when interpreting these reports because the conversion is sometimes not included (e.g., the Channel [1998b] consultative letter reports results in units of the test concentrations rather than the dose converted to milligrams per kilogram per day). Thyroids were weighed, histopathology and morphometry performed, and thyroid hormone levels were measured with a radioimmune assay technique.

The consultative letter of Channel (1998b) provides results and comments on a histopathological analysis of the rat thyroids from the Caldwell et al. (1995) 14-day study that was performed by the Air Force Research Laboratory/Human Effectiveness Directorate (AFRL/HEST) and never officially published (Eggers, 1996, as cited in Channel, 1998b). As part of the previous assessment, EPA requested from the AFRL/HEST the previously unpublished histopathology data from the 14-day oral dosing study performed by Caldwell et al. (1995). The histopathology was discussed in the paper on the study design (Caldwell and Mattie, 1995) but had not been published in either Caldwell et al. (1995) or King (1995). The histopathology data discussed herein were provided in a consultative letter from the AFRL/HEST (Channel, 1998b). The EPA also performed a reanalysis of the thyroid hormone data (T4, T3, rT3, TSH, and thyroglobulin [hTg]) found in the Caldwell et al. (1995) and King (1995) reports (Crofton, 1998a). Because these individual data were supplied only electronically on Microsoft Excel® spreadsheets and not submitted formally to EPA, Crofton, (1998a) represents the official publication of these data. These histopathology data and reanalyses of effect levels using the PWG results and new hormone analyses are found in the following sections.

5.2.2.1 Thyroid Histology Data

Channel (1998a) submitted that the incidence of thyroid follicular cell hypertrophy determined by standard histology was significantly different from control at a lower dose (0.44 0.47 mg/kg-day) than for the incidence of decrease in follicular lumen size (2.26 3.06 mg/kg-day), but the statistics indicate a NOAEL at 0.11 0.12 mg/kg-day. However, the documentation of the statistics was not provided, and Eggers (1996) apparently combined both sexes for the analyses. It is recommended in the report (Channel, 1998a), and EPA concurred, that a re-analysis was warranted for a number of reasons. First, there was a gender-by-treatment interaction observed in the thyroid hormone analyses (see Section 5.2.2.2). Secondly, there was an apparent dose trend, despite the limited sample size, in the incidence of response: male and female combined was 7/12, 6/11, 11/12, 10/12, 12/12, 12/12, 12/12, and 12/12; male only was 3/6, 4/6, 5/6, 5/6, 6/6, 6/6, 6/6, and 6/6; and female only was 4/6, 2/5, 6/6, 5/6, 6/6, 6/6, 6/6, and 6/6 for the 0, 0.1, 1.0, 5.0, 10, 20, 50, and 100 mg/kg-day groups, respectively. Finally, the analysis did not combine severity and incidence data for the decrease in lumen size, but the mean severity scores alone were statistically significant from control above the 0.44/0.47 mg/kg-day

group. A separate computerized morphometric analysis of follicular lumen size was performed by AFRL/HEST for the 0, 0.11/0.12, 1.11/1.23, 4.32/4.91, and 22.16/24.86 mg/kg-day groups, and a statistically significant difference in the incidence of decrease in lumen size was evident in the males at the 1.11 mg/kg-day dose and, in females, at the 4.91 mg/kg-day dose; however, the gender-by-treatment effect was not taken into account. Relative thyroid weights were significantly increased in 11.44/11.47 and the 22.16/24.86 mg/kg-day dose groups compared to controls.

Results of the PWG analysis can be found in Wolf (2000; 2001; Tables 1 and 2). Female rats appeared to be slightly more sensitive in this study with a NOAEL designated at 1.23 mg/kg-day; whereas, in males it was somewhat difficult to ascertain. This may be due to the difficulty that the PWG had in reading the slides from this study due to the non-routine staining method (periodic acid shift [PAS] reaction with a green counterstain) as noted in Wolf (2000). BMD analysis (Table 5-1) for the combined female and male data results in BMDL values for a 10% increase in incidence at 0.72 mg/kg-day for colloid depletion and 0.78 mg/kg-day for hyperplasia. The difficulty noted above with the staining for this study was most prominent in evaluating hypertrophy (Wolf, personal communication), so that these estimates were not calculated. Re-analysis of the morphometry on thyroid follicular lumen size identified a NOAEL at the 0.44/0.47 mg/kg-day dose.

5.2.2.2 Thyroid and Pituitary Hormone Analyses

The thyroid and pituitary hormone data were reanalyzed using five two-way analysis of variance (ANOVA) tests, one each for all of the hormones (Crofton, 1998a). Data from dependent measures (T3, T4, rT3, TSH, and hTg) were subjected to separate two-way ANOVAs, with gender (male and female), and treatment (dose) as independent, between-subject variables. Step-down ANOVA tests were conducted as indicated by significant interactions and discussed in Crofton and Marcus (2001) and Marcus (2001). Mean contrasts were performed using Duncan's Multiple Range Test. Results of these reanalyses are similar to those stated in the Caldwell et al. (1995) and King (1995) reports with some notable exceptions. Figure 5-3 shows the dose-dependent effects on T3, T4, and TSH.

There was a significant gender-by-treatment interaction on total serum T3, and subsequent step-down ANOVA tests showed significant treatment effects for both genders. Figure 5-3(A)

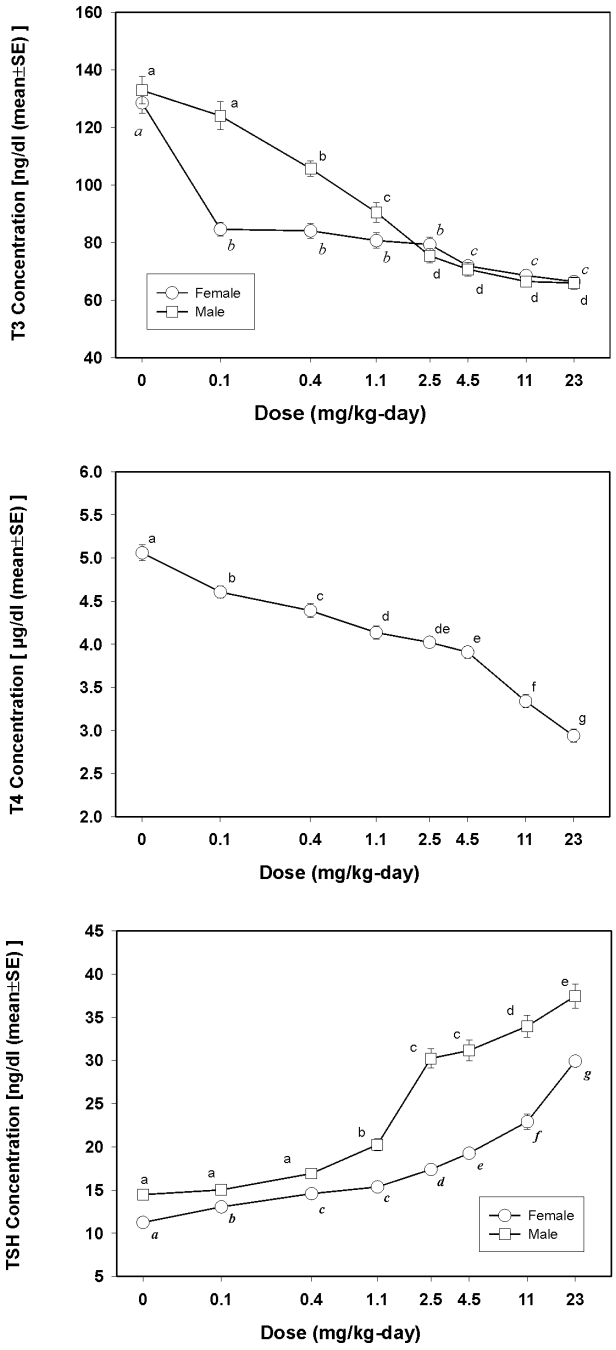

Figure 5-3. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 (A), T4 (B), and TSH (C) concentrations (ng/mL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were significantly different ($p < 0.05$). Data of Channel (1998b) and Crofton (1998a). Daily dose was estimated from water consumption data.

illustrates dose-dependent decreases in T3 for both genders while females were slightly more sensitive compared to males. The overall gender-by-treatment interaction was not significant for T4, but there was a significant main effect of treatment (Figure 5-3(B)). Perchlorate also decreased T4 in a dose-dependent manner. There was a significant gender-by-treatment interaction on total serum TSH, and subsequent step-down ANOVA tests showed significant treatment effects for both genders. Dose-dependent increases in TSH were observed for both genders; however, females were slightly more sensitive compared to males.

The Caldwell et al. (1995) study is the only one in which an additional thyroid hormone, rT3, and hTg were assayed (Tg in rats was assayed with a human RIA kit, thus the notation "h"). There was no significant gender-by-treatment interaction for rT3. Figure 5-4(A) clearly indicates that perchlorate increases rT3 in a dose-dependent manner. There was a significant gender-by-treatment interaction on hTg, and subsequent step-down ANOVA tests showed significant treatment effects for both genders. Figure 5-4(B) illustrates the dose-dependent increases in hTg for both genders. Both genders were equally sensitive, with males exhibiting a slightly greater response to the lowest dosage.

Perchlorate exposure decreased circulating T3 and T4 and increased TSH. This report also provides evidence that rT3, formed mostly in extrathyroidal tissues, was increased by this exposure. Thyroglobulin also was increased. The NOAELs and LOAELs are summarized in Table 5-2. A NOAEL for TSH was observed in males only at 0.44 mg/kg-day and at 0.11/0.12 for rT3. Note that free-standing LOAELs (i.e., effects at the lowest dosage tested) were found at 0.11/0.12 mg/kg-day for T3 in females, for T4 and hTg in both sexes, and for TSH in females.

5.2.3 The 90-Day Testing Strategy Bioassay in Rats

The 90-day study that was part of the testing strategy consisted of oral administration of ammonium perchlorate via drinking water to male and female Sprague-Dawley rats at doses of 0, 0.01, 0.05, 0.2, 1.0, and 10 mg/kg-day (Springborn Laboratories, Inc., 1998). This study has also been reported in the literature (Siglin et al., 2000), but because that manuscript did not use the thyroid histopathology as reported by the PWG (Wolf, 2000) it will not be discussed further in this document. A 14-day sacrifice also was included in the study for comparison with the Caldwell et al. (1995) study of that same duration. Ten rats/sex/dose were used, and an additional 10 rats/sex/dose were sacrificed after the 30-day recovery period following cessation

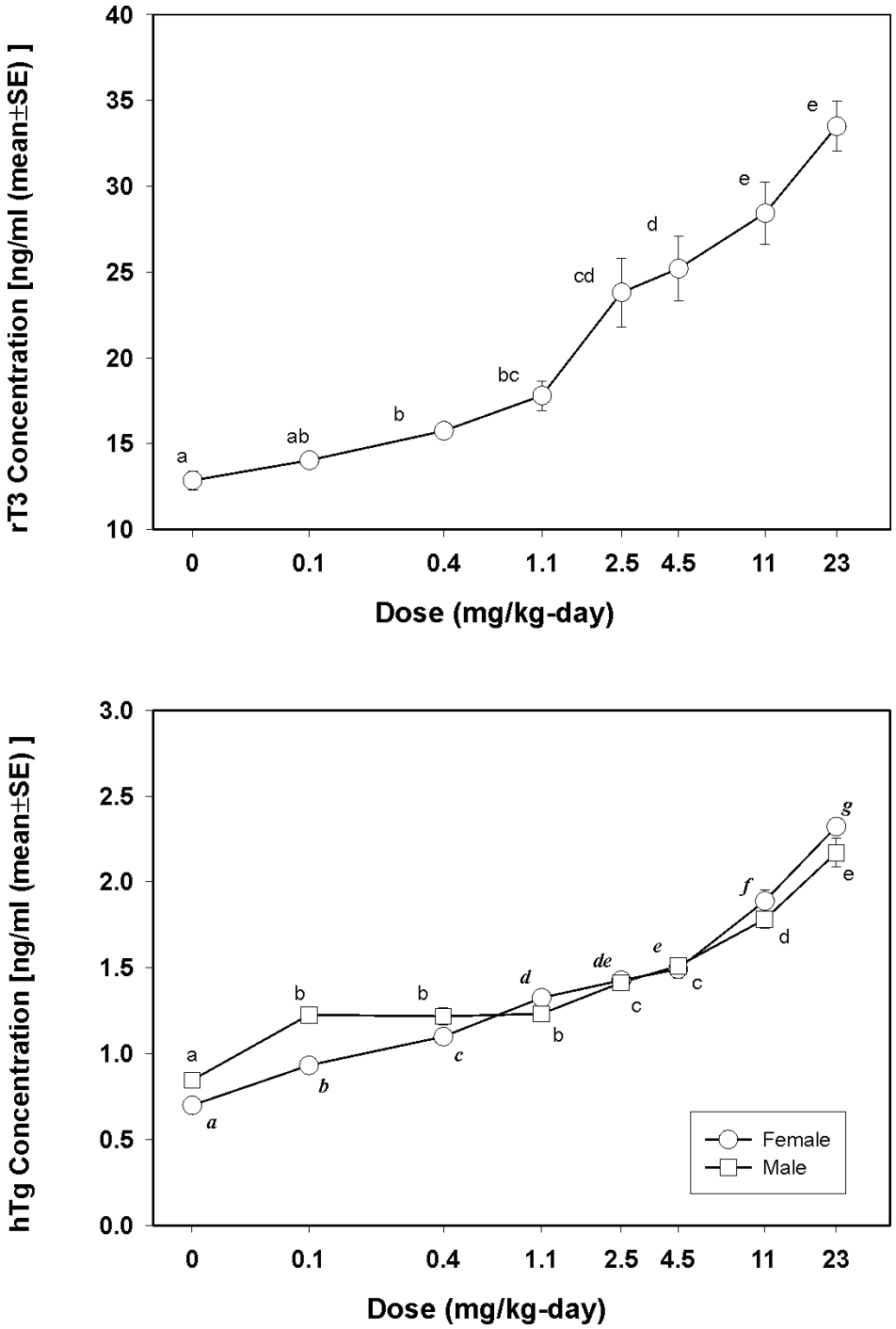

Figure 5-4. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum rT3 (A) and hTg (B) concentrations (ng/mL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Channel (1998b) and Crofton (1998a). Means with different letters were significantly different ($p < 0.05$). Daily dose was estimated from water consumption data.

of the 90-day exposure at doses of 0, 0.05, 1.0, and 10 mg/kg-day to evaluate reversibility of any observed lesions.

The stock solution of the test article was diluted with reverse osmosis (RO) water and prepared fresh five times during the study (at least once every 5 weeks). Stability analyses were performed by the sponsor (AFRL/HEST) and showed that ammonium perchlorate solutions were stable for 109 days (Tsui et al., 1998). The sponsor also confirmed that the stock and dosing solutions were within an acceptable concentration range (Springborn Laboratories, Inc., 1998; Appendix B). Control drinking water solutions were analyzed by the sponsor to confirm no contamination of detectable nitrate, an ion that could cause possible interference to estimating the dose of test article. Dosing solutions were prepared fresh for each week, and the administered concentrations were adjusted based on measured body weights and water intake.

The parameters evaluated included clinical observations, body and organ weights, food and water consumption, hematology, clinical chemistry, ophthalmology, and gross necropsy. Histopathology was performed on all tissues from the control and high-dose groups. The liver, kidneys, lungs, thyroid/parathyroid, and gross lesions from all intermediate dose groups and for the recovery groups also were examined microscopically. Evaluation of additional reproductive parameters, i.e., estrous cyclicity in females and sperm motility and morphology in males, also was performed. Thyroid hormone analyses were performed at the 14-, 90-, and 120-day sacrifices. Only the 0, 0.05, 1.0 and 10.0 mg/kg-day groups were continued until the 120-day time point. All hormone and tissue collection was balanced over time-of-day to control for the circadian rhythms of hormones.

5.2.3.1 General Toxicity, Thyroid Histopathology Results, and Satellite Reproductive Assay

There were no clinical signs of toxicity observed during the treatment or recovery periods. All rats survived to scheduled sacrifice except one female rat in the 0.05 mg/kg-day group that was found dead during the recovery period. However, this death was considered unrelated to treatment because no deaths occurred in any of the higher dose groups, and the histopathologic evaluation for cause of death was inconclusive. No statistically significant or remarkable findings were observed among the groups with respect to clinical observations, body weights, food or water consumption, ophthalmology, hematology, or clinical chemistry. Miscellaneous

lesions that occurred with equal incidence and severity in all dose groups and controls included extramedullary hematopoiesis in the livers, inflammation in the lungs, minimal nephropathy in the kidneys and inflammation of the heart. Because none of these lesions demonstrated a dose response, and some are commonly seen in young rats, they were not considered treatment-related. The only treatment-related lesions observed at gross necropsy were reddened thyroids, attributed to minimal congestion of the blood vessels.

Absolute thyroid weight and thyroid weight relative to both final body weight and brain weight were increased significantly in males of the 10 mg/kg-day dose group after 14 and 90 days of treatment and in females at the 10 mg/kg-day dose group after 90 days indicating LOAEL at 10 and a NOAEL at 1 mg/kg-day. These thyroid weight measures were comparable to control values in both males and females of the 10 mg/kg-day group at the end of the 30-day recovery period. Histopathology was evaluated on Days 14, 90, and 30 postexposure (120 days). The corresponding PWG review results can be found in Wolf (2000; 2001, Tables 3 through 8). Male rats appeared to be slightly more sensitive, exhibiting follicular cell hyperplasia by Day 14 and not recovering fully for any of the thyroid histopathological indices by 30 days post exposure. On Day 14, females showed decreased colloid and follicular cell hypertrophy at 10 mg/kg-day. Males also showed a significant increase in these two thyroid response measures at this dose but also exhibited changes at lower doses and in addition showed hyperplasia. By 90 days, all three response measures (colloid depletion, follicular cell hypertrophy, and follicular cell hyperplasia) in both sexes were significant at 10.0 mg/kg-day, again indicating a LOAEL at 10 and a NOAEL at 1 mg/kg-day. Recovery of the thyroid histopathological changes was essentially complete by 30 days post-exposure although the males did have some indication of residual toxicity.

The BMD analyses for these data are found in Table 5-1 and Figures 5-1 and 5-2. Data for females and males were combined. The BMDL for colloid depletion and hypertrophy at 14 days were 0.28 and 0.017 mg/kg-day, with no estimate for hyperplasia. By 90-days, the BMDL values decreased for colloid depletion and hypertrophy to 0.03 and 0.008 mg/kg-day. The BMDL value for hyperplasia was 2.09 mg/kg-day. No observed effect was estimated for the 120 day value.

Estrous cyclicity was evaluated for 3 weeks prior to sacrifice in all females of the 90- and 120-day termination groups by examining daily vaginal smears. The number and percentage of females cycling and the mean cycle length were determined for each group. There is an apparent

dose-related response for the absolute number and proportion of females with an abnormal estrous cycle (defined as less than 3 or more than 5 days). The number and percentage of females with at least one abnormal cycle in those females cycling was 1/10 (10%), 1/10 (10%), 5/9 (56%), 6/9 (67%), 0/8 (0%), and 0/10 (0%) at the 0, 0.01, 0.05, 0.2, 1.0, and 10-mg/kg-day doses. The proportion began to increase at the 0.05 mg/kg-day dose level, peaked at the 0.2 mg/kg-day dose level, and then declined at the two higher doses. This suggests the possibility of an inverted U-shaped dose-response pattern. Examination of the 120-day data (after 30-day recovery) also revealed changes in cyclicity with 1/5 (20%), 1/7 (14%), 1/6 (16%), and 4/6 (67%) females not cycling in the 0.0, 0.05, 1.0, and 10-mg/kg-day groups, respectively. Because the number of rats in the add-on groups (n = 10) did not provide the level of statistical power that would be desired, this indication of an effect in a study with limited power was of concern in 1998, but the results of the two-generation reproductive study completed in 1999 did not indicate any effects on this endpoint (Section 5.5.1).

Sperm samples were obtained from all male rats terminated after 90 or 120 days for evaluation of sperm count, concentration, motility, and morphology. The mean percentage of normal sperm was calculated for each group. There were no treatment-related effects on sperm parameters noted although again the number tested is small. The effects on the percentage of normal sperm appear to be artifacts because of a single outlier in each of the two groups with lower means. These occurred at different dose levels in the exposure versus recovery phases.

5.2.3.2 Thyroid and Pituitary Hormone Analyses

The assays for T4, T3, and TSH were performed using RIA kits according to the manufacturer's standard procedures. Assay kits from the same batch number and with the same expiration date were used for each animal termination period (Study Days 14, 90, or 120). Samples and standards were run in triplicate. The Springborn Laboratories report included an appendix (Springborn Laboratories, Inc., 1998; Appendix I) containing the results of these thyroid hormone assays. The Springborn report used a series of individual ANOVA tests to determine main effects of treatment for all three hormones in both genders and at three time points during the study (Day 14, Day 90, and Day 120 a [30-day recovery time]). As part of its 1998 assessment, EPA reanalyzed these thyroid hormone data using three-way ANOVA tests, one for each of the three hormones, to allow for a statistical comparison of the interaction

between gender, time, and treatment (Crofton, 1998b). The Crofton (1998b) analysis also contains a printout of all of the individual animal data, an omission from Springborn Laboratories, Inc. (1998). As suggested in the external peer review (Research Triangle Institute, 1999), EPA reanalyzed these data from each hormone at each time point (Day 14, Day 90, and Day 120) with two-way ANOVA tests. Gender and treatment (dose) were used as independent between-subject variables. Dependent variables were T3, T4, and TSH. Step-down ANOVA tests were conducted as indicated by significant interactions (Crofton and Marcus, 2001; Marcus, 2001). Mean contrasts were performed using Duncan's Multiple Range Test.

Results of the EPA reanalyses, shown in Table 5-2 and illustrated in Figures 5-5 through 5-7, are similar to those stated in the contract report (Springborn Laboratories, Inc., 1998) with a few notable exceptions. First, there is only a marginal interaction between gender and treatment, resulting from a slight difference in magnitude of effects between genders. However, no differences in LOAELs between genders were observed (with minor exceptions likely caused by small changes in variance between groups, which are probably not biologically significant [see below]). Results of the analyses for each thyroid hormone and TSH are discussed individually.

There were significant day-by-gender-by-treatment interactions for T3 on Day 14 and Day 90. Therefore, separate ANOVA tests were conducted on each gender to test for a main effect of treatment. Lack of a significant gender-by-treatment interaction on the 120-day data led to one subsequent ANOVA to test for a main effect of treatment. Data from Day 14 revealed a LOAEL of 0.01 mg/kg-day for males (see Figure 5-5). There was a NOAEL of 10 mg/kg-day for T3 in females. The low potency of perchlorate on T3 in females at the 14-day time point may be artifactual. Not plotted on the figure for Day 14 are all the available data from control female rats from this laboratory, including the Day 90 and Day 120 time points, and the data from two other studies. These historical data show that the group mean for females in Figure 5-5 for the 14-day time point may be artificially low relative to some of the other data from the AFRL/HEST laboratory. Thus, the biological significance of this gender-dependent effect of perchlorate after 14-days of exposure is suspect. Consistent with this conclusion is the significant dose-dependent decrease in T3 concentrations in female rats exposed to 0.125 to 250 mg/kg-day perchlorate in a previous 14-day exposure study by this same laboratory (Caldwell et al., 1995). The LOAEL for effects on T3 for both males and females was 0.01 on Day 90. The NOAEL for effects on T3 at

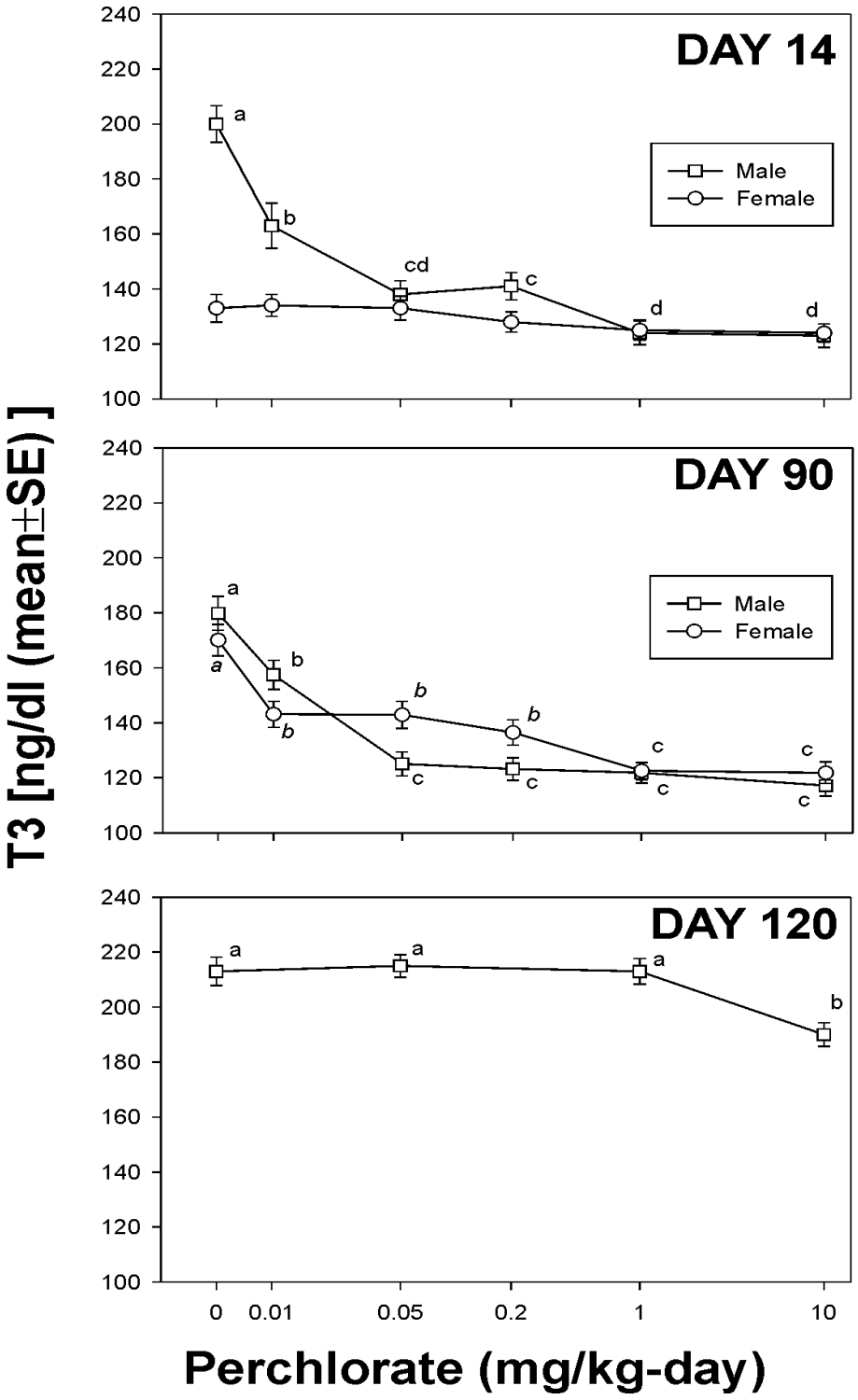

Figure 5-5. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 concentrations as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were statistically different ($p < 0.05$). The 120-day time point is 30 days after cessation of exposure.

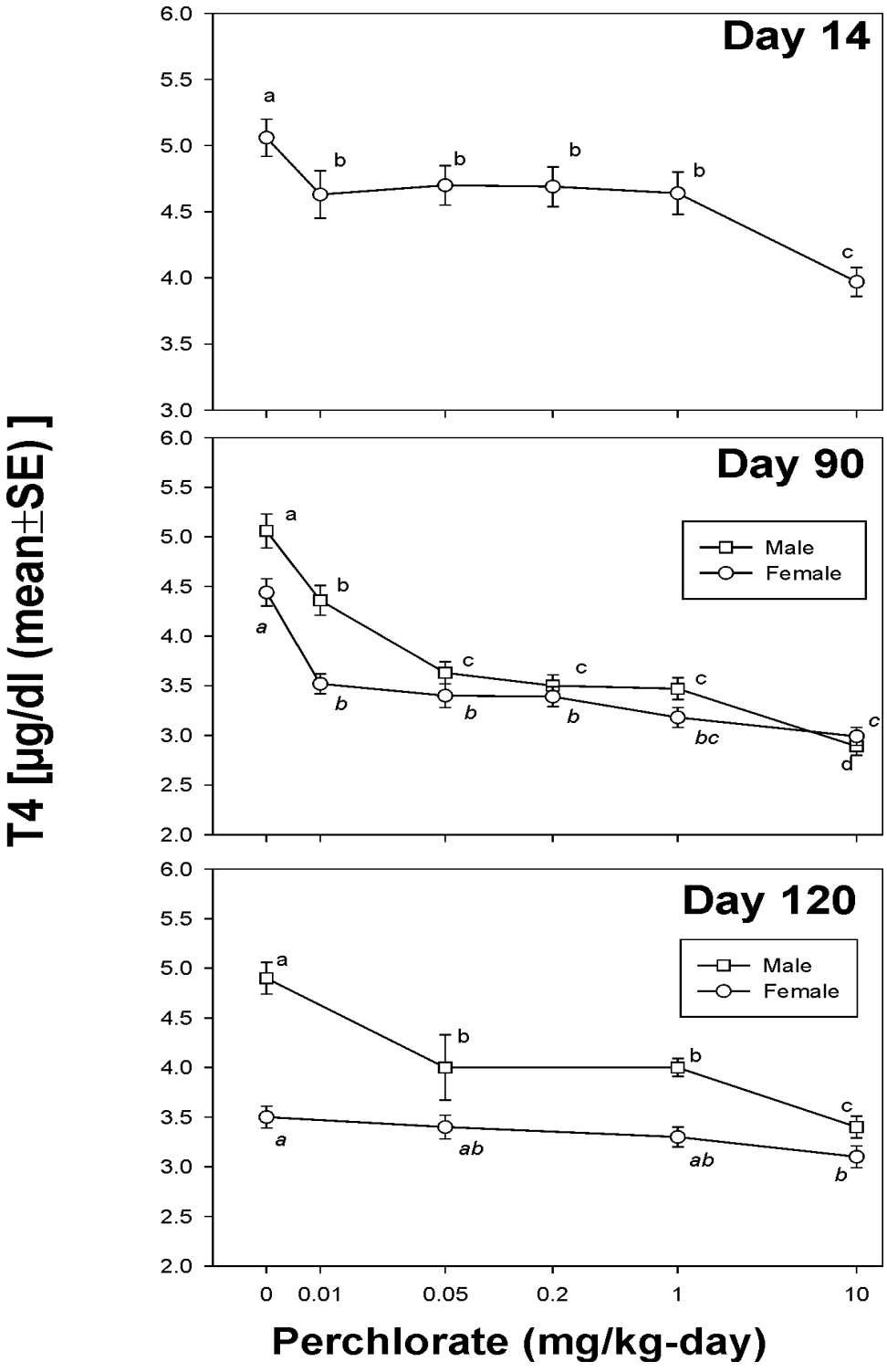

Figure 5-6. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T4 concentrations as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were significantly different ($p < 0.05$). The 120-day time point is 30 days after cessation of exposure.

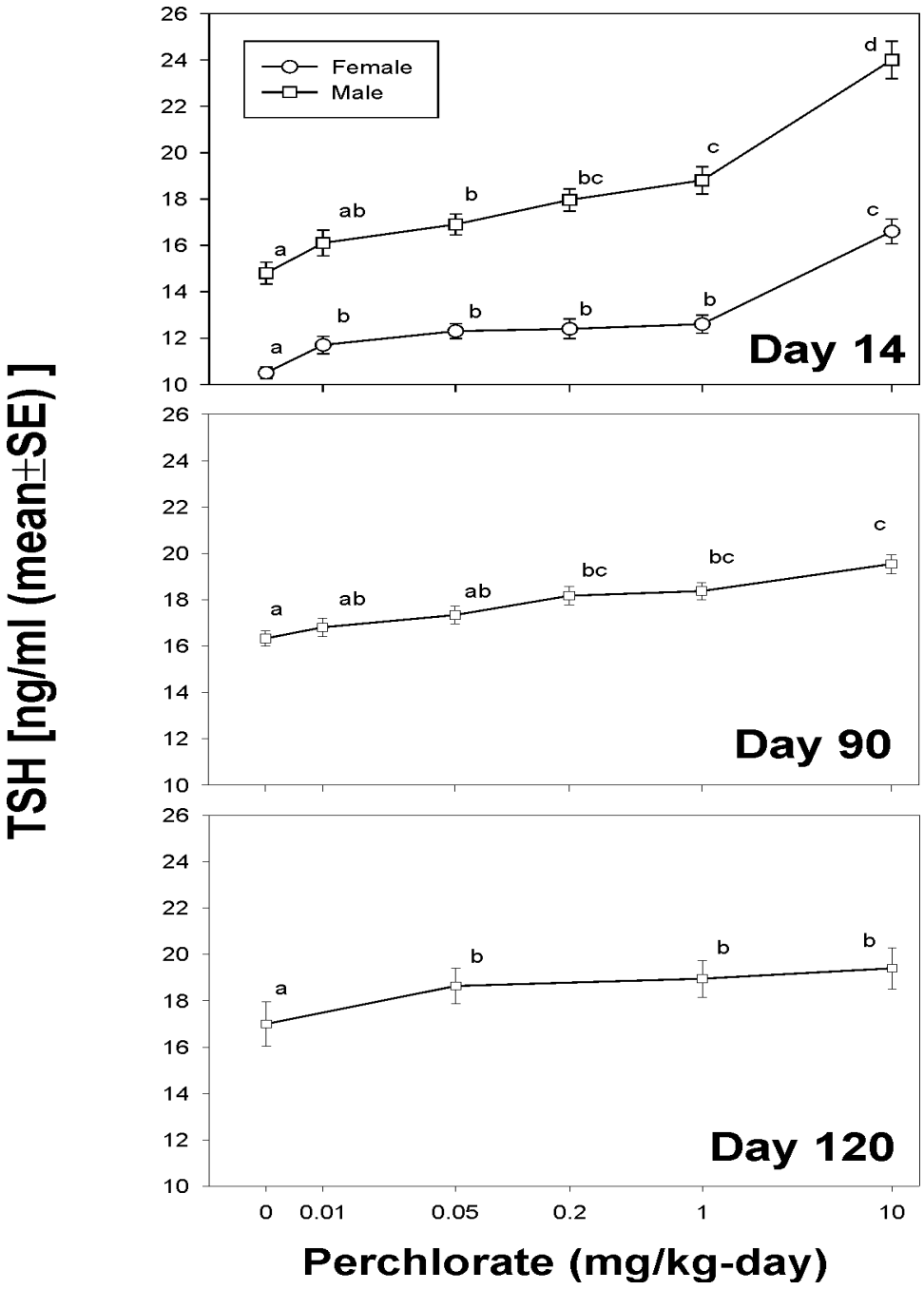

Figure 5-7. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total TSH as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Springborn Laboratories, Inc. (1998). A main gender-by-treatment interaction was observed for Day 14, but not Days 90 and 120; therefore, data are presented separately for males and females on Day 14 and collapsed across gender for Days 90 and 120. Means with different letters were significantly different ($p < 0.05$). The 120-day time point is 30 days after cessation of exposure.

Day 120 was 10 mg/kg-day, indicative of a recovery of T3 concentrations after cessation of treatment.

There were significant day-by-treatment interactions for effects on T4 at the 90- and 120-day time points but not at the 14-day time point. Mean contrast tests for Day 14 data revealed a free-standing LOAEL of 0.01 mg/kg-day for effects on T4 in both sexes. The 0.01 mg/kg-day dosage was also a free-standing LOAEL on Day 90 for effects on T4 in both sexes. Analysis of the data from the 30-day recovery period (the Day 120 time point) revealed a free-standing LOAEL of 0.05 mg/kg-day in males and a NOAEL of 1.0 mg/kg-day in females for effects on T4.

There was a significant day-by-gender-by-treatment interaction for TSH only on Day 14. Therefore, separate ANOVA tests were conducted on each gender to test for a main effect of treatment for the Day 14 time point. Lack of a significant gender-by-treatment interaction for the 90- and 120-day data led to subsequent one-way ANOVA tests at each time point to test for a main effect of treatment. Perchlorate caused a dose-dependent increase in TSH that was apparent at the Day 14 and Day 90 time points (see Figure 5-7). The NOAEL for effects on TSH at Day 14 data was 0.01 mg/kg-day in the males. The 0.01 mg/kg-day dose was a free-standing LOAEL in the females. This small difference between males and females likely is caused by small changes in variance between groups rather than by a biologically significant difference (the absolute increase relative to the control mean in the 0.05-mg/kg-day female group is actually smaller than the same comparison in the males). The TSH concentrations did not recover to control values 30 days after cessation of treatment with a free-standing LOAEL at 0.05 mg/kg-day in both sexes.

The data demonstrate a dose- and time-dependent effect of perchlorate on thyroid hormones and TSH. There was no LOAEL established in this data set due to multiple effects at the lowest dose of 0.01 mg/kg-day. There was some evidence of recovery at the Day 120-evaluation (30 days after cessation of treatment). The NOAEL for effects on T3 increased to 1.0 mg/kg-day. However, the omission of the 0.01 mg/kg-day dose group at the 120-day time point make it difficult to conclude about a recovery of effects on T4 and TSH.

5.3 DEVELOPMENTAL NEUROTOXICITY STUDIES

Concern for potential neurodevelopmental sequelae was warranted given the established mode of action for perchlorate, and the original 1997 testing strategy included a developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a). Results of that study raised additional issues and concerns so that the external peer review convened in 1999 recommended additional testing. This section describes results of the available studies that tested neurodevelopmental indices per se. The 1998 neurodevelopmental study is reviewed in Section 5.3.1. Results of the new study on motor activity are reviewed in Section 5.3.2. The "Effects Study" repeated the study of brain morphometry as a measure of neurodevelopmental toxicity and is reviewed in Section 5.3.3.

5.3.1 The 1998 Developmental Neurotoxicity Study

The neurobehavioral developmental study of ammonium perchlorate that was part of the original 1997 testing strategy was performed by drinking water administration in Sprague-Dawley rats (Argus Research Laboratories, Inc., 1998a). A schematic of this study design is provided as Figure A-1 (Appendix A) of this document to aid understanding of terminology and the protocol. It should be noted that Argus Laboratories identifies the day of birth as PND1; therefore, the age of PND10 and PND22 actually correspond to PND9 and PND21 in this study. The description of the study design will use the Argus nomenclature in order to readily compare with the contract report. Subsequent supplemental data submittals and additional analyses pertaining to this were requested by EPA and provided by Argus Laboratories study (York, 1998a,b,c,d,e).

Female rats (25/dosage group) were administered target doses of 0, 0.1, 1.0, 3.0, and 10 mg/kg-day by continual access to ammonium perchlorate in nonchlorinated RO deionized water beginning on gestation day zero (GD0) and ending at scheduled sacrifice. Test substance concentrations were evaluated weekly, based on actual water consumption levels recorded the previous week and adjusted as necessary to more closely achieve the target dose levels. Test solutions were prepared weekly. The stability of the stock solution and that concentrations agreed well with nominal concentrations were determined by AFRL/HEST (Argus Research

Laboratories, Inc., 1998a; Appendix J). Feed and water consumption were recorded daily during exposure.

After acclimation for 14 days, virgin female rats were cohabited with breeder male rats (one male rat per female rat) for a maximum of 7 days. Female rats with spermatozoa observed in a vaginal smear or a copulatory plug observed in situ were considered to be at GD0. The F0-generation dams were examined at approximately the same time each day during the exposure period for signs of maternal behavior, autonomic dysfunction, abnormal postures, abnormal movements or behavior patterns, and unusual appearance. Pregnancy outcome measures evaluated at birth included pregnancy rate, duration of gestation, number of implantation sites, gestation index (number with live pups/number pregnant), number of pups/litter, sex ratio of pups, and viability and lactation indices. Maternal body weight was recorded on GD0, daily during the exposure period, weekly during the post-weaning period, and at sacrifice. The same set of signs as examined during exposure were evaluated on a weekly basis during post-weaning. Thyroids from all F0-generation rats were weighed and evaluated histologically. Five dams per group were selected for sacrifice and blood collection on post-natal day 10 (PND10) from those with no surviving pups or with litters of less than eight pups. Thyroid and pituitary hormone analyses (T3, T4, and TSH) were done on the blood (see Section 5.3.1.3). All dams not selected for continued observation were sacrificed on PND22.

Pups (F1-generation) were counted and clinical signs were recorded once daily during pre-and post-weaning. Body weight was recorded on PNDs 1, 5, 8, 12, 14, 18, and 22 and then weekly during post-weaning. Feed consumption values were recorded weekly during post-weaning. Pups that appeared stillborn and those that died before initial examination on PND1 were examined for vital status, and the gross lesions were preserved. Pups that were not selected for continued observation were sacrificed and necropsied on PND5. Blood was sampled for thyroid and pituitary hormone analysis, and the thyroids were examined histologically. The F1-generation pups not selected for continued observation on PND10 (n = 102) were sacrificed and examined for gross lesions. Post-weaning pups that were selected for continued observation were given ammonium perchlorate in RO deionized water with chlorine (added at a maximum of 1.2 ppm as a bacteriostat).

Other pups (F1-generation) were assigned to four different subsets for additional evaluations. The first male and female pup (1/sex/dose; total of 97 male and 100 female pups)

were assigned randomly to Subset 1 for brain weight and neurohistological examination (including morphometric measurements). All pups were selected for fixed brain weights on PND12; 6/sex/dose (total of 30 male and 30 female pups) were selected for neurohistological examination. The second male and female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 2 for passive avoidance testing on PNDs 23 to 25 and PNDs 30 to 32; water maze testing on PNDs 59 to 63 and PNDs 66 to70; and scheduled sacrifice at PNDs 90 to 92, with blood collection for thyroid and pituitary hormone analysis. The third male and female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 3 for motor activity evaluation on PNDs 14, 18, 22, and 59; auditory startle habituation on PNDs 23 and 60; and scheduled sacrifice on PNDs 67 to 69. The fourth male and female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 4 for regional brain weight evaluation on PNDs 81 to 86 (6/sex/dose; total of 30 male and 30 female rats) and neurohistological examination on PNDs 82 to 85 (6/sex/dose; total of 30 male and 30 female rats). Female pups also were evaluated for the age of vaginal patency beginning on PND28, and male pups were evaluated for the age of preputial separation beginning on PND39. A few of these measurements inadvertently went unrecorded, but the laboratory asserted that this did not affect the results because a sufficient amount of data on other rats was recorded.

5.3.1.1 Results of General Toxicity Measures, Neurohistology, and Morphology

Results in the dams (F0-Generation) revealed no treatment-related effects on food or water consumption (Argus Research Laboratories, Inc., 1998a; Appendix B, Tables B7 through B14), mortality (Appendix B, Tables B2 and B18), clinical signs (Appendix B, Table B2), necropsy (Appendix B, Table B18), body weight (Appendixes A and B, Figure A1 and Tables B3 through B6), or pregnancy outcome measures (Appendix B, Tables B15 through B16). Effects on thyroid weight, histopathology, and thyroid and pituitary hormone analyses will be discussed below in Sections 5.3.1.2 and 5.3.1.3.

Results in the pups (F1-generation) revealed no treatment-related effects on feed consumption (Argus Research Laboratories, Inc., 1998a; Appendix C, Tables C18 and C19), mortality (Appendix C, Tables C1 and C2), clinical signs (Appendix C, Tables C1 and C2), body weight (Appendixes A and C, Figures A2 and A3 and Tables C3 through C6), or sexual

development landmarks (Appendix C, Table C11). No treatment-related effects were observed on mortality, brain weight, or body weight in the pups of Subset 1 at PND12 (Argus Research Laboratories, Inc., 1998a; Tables D1 and D2), Subset 2 at PNDs 90 to 92 (Tables E3 and E4), or Subset 3 at PNDs 67 to 69 (Tables F5 and F6). Results of the neurobehavioral tests from Subsets 2 and 3 will be discussed in Section 5.3.1.4.

In the Subset 1 subgroup subjected to neurohistological examination (the F1 pups sacrificed on PND12), morphometric analyses revealed a 23.4% increase in the size of the corpus callosum in females and a 30.2% increase in males (not significant) at the high dose (10 mg/kg-day). Slight decreases in brain weight also were noted at the highest dose in females. In Subset 4 (the F1 pups sacrificed on PND82), there was a continued effect on the size of the corpus callosum (20.9% increase) in males, but no effect in females at the highest dose. There was also a 3.4% increase in the brain weight in males and increases in the size of the frontal cortex (9.2%) and the caudate putamen (10.2%). The EPA concluded that the effects may be significant and that analyses of the next lower dose (or, at least, historical control data for the affected endpoints) were warranted and requested additional analyses from the sponsor (PSG). York (1998d) responded with morphometry analyses of the next lower dose (3.0 mg/kg-day) of the Subset 1 F1 pups at PND12. The new analysis noted, in addition to previous findings, a statistically significant increase in the anterior/posterior cerebellum size, a statistically significant decrease in the caudate putamen for the F1 PND12 female pups, and a statistical significant decrease in the hippocampal gyrus size for the F1 PND12 male pups. These effects were not considered treatment-related by the Primedica/Argus pathologist because they were not dose dependent.

A preliminary reanalysis by EPA (Crofton, 1998c) of the control, 3- and 10-mg/kg-day groups (York 1998d) was restricted to the corpus callosum because this was the area with the largest effect. The analysis revealed no interaction of gender and treatment; however, there was a significant effect of treatment ($F[2,30] = 7.65$, $p < 0.0021$). There was a significant increase in the size of the corpus callosum only in the 10-mg/kg-day group. Group means were 288, 278, and 366 μm for the controls and 3- and 10-mg/kg-day groups, respectively. Incorporation of historical control data from both PND10 and PND12 (mean for controls = 264 μm for PNDs 10 and 265 μm for PND12; York, 1998a) supports the conclusion that the control values for corpus callosum size in the data set are within the "normal" range (York 1998a; see also Argus Research Laboratories, Inc., 1998a).

EPA did not agree with the argument put forth by Argus Research Laboratories, Inc. (1998a) that these effects were "not suggestive of a neurotoxic effect" because of "an unknown biological significance." EPA considers a 27% increase in the size of any brain region to be a potentially adverse effect (U.S. Environmental Protection Agency, 1998e), and designated 10 mg/kg-day as the LOAEL and the NOAEL at 3 mg/kg-day for these changes in brain histology. No additional evaluation of the brains from the neurohistological examination of Subset 4 pups (PND82 to PND85) were ever submitted to EPA although it was suggested again that the next lower dose group be analyzed because of the significant increases in brain weights and in the frontal cortex and corpus callosum measurements for the males in the high-dose group.

Additional analyses of the brain morphometry were provided by the EPA at the 1999 external peer review (Geller, 1999a) that corroborated the preliminary finding of Crofton (1998c). The data were analyzed using a 2-way ANOVA, with dose and sex as independent variables. To correct for multiple comparisons, the acceptable alpha for significance (for all interaction main effects) was corrected to 0.016 (alpha of 0.05 divided by the square root of the number of ANOVA tests).

Significant effects of dose were found in corpus collosum, hippoacampal gyrus, anterior and posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate putamen. The effect on corpus callosum was confirmed and showed an increase in size at the 10 mg/kg-day dose. Hippocampal gyrus (12% less than control) and caudate putamen (7.3% less than control) showed a decrease in size at the 3 mg/kg-day dose, with no significant difference between control and high dose, yielding a U-shaped dose response. The anterior and posterior cerebellum showed a significant increase in size at the 3 mg/kg-day group (13%).

Because of concern for this effect voiced at the 1999 external peer review, the blocks of brain tissue were evaluated to determine if they could be refaced and additional sections evaluated. It was determined that the remaining materials were of insufficient quality for additional sectioning and histological evaluation (Harry, 2001). As an alternative, brain morphometry measurements were included in the "Effects Study", described below in Section 5.3.3, to determine if the alteration in brain morphometry could be repeated.

5.3.1.2 Evaluation of Thyroid Histopathology

Appendix O of the Argus Research Laboratories, Inc. (1998a) neurodevelopmental study presents thyroid histopathology data provided by the sponsor (AFRL/HEST). Note that the data analyzed by EPA in the 1998 document for PND5 F1-generation rat pups are from the final report for the PND5 time point (Channel, 1998c). Channel (1998c) reported that the decrease in follicular lumen area in these pups at PND90 to PND92 showed no significant differences between dose groups and controls for either females or males based on t-test or Mann-Whitney Rank Sum Test (M-W RST). These data suggest a recovery from the effects observed in the thyroids of the pups at PND5.

The report also contained measurements, performed by Dr. William Baker of AFRL/HEST, of both follicular epithelial cell height and the follicular lumen diameter. These data were subsequently formally transmitted to EPA by consultative letter (Channel, 1998c) in Microsoft Excel® spreadsheets. For the final morphometric study (Channel, 1998c), the arbitrary decision based on ease of detection of this region in digitized images was made by Dr. William Baker to focus on only a lumen area measurement because of time constraints (Jarabek, 1998). The mean follicular lumen area represents the mean area of all follicular lumens measured from the three histological sections sampled from each rat and is expressed in microns. In the opinion of Dr. Charles Capen of Ohio State University (Crofton, 1998d), the measurement of follicular height is usually more sensitive than those of follicle diameter and lumen area. In support of this opinion, data collected by Dr. Baker (Argus Research Laboratories, Inc., 1998a; Appendix O) demonstrated significant increases in males rats in the incidence of follicular epithelial cell hypertrophy at doses much lower than those doses that increased the incidence of decreased lumen area. The difference observed between standard histopathology as originally reported by Argus Research Laboratories, Inc. (1998a) and the thyroid morphometry performed by Dr. Baker was analyzed extensively by the EPA in its 1998 assessment. The results indicated that the morphometry performed on lumen size was a less sensitive measure of thyroid histopathology. The analyses of the thyroid morphometry are retained in this reassessment; whereas, the PWG review results will be presented below for the histopathology.

Data from the dependent measure (follicle lumen size) based on the morphometric analyses (Channel, 1998c) were available for pups sacrificed at ages PND5 and PND90. These data were reanalyzed by EPA (Crofton and Marcus, 2001). Because there was only one block of animals at

PND90 compared to two blocks of data at PND5, and because the slides for PND90 were processed at a much later time, the data for the two ages were analyzed separately. Data from PND5 pups were subjected to three-way ANOVA tests with gender, treatment (dose), and block (two separate analyses of separate blocks of data) as independent between-subjects variables. Data from PND90 were subjects to a two-way ANOVA with gender and treatment (dose) as independent between-subjects variables. Step-down ANOVA tests were conducted as indicated by significant interactions and recalculated by Crofton and Marcus (2001) and Marcus (2001). Mean contrasts were performed using Duncan's Multiple Range Test. Note that in the Crofton and Marcus (2001) memorandum the 0.1 mg/kg-day dose is incorrectly labeled as 0.3 mg/kg-day. There was a significant main effect of treatment on lumen size for all doses at PND5, resulting in a free-standing LOAEL of 0.1 mg/kg-day. The data are plotted in Figure 5-8. There was no significant effect of perchlorate on lumen size at PND90.

The thyroid histopathology as reviewed and reported by the PWG can be found in Wolf (2001; Tables 9 through 13). This report includes corrections for slides sent to EPA that contained animals with autolysis and those necropsied at different times than indicated for the study protocol or to exclude dams that did not have litters.

The F0 generation dams (Wolf, 2001: Table 9) exhibited decreased colloid and increases in both hypertrophy and hyperplasia. A clear dose-response was not evident, however, with the possible exception of colloid depletion at levels above 0.1 mg/kg-day.

Thyroid histopathology in the pups on PND4 (Wolf, 2001: Tables 10 and 11) was more pronounced, with colloid depletion and increases in hypertrophy at 0.1 and 3 mg/kg-day. Hyperplasia appeared to be effected at 3 mg/kg-day. The BMD analyses presented in Table 5-1 support these levels with BMDL estimates for colloid depletion at 0.33, increased hypertrophy at 0.88, and increased hyperplasia at 3.62 mg/kg-day. These results were obtained with a constrained model, but an adequate fit is obtained by fitting the model without restricting the exponent on dose to be ≥ 1 and results in a BMDL for pups on PND4 in this study at 0.009 for colloid depletion (Geller, 2001a).

The argument for the lack of biological plausibility of unrestricted functions is based on cancer modeling theory from the early 1960s (Mantel and Bryan, 1961) that attempted to derive a default procedure for modeling tumor data at the time when cancer was thought to be a one-stage process and many bioassays used only 1 dose and control. Given the increased sophistication of

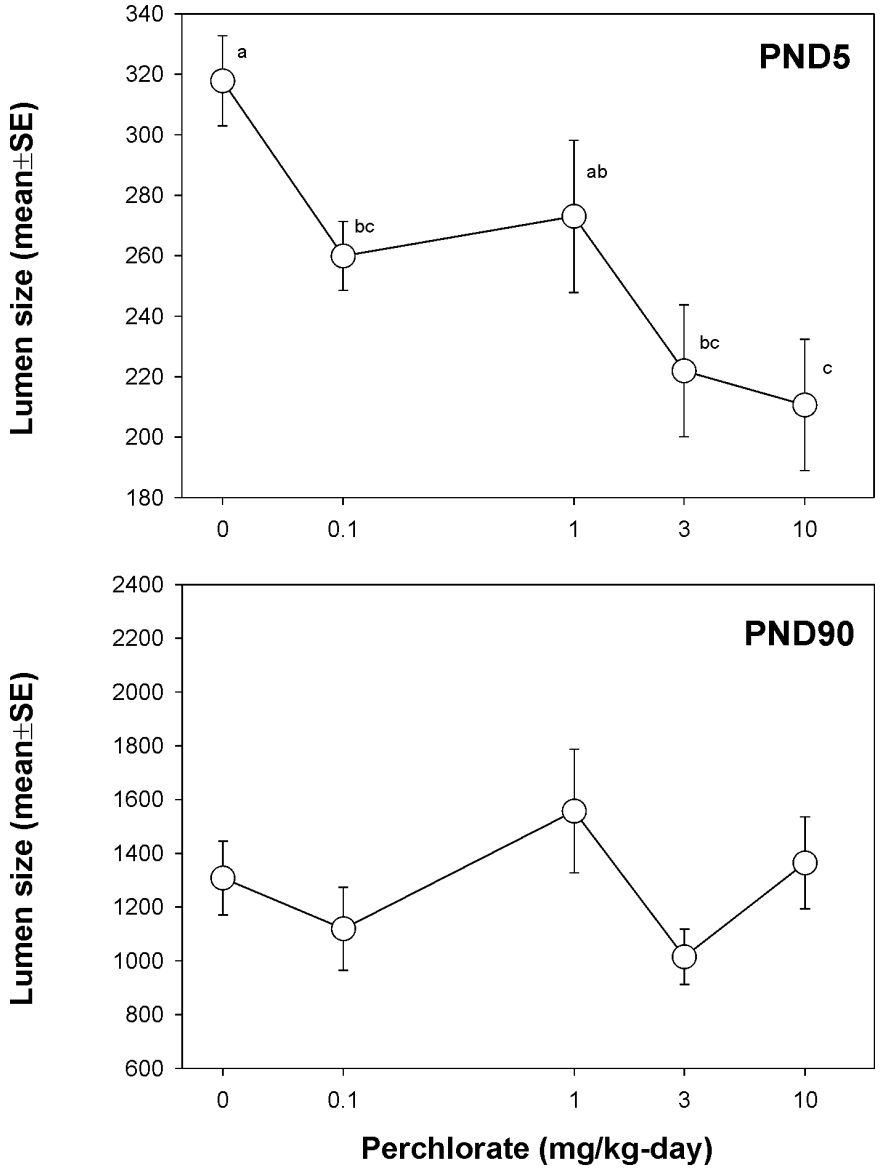

Figure 5-8. Effects from maternal drinking water administration of ammonium perchlorate to SD rats on thyroid gland follicular lumen size in F1-generation offspring on PND5 as recalculated in Crofton and Marcus (2001). Data of Channel (1998c) and Argus Research Laboratories, Inc. (1998a). Means with different letters were significantly different ($p < 0.05$). Daily dose was estimated from water consumption data.

contemporary bioassays and the level of organization at which effects are now being identified (i.e., precursor events at the cellular and molecular levels), Hasselblad et al. (1995) have argued that restricting the slopes of fits to the data prioritizes mathematical convenience over fitting the data. The thyroid hormone data show exquisite sensitivity to very low doses of perchlorate. This suggests that models fit with nonsupralinear slopes and lower doses need to be tested. It is interesting to note that PWG results for colloid depletion are very similar to the 1998 EPA analysis on the previous histopathological read by Argus Laboratories, Inc. (1998a) for hypertrophy/hyperplasia that resulted in a BMDL of 0.1 mg/kg-day.

Histopathology in the animals from PND90 and PND92 (Wolf, 2001: Tables 12 and 13) indicated variable effects on colloid depletion, hypertrophy, and hyperplasia. As indicated in Table 5-1, a BMDL was only calculated with confidence for colloid depletion with a resultant estimate of 0.03 mg/kg-day.

Evaluation of the histopathology in this study indicate that the pups are the most sensitive with a BMDL between 0.009 and 0.33 mg/kg-day.

5.3.1.3 Thyroid and Pituitary Hormone Analyses

Serum was collected and thyroid hormone analyses performed as part of the neurodevelopmental study (Argus Research Laboratories, Inc., 1998a; Crofton, 1998f)). The following is a statistical analysis of the thyroid and pituitary hormone data (T4, T3, and TSH) found in that report (Crofton and Marcus, 2001). At the time of this assessment, individual animal data were available from both the F1-generation pups (male and female samples were pooled for each litter) on PND5 and the F0 generation (parents) on post-partum Day 10 (PP10). Only the F1 data were reanalyzed because of the very limited (n = 2 to 5/group) data for the parental F0 PP10 group.

All data were supplied in Microsoft Excel® spreadsheets via E-mail by Dr. David Mattie (AFRL/HEST). Data for dependent measures (T4, T3, and TSH) were subjected to separate one-way ANOVA tests. Treatment (dose) was used as the independent, between-subjects variable. Mean contrasts were performed using Duncan's Multiple Range Test.

There were significant main effects of treatment for all the hormones. The data are plotted in Figure 5-9. Results of these reanalyses are similar to those stated in the report (Argus Research Laboratories, Inc., 1998a). There was a significant decrease in both T3 and T4, as well

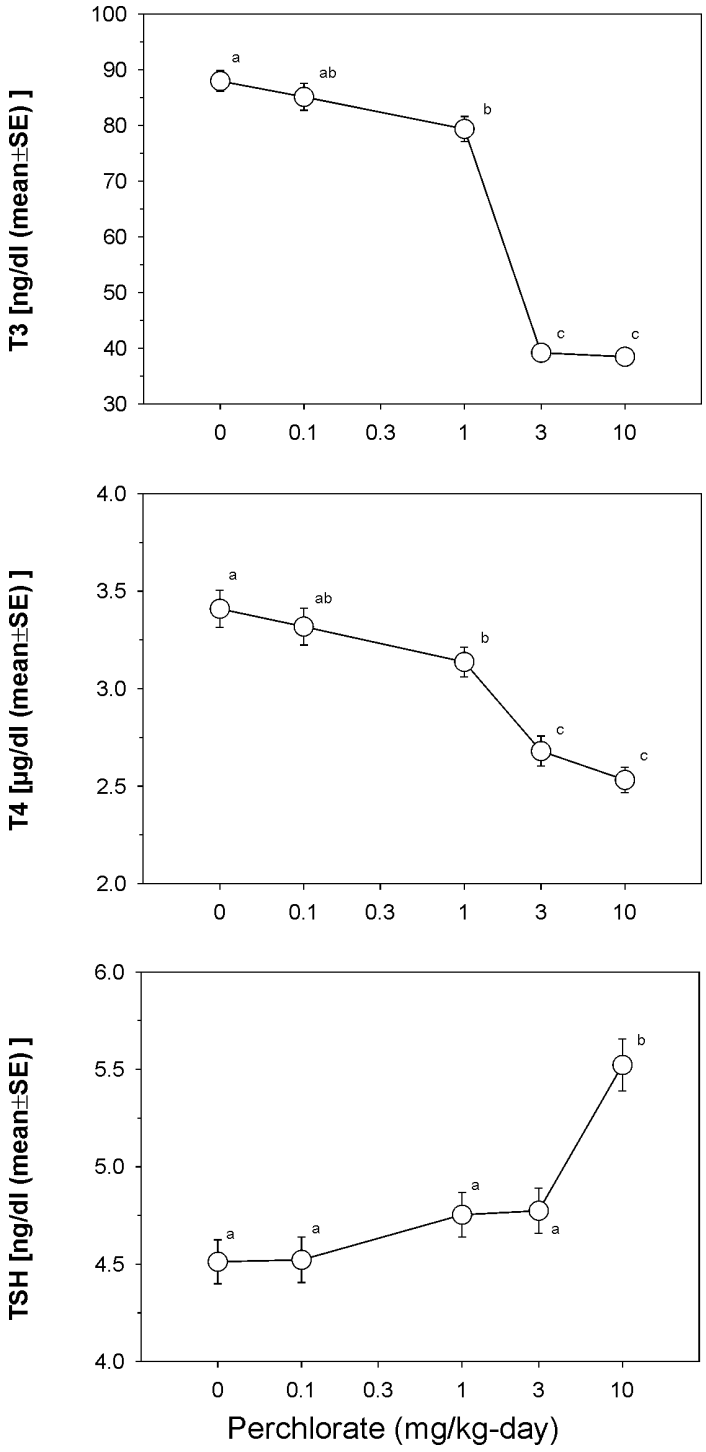

Figure 5-9. Effects from maternal drinking water administration of ammonium perchlorate to SD rat F1-generation pups on serum total T3 (A), T4 (B) and TSH (C) concentrations (ng/dL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Argus Research Laboratories, Inc. (1998a). Means with different letters were significantly different ($p<0.05$). Daily dose was estimated from water consumption data.

as the expected increase in TSH. The NOAEL for the effects of perchlorate on T3, T4, and TSH are 0.1, 0.1, and 3.0 mg/kg-day, respectively. These results are consistent with the known mechanism-of-action of perchlorate (inhibition of thyroid hormones). The increased TSH is likely a result of the activation of the pituitary-thyroid feedback mechanism.

5.3.1.4 Behavioral Evaluations

The 1998 EPA review of the behavioral evaluations performed on Subset 3 pups agreed with the Argus Research Laboratories, Inc. (1998a) report with one exception regarding an increase in motor activity in male rats on PND14 that no perchlorate-induced changes were detected in any of the other behavioral indices (i.e., passive avoidance, water maze, auditory startle). The EPA disagreed with the Argus Research Laboratories, Inc. (1998a) report and subsequent submissions (York, 1998a,b,c,d,e) with regard to the significance of the motor activity changes.

The data originally were analyzed using two separate three-way ANOVA tests (age, treatment, and habituation block), one for each gender (Argus Research Laboratories, Inc., 1998a). This analysis demonstrated a significant decrease in the amount of habituation in the two highest dose groups on PND14 in the male pups. There were no changes detected at any other ages (i.e., PND18, PND22, PND59). On initial review by EPA, it was recommended to the sponsor (PSG) that an additional analysis of the data be conducted using gender as a within-subject variable, or alternatively, to use a nested design with gender nested under litter (see Holson and Pearce [1992] and Cox [1994], for a review of statistical methods used in developmental studies and the importance of using litter as the unit of measure). The EPA also questioned why the method or statistics did not detect significance for the dose-dependent increase in total session counts that amounted to a 95% increase over controls in the highest dosage group (see Figure 5-10). The response from Argus Laboratory (York, 1998b) included a new analysis in which gender was used as a between-subjects variable. No interactions with, or main effects of, treatment were found in this analysis.

EPA remained concerned that Argus Research Laboratory and the sponsor (PSG) failed to respond adequately to the request for an explanation of why the analysis failed to detect significance in the PND14 motor activity for the male rats. Figure 5-10 illustrates the clear dose-dependent increase in two different measurements of motor activity: (1) time-spent-in-

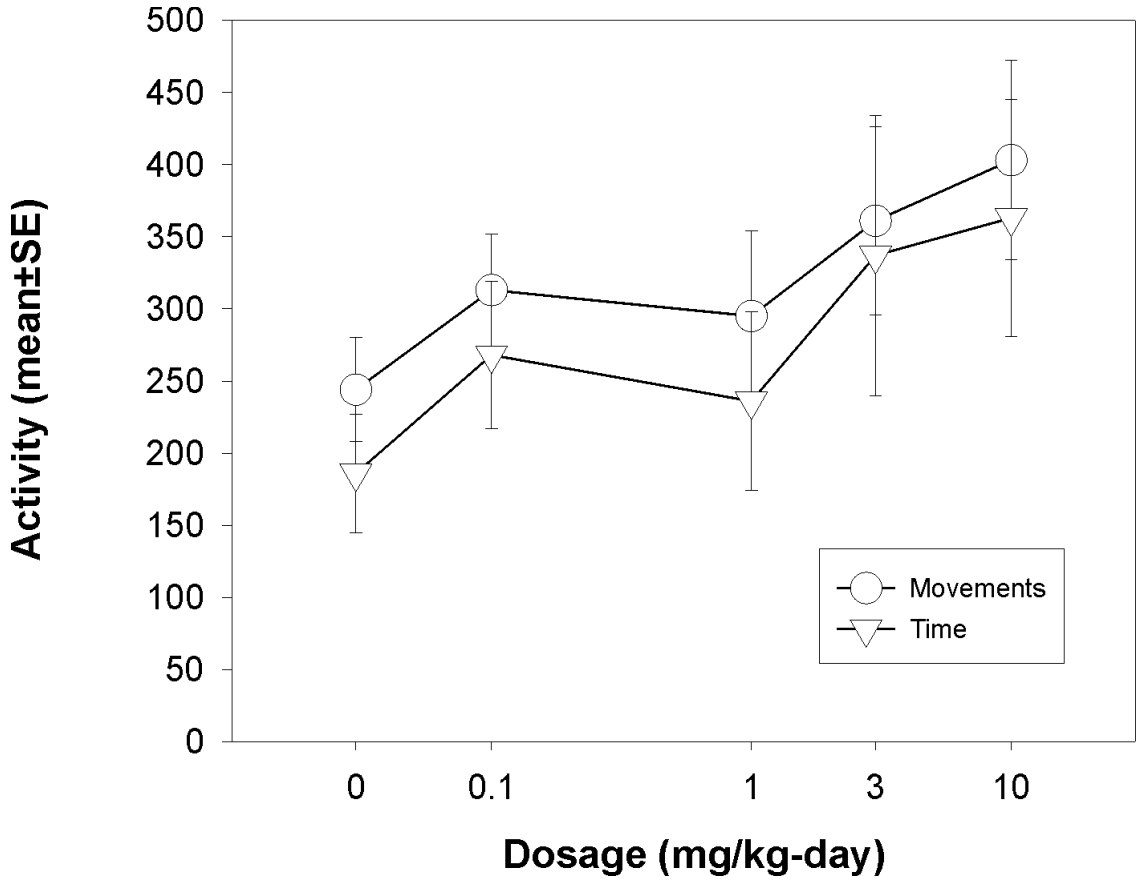

Figure 5-10. The effects of developmental exposure to perchlorate on motor activity in male rats on PND14. Data of Argus Research Laboratories, Inc. (1998a). The dose-dependent increases in both number of movements and time spent in movement were not statistically different, even though the increases were substantial at the higher dosages.

movement ("time") and (2) total number of movements ("movements"). The time variable increased over 95% at the highest dose relative to controls (group means of 363 and 186, respectively). The number-of-movements variable increased approximately 65% relative to controls. Expert opinion of EPA neurotoxicologists was sought, and it was their opinion that increases in motor activity over 50%, especially in developing animals, were clearly of concern from a biological perspective (Crofton et. al., 1998). The critical issue for evaluation of these motor activity data was how to resolve the difference between what is a clearly a biologically significant alteration in behavior with a lack of statistical significance. In an attempt to resolve the issue, EPA also requested positive control data from the testing laboratory for this device that

was not provided in the original report, as well as any available historical control data. York (1998a) replied with a number of positive control studies and a limited amount of historical control data from PND14 pups.

The positive control data were requested to help understand the sensitivity of the device in detecting increases in motor activity (i.e., what is the smallest increase in motor activity that has been detected by this device). Unfortunately, the positive control data were of limited use in interpreting the sensitivity of the device. The submission (York, 1998a) contained data from experiments with amphetamine and triadimefon in adult rats. The smallest increase in activity that was induced by either chemical was a 109% increase relative to controls. Although these effects were statistically significant, they are greater than the effects produced by the highest dosage of perchlorate in the PND14 pups. There were also positive control data from chlorpromazine-treated animals that showed significant decreases ($\geq$32%) in activity. However, ability to detect decreases does not necessarily translate to the detection of increases.

The historical control data from PND14 rats were requested to help understand the variability normally found in control animals. Unfortunately, the historical control data submitted were only useful in that the data raised more suspicion that the degree of experimental control over this behavior by the testing facility was inadequate. For the time data, the control mean for the perchlorate data set was 186 sec. For the three relevant historical control data sets, the means were 1026, 965, and 458 sec. Either the lab had very little control over the behavior, or the data were from a different test apparatus or from a different usage of the same apparatus. In any case, the data were of no use in helping EPA determine the historical profile of control animal behavior in this test apparatus.

In lieu of the absence of useful positive control and historical control data, EPA was left with the issue of ascertaining statistical versus biological significance. There were a number of reasons for the lack of statistical significance. The first reason was the extremely large within-group variability exemplified by coefficients of variation (CV) greater than 100%. It was the opinion of Crofton et al. (1998) that this was likely caused by the inability of the testing laboratory to gain adequate control over the behavior being tested. This large variability results in very little statistical power and increases the potential for Type II errors. Normally, an increase in sample size (by additional testing) allows for adequate power to refute or support the conclusion of an effect. Given the CVs of about 100%, simple power calculations (see Cohen,

1987) for detecting a 40% change in one group out of five results in needed group sizes of about 70 to 90 animals per group. The second reason was that the effect, a 95% increase, while rather large from a biological perspective, occurs in only one gender on only 1 day out of 4 test days. The large variability coupled with the complicated design (treatment, age, gender, and block) would tend to mask anything other than extremely large effects. This conclusion is consistent with the content of a phone conversation (Crofton, 1998g) with Dr. Simon Mats. Dr. Mats was the statistician from the contract laboratory (Primedica/Argus) who conducted the revised statistical analysis of these data. Lastly, the effect seen in the males on PND14 may indeed be a Type I error and may not be found again if this experiment was repeated.

The assignment of biological significance to the effect seen was supported by both the underlying mode of action of perchlorate and the effects of other chemical and physical insults on the motor activity of post-natal rats. The hypothesis that a thyrotoxic chemical would induce a delay in any aspect of nervous system development is highly plausible. A delay in the onset of habituation would be evidenced by an increase in overall counts, as well as a decrease in the rate of a habituation (Ruppert et al., 1985a,b). This delay could be quite transient. Other agents that interfere with thyroid hormones during development are known to induce delays of a few days magnitude in developmental landmarks such as eye opening (Goldey et al., 1995a,b). This is the type of effect seen on PND14 in the Argus Research Laboratories, Inc. (1998a) report.

Developmental exposure to numerous hypothyroid-inducing agents (e.g., propylthiouracil, methimazole) are known to result in delays in the ontogeny in many behaviors (cf., Comer and Norton, 1982; Goldey et al., 1995a,b; Schneider and Golden, 1986; Tamasy et al., 1986), including the development of habituation. However, effects of these chemicals on total motor activity counts vary from increased to decreased, depending on the chemical and age of testing. Rice (2000) has noted parallels between the features of attention deficit hyperactivity disorder (ADHD) and the behavior of monkeys exposed to polychlorinated biphenyls (PCBs). The mechanism for the gender-dependent nature of the effect of perchlorate also remains to be determined. In addition, there are numerous reports from the literature that support the biological significance of a 40 to 50% increase in motor activity in postnatal rats (cf., Campbell et al., 1969; Ruppert et al., 1985a,b).

In summary, EPA maintained that the increase in activity should be considered biologically significant until additional data could be marshaled to suggest or prove otherwise. The

inadequacy of standard parametric statistics to detect a significant difference suggested that alternative analyses should be used on these data, such as the benchmark approach. This type of statistical approach may be useful because of the inverse relationship between the data variability and the benchmark dose (BMD). The BMDL estimates were calculated for data on the movement (number of movements) and time (time spent moving) measures from the motor activity test from PND14 pups. These data were fit by a linear function with fairly shallow slope, yielding BMD estimates for movement and time of 1.94 and 1.33 mg/kg-day and BMDL estimates of 1.04 and 0.66 mg/kg-day, respectively. These BMD and BMDL estimates could serve as estimates of LOAEL and NOAEL for this data set. The estimates are in accord with doses with activity values that may have emerged as significantly different from control had the data set not had its unusually high variability. These BMD analyses bring the motor activity NOAEL more within the range of the T3 and T4 NOAEL and below that for TSH.

5.3.2 Motor Activity Study (Bekkedal et al., 2000)

In response to recommendations at the 1999 peer review for an additional study, the United States Navy (USN) performed a study that included evaluation of motor activity in Sprague Dawley rats of both sexes (Bekkedal et al., 2000). Female Sprague-Dawley rats were dosed with ammonium perchlorate for two weeks at 0, 0.1, 1.0, 3.0 or 10.0 mg/kg-day prior to mating with the breeder males and through PND10. PND1 was counted as the day when the first pup was observed in the cage. All pups within a litter were weighed on PND5 when the litters were culled to eight pups of 4 males and 4 females or as close as possible to that combination. Pups and dams from any litters with less than 8 pups were eliminated. On PND14, one male and one female were randomly selected from each litter to be used in the motor activity testing. These same animals were tested on PND14, PND18 and PND22. Nine different measures of motor activity were automatically recorded using Opto-Varimex activity meters at ten minute intervals. The measures included: frequency and time of ambulatory movements, frequency and time of sterotypic movements, frequency of movements in the horizontal plane, distance traveled in the horizontal plane, frequency of rears, total number of horizontal movements made while in the rearing position (vertical plane movements), and time spent resting.

Bekkedal et al. (2000) analyzed each of the nine measures of motor activity separately using a univariate repeated-measures ANOVA. The between subjects variable was perchlorate

dose, with 5 levels. The three within-subject variable were sex (2 levels), age (3 levels), and time block (9 levels). Due to violation of the sphericity assumption, the Greenhouse-Geisser test was employed with a fiducial limit set at $p < 0.05$. No statistically significant differences were found for the main effect of perchlorate exposure for any of the 9 measures nor any reliable interactions related to dose. The authors do note, however, a general pattern of dose-dependent changes in the later sessions (90-minute). They also note that this pattern, as in the previous Argus Laboratories, Inc. (1998a), suggest that exposed pups have a slightly slower rate of habituation and thus maintain a higher level of activity as compared to untreated pups. Additional follow-up tests were suggested.

5.3.2.1 EPA and NIEHS Statistical Analyses of Motor Activity Effects

Because EPA was concerned about effect on motor activity in the original study and it appeared that a similar pattern of effects was again emerging in the study repeated by Bekkedal et al. (2000), EPA requested that NIEHS perform a statistical evaluation that could formally integrate the various measures together as well as statistically compare the two studies with each other (Dunson, 2001a). A Bayesian hierarchical model (Gelfand et al., 1990) was chosen to assess the weight of evidence of a dose-response trend in motor activity. A linear mixed-effects regression model (Laird and Ware, 1982) related dose, sex, age, habituation time and a habituation time x dose interaction term to the expected number of ambulatory movements, with an animal-specific intercept included to account for within-animal dependency. To complete a Bayesian specification of the model, a vague (or uninformative) but proper prior distributions for each of the unknown parameters was chosen. In particular, the prior for the parameters that related dose to motor activity was centered on a value corresponding to the null hypothesis of no effect of perchlorate. The model was fit using BUGS, a widely-used software package for Bayesian analyses (Gilks et al., 1994).

The analyses were conducted under a variety of different choices of prior variance for the dose parameters and prior means and variances for the other parameters in the model. The dose level associated with a 10% increase in the number of ambulatory movements by inverse estimation (refer to Appendix A in Dunson, 2001a). The choice of 10% as the benchmark level is consistent with standard practice for dichotomous outcomes. The 5% level often used for

continuous outcomes was judged to be too low for measuring a biologically significant increase in motor activity. Conclusions were consistent across the analyses.

As noted by Bekkedal et al. (2000), the effect of ammonium perchlorate on the number of ambulatory movements was found to increase significantly with habituation time (posterior probability = 0.98). In the first habituation interval there was modest evidence of an increase in motor activity with dose (posterior probability = 0.79), while in the final interval there was clear evidence of an increase in motor activity with dose (posterior probability > 0.99). The posterior density for the expected increase in the logarithm of the number of ambulatory movements at the final habituation time per unit (mg/kg-day) increase in dose of ammonium perchlorate is plotted in Figure 5-11 for the USN study (Bekkedal et al., 2000). The posterior density is centered on a positive slope and assigns low probability to a negative slope, suggesting a clear increase in motor activity with dose. The dose estimated to increase the mean number of ambulatory movements at the final habituation time by 10% is 1.62 with a 95% credible interval of (0.90, 7.87). There was no evidence of an interaction between age and dose, nor of any effect of gender.

The previous study of Argus Laboratories, Inc. (1998a) was also analyzed in this fashion and results were very similar (Figure 5-11). In contrast to the Bekkedal et al. (2000) study, dosage began at the first day of gestation and continued through parturition and up to lactation day 10 (PND10). Dams were dosed at 0, 0.1, 1.0, 3.0 and 10.0 mg/kg-day. Movements of each pup were monitored by a passive infrared sensor. Each test session was 90 minutes in duration. The number and time spent in movement was tabulated at each five-minute interval. In order to be comparable with the USN analysis, every two of the five-minute intervals were combined into a ten-minute interval. However, the Bekkedal et al. (2000) study did not have data for PND59, so the results are not entirely comparable. Again, there was evidence of an increase in the effect of ammonium perchlorate on motor activity at the later habituation times (posterior probability = 0.93). In the first habituation interval there was no evidence of an increase in motor activity with dose (posterior probability = 0.58), while in the final interval there was moderate evidence (posterior probability = 0.94). The dose estimated to increase the average of ambulatory movements in the final habituation time by 10% is 4.60 with a credible interval of (2.18, infinity). This interval was wider than the interval observed in the Bekkedal et al. (2000) study;

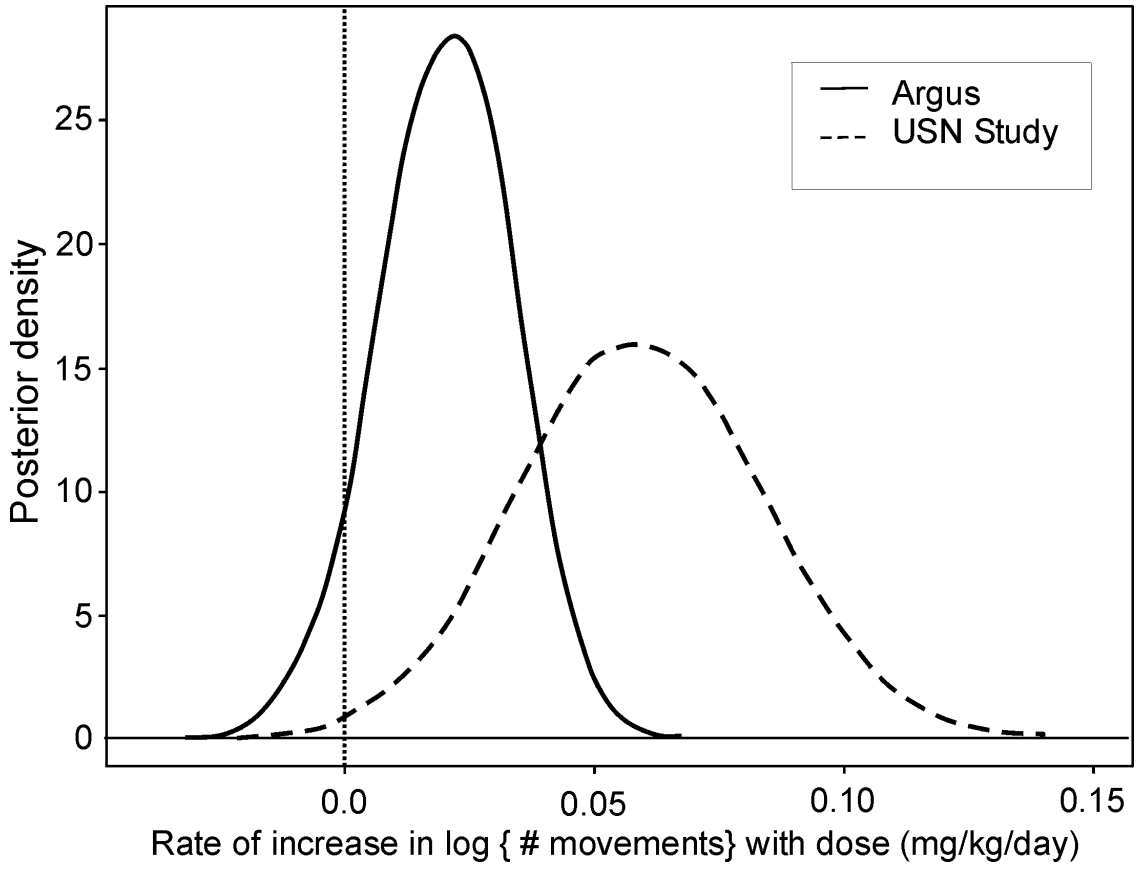

Figure 5-11. Bayesian estimates of the posterior densities for the expected increase in the logarithm of the number of ambulatory movements at the final habituation time per unit dose (mg/kg-day) increase of ammonium perchlorate (Dunson, 2001a). A separate analysis for the Argus Research Laboratories, Inc. (1998a) and United States Navy (Bekkedal et al., 2000) was performed.

possibly due to greater variability in the Argus data as noted in 1998 by EPA**.** This result is slightly higher than the BMD analysis (Section 5.3.1.4) estimate of 1.04 mg/kg-day.

One of the advantages of Bayesian analysis is that it provides for formal combination of data from different studies. To perform a combined analysis of data from the USN Study (Bekkedal et al., 2000) and the Argus (1998) study, a modification of the model described above was used (Dunson, 2001a). The number of ambulatory movements was first standardized by subtracting the overall mean and dividing by the standard deviation. A linear mixed-effects regression model that incorporated distinct baseline parameters (i.e., intercept, age-effects,

habituation time effects, error variances) for the two studies was then fit, assuming common slope parameters. This approach allowed the different studies to have distinct baseline parameters, including aging effects.

Figure 5-12 shows the posterior density from the combined analysis of the Argus Research Laboratories, Inc. (1998a) study and the Bekkedal et al. (2000) study. In this combined analysis, the posterior probability of an increase in motor activity with dose was 0.99. For rats that averages 34.09 ambulatory movements at the final habituation time in the absence of exposure (the average value in the Argus study), the estimated dose needed to increase this average by 10% is 3.33 [95% credible interval = (1.91,12.78)].

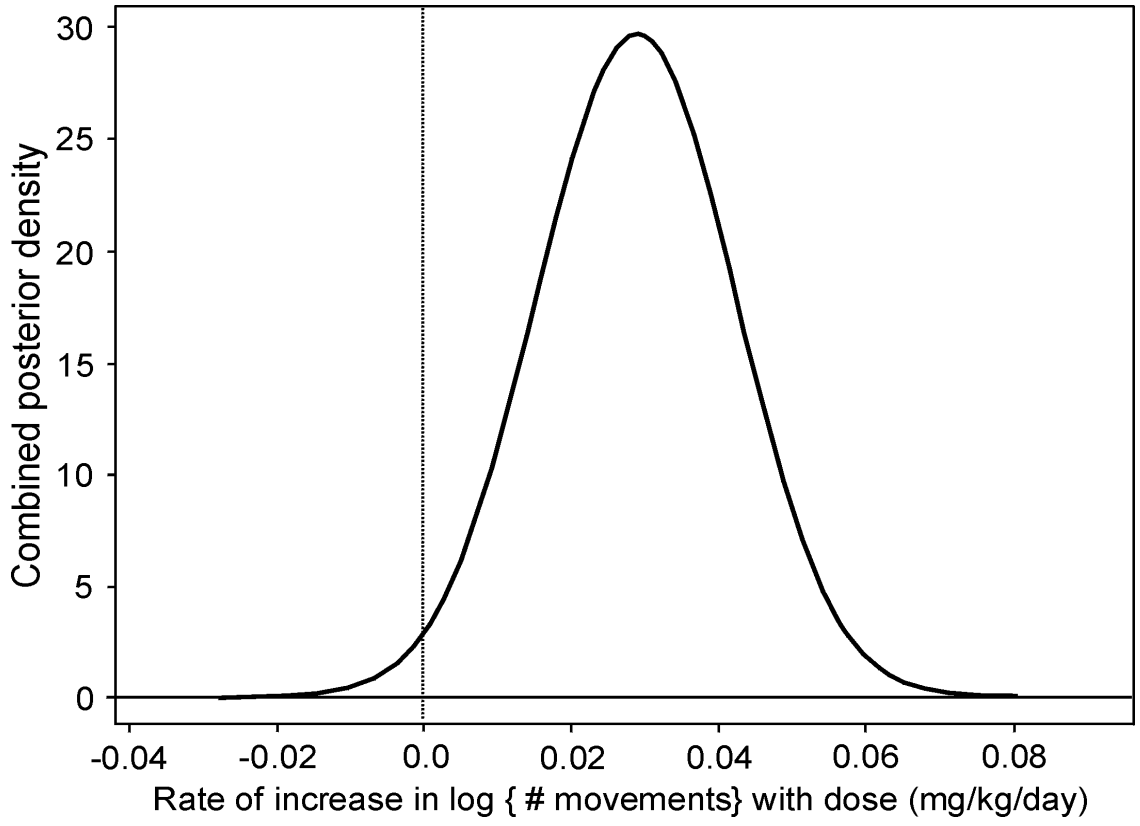

Figure 5-12. Bayesian estimate of the posterior density for the expected increase in the logarithm of the number of ambulatory movements at the final habituation time per unit dose (mg/kg-day) increase of ammonium perchlorate for the combined data from the two studies of motor activity effects shown in Figure 5-12 (Dunson, 2001a).

There was evidence of an increasing dose-response trend in motor activity in both the Argus Research Laboratories, Inc. (1998a) and Bekkedal et al. (2000) studies, although the effect in the Argus study was less pronounced, likely due to the variability in the data previously noted. Given this, it is remarkable that the two studies showed such similar results. The Bayesian analysis can be applied to risk assessment in an analogous fashion to the benchmark dose analysis (Hasselblad and Jarabek, 1996). The lower limit on the estimated dose corresponding to a 10% increase in motor activity relative to control can be used as a surrogate for the NOAEL for the point of departure for reference dose derivation. For the Argus Research study, the lower limit of the 95% credible interval for the dose was 2.18, while for the Bekkedal et al. (2000) study the corresponding estimate was 0.90. In the combined analysis, the lower limit was 1.91. Because of the variability in the Argus Research Laboratories, Inc. (1998a) study, a NOAEL that relied on the Bekkedal et al. (2000) was chosen at 1.0 mg/kg-day to represent effects on motor activity from these combined data.

5.3.3 The 2001 "Effects Study"

The Argus Research Laboratories, Inc. (2001) study was performed in response to recommendations made at the 1999 external peer review (Research Triangle Institute, 1999) for additional analyses of the thyroid and brain effects during gestation and post-natal days. Because Argus Laboratories identified the day of birth as PND1, the age nomenclature of PND5, PND10, and PND22 (Argus, 2001) is off by one day as referenced by EPA definition. These ages are therefore referred to as PND4, PND9, and PND21.

It should be noted that exposure in this study started two weeks prior to the start of cohabitation. The rationale was to ensure a hypothyroid state, but given the response of the rat system to perturbation, it is more likely that this resulted in the dams already compensating for the effect of perchlorate prior to pregnancy by upregulation of the NIS, making comparison with the 1998 developmental neurotoxicity study (Section 5.3.1) more difficult.

The thyroid and brain from one male and one female pup per litter were selected for histological and morphometric evaluation, with one set evaluated on PND4, PND9, and PND21.

5.3.3.1 Results of General Toxicity Measures

There were no remarkable clinical or necropsy observations. Average body weights and body weight changes for female rats were comparable among the five exposure groups through the pre-cohabitation and gestation periods. Body weight gains for female rats in the 1.0 and 30.0 mg/kg-day target dosage groups were significantly increased on PND12 to PND15 compared to the carrier group. These increases were not considered treatment-related because they were a singular occurrence and were transient.

5.3.3.2 Evaluation of Thyroid Histopathology

The thyroid histopathology in this study was evaluated using the same scoring system as developed for the PWG review and was performed by one of the pathologists who served on the PWG. A second read of these slides has not occurred. The data will be discussed individually for each of the time points. Benchmark dose analyses conducted by EPA will be presented in Section 5.3.3.2.1.

Absolute thyroid weights were increased significantly in the 30.0 mg/kg-day group in the dams on GD21 and decreased colloid; increased hypertrophy and increased hyperplasia were also noted at this dose. Thyroid weights were not collected for fetuses on GD21, but colloid depletion was noted in both male and female fetuses at both the 1.0 and 30.0 mg/kg-day doses.

Thyroid weight in pups was measured on PND4, and the absolute weight was significantly effected at 30 mg/kg-day, suggesting a NOAEL at 1.0 mg/kg-day. Histopathology was evident at lower doses, suggesting a NOAEL at 0.1 for colloid depletion; however, no real dose-related trend in either hypertrophy or hyperplasia was evident.

Thyroid weight in dams on PND9 continued to be effected significantly at 30 mg/kg-day, with histopathology noted at lower doses. The pups on PND9 were more sensitive than the dams, exhibiting statistically increased absolute thyroid weights at 0.1 mg/kg-day and higher doses and suggesting a NOAEL at 0.01 mg/kg-day. A dose-related trend in histopathology in this same range of doses was noted in the pups, especially for colloid depletion.

Thyroid weight in dams on PND21 remained significantly effected at 30 mg/kg-day, with a clear dose-related trend in colloid depletion, hypertrophy and hyperplasia. All three histopathological indices were increased at 30 mg/kg-day, and hyperplasia was also significantly increased at the 1 mg/kg-day dose. It is interesting to note that hyperplasia was more sensitive

than both hypertrophy and colloid depletion in the dams at this time point, perhaps indicating a system coming into the chronic phase of compensation described in Chapter 6.

Pups on PND21 also continued to exhibit increased thyroid weights at both 1 and 30 mg/kg-day (females only at 1.0 mg/kg-day). Colloid depletion was clearly significant at 30 mg/kg-day, and hyperplasia was noted although not designated as significant. Despite the assertion by Argus Research Laboratories, Inc. (2001) that there was no dose-related trend in hyperplasia, a BMD analysis indicated otherwise (see below). Hypertrophy was not noted, again indicating an overlap among the three diagnostic indices of thyroid effects used by the PWG.

Benchmark dose analyses performed by EPA are presented in Table 5-3 (Geller, 2001b). A benchmark response level of a 10% increase in incidence over controls, i.e., BMD10 and BMDL10, was adopted for all studies. Data were fit with a log-logistic function constrained such that the slope was ≥ 1.

5.3.3.2.1 Benchmark Dose Analyses of Thyroid Histopathology

BMDL values in the dams on GD21 were 1.01, 1.19, and 8.51 mg/kg-day for colloid depletion, hypertrophy, and hyperplasia. By PND9, these values decreased to 0.13, 1.01, and 0.92 mg/kg-day. Similar values for dams on PND21 were 0.62, 1.24, and 0.99 mg/kg-day for colloid depletion, hypertrophy, and hyperplasia. Of note is the overlap between the estimates for hypertrophy and hyperplasia.

The effects of ammonium perchlorate on the pups' thyroid glands are largely limited to colloid depletion. The dams show additional dose-related effects on thyroid histopathology that were evaluated as thyroid hypertrophy and hyperplasia. The low incidence of these latter two endpoints in pups may be related to the duration of exposure compared to the dams and the adult rats examined in earlier studies (Geller, 2001a). Alternatively, hyperplasia and hypertrophy may be have been difficult to detect in the smaller thyroid glands from the young pups.

The BMDL10 is lowest in the GD21 pups and is estimated at 0.12 mg/kg-day for the male and female pups combined, or for male pups alone, and for female pups alone at 0.04 mg/kg-day. The BMDL10 increases with age (Figure 5-13), suggesting that the thyroid gland may be most susceptible to the effects of perchlorate during gestation or at the time of parturition (Geller, 2001b). This is likely due to the double effects of perchlorate inhibition of thyroid function in

TABLE 5-3. BENCHMARK DOSE (BMD)[a] AND BENCHMARK DOSE LOWER CONFIDENCE LIMIT (BMDL)[a] ESTIMATES FROM THYROID HISTOPATHOLOGY IN THE "EFFECTS STUDY" (Argus Laboratories, Inc., 2001; Geller, 2001b)

Study Population "Effects" Study (Argus, 2001)	Colloid Depletion				Hypertrophy				Hyperplasia			
	BMD	BMDL	χ^2 [b]	Exp [c]	BMD	BMDL	χ^2 [b]	Exp [c]	BMD	BMDL	χ^2 [b]	Exp [c]
GD 21 Dams	5.10	1.01	1.00	17.90	15.46	1.19	1.00	6.25	28.54	8.51	1.0	5.03
GD 21 Male pups	0.69	0.12	1.00	8.82	NOE [4]	NOE	NOE	NOE	NOE	NOE	NOE	NOE
GD 21 Female pups	0.18	0.04	0.60	2.08	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
GD 21 M + F pups	0.65	0.12	0.16	7.80	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 Male pups	0.88	0.29	0.12	7.37	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 Female pups	0.82	0.18	0.12	7.78	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 M + F pups	0.84	0.33	0.02	7.50	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 Dams	0.62	0.13	0.59	2.65	2.65	1.01	0.22	17.86	2.24	0.92	0.49	1.0
PND9 Male pups	1.29	0.71	0.59	6.40	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 Female pups	0.33	0.13	0.61	1.30	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 M + F pups	0.93	0.48	0.36	3.77	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND21 Dams	1.21	0.62	0.34	4.90	15.60	1.24	1.0	6.34	3.59	0.99	0.66	1.0
PND21 Male pups	17.33	1.36	1.0	5.85	NOE	NOE	NOE	NOE	26.97	5.45	0.58	5.06
PND21 Female pups	16.42	1.24	1.00	5.94	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND21 M + F pups	17.32	2.17	1.0	5.92	NOE	NOE	NOE	NOE	54.17	13.70	0.24	1.0

[a] Units of mg/kg-day.
[b] χ^2 goodness of fit criterion
[c] Exponent in log-logistic function restricted to be ≥ 1.0.
[d] NOE = No observed effect.

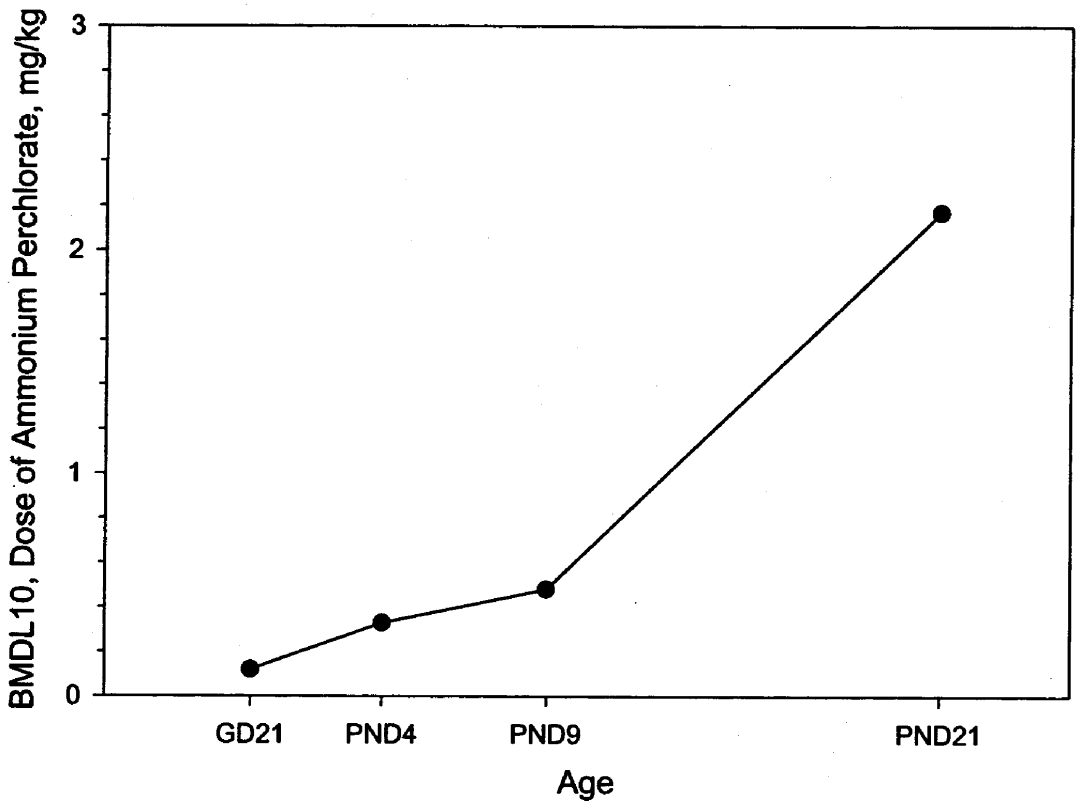

Figure 5-13. Lower confidence limit on the dose of ammonium perchlorate in drinking water that produced a 10% increase in the incidence of colloid depletion in the thyroid gland as a function of post-natal age of rat pups. Data of Argus Laboratories, Inc. (2001). Male and female data combined (Geller, 2001b).

the pup and the lack of protection of the pup by the dam because of her own compromised thyroid function. After 21 days of post-natal exposure, the male pups also show follicular cell hyperplasia.

The BMD and BMDL estimates of 0.84 and 0.33 mg/kg-day for the PND4 male and female pups in this study (Table 5-3) do corroborate the BMD and BMDL for colloid depletion for the PND4 pups from the 1998 Neurobehavioral Developmental study of 0.53 and 0.33 mg/kg-day (Table 5-1). However, it should be noted that an unrestricted model also fits those data adequately and results in a BMD and BMDL estimate of 0.45 and 0.009 mg/kg-day, suggesting variability in those analyses (Geller, 2001b). Again, the lower estimates based on the 1998 data at this time point (PND4) may be due to differences in the dosing of the dams between the two studies.

The BMD and BMDL estimates of 17.32 and 2.17 mg/kg-day for the PND21 male and female pups in this "Effects Study" (Table 5-3) are somewhat higher than the previous 1999 two-generation reproductive toxicity study estimates of 2.51 and 0.80 mg/kg-day (Table 5-1). However, comparison of the results of the two-generation reproductive toxicity study to the current results may be difficult because of differences in the spacing of doses tested.

5.3.3.3 Thyroid and Pituitary Hormone Analyses

Thyroid (T3 and T4) and pituitary (TSH) hormones were also analyzed in the "Effects Study" at various time points. Thyroid hormones and TSH were evaluated in the dams and fetus on GD21, in the dams on PND10 and PND22, and in neonates on PND5, PND10, and PND22 (corresponding to PND4, PND9, and PND21 according to EPA nomenclature as explained earlier). Table 5-4 presents the results of ANOVA analyses performed by EPA (Crofton, 2001b). Maternal serum measures of the hormones were subjected to separate two-way ANOVA. Treatment (dose) and age (GD21 or PND5, PND10 or PND22) were the independent between-subjects variables. Two separate approaches were used to address the offspring data due to differences in experimental design. The data from GD21, PND5 and PND10 were obtained from litter-pooled samples due to the small volumes of blood and no gender analyses were possible. These data were subjected to separate two-way ANOVA with age (GD21, PND5, or PND10) and treatment (dose) as between-subjects variables. Blood samples from PND22 were not pooled so that the data from this age were subjected to separate two-way ANOVA with gender and treatment (dose) as independent variables. Mean contrasts were performed using Duncan's Multiple range test. Significant two-way ANOVA were followed by step-down one-way ANOVA to determine the main effects of treatment. If the interaction term was not significant, then the model was refit if main effects were found. A reduced model was then fitted to the data retaining only the main effects found significant previously, described as the "liberal" approach in Crofton and Marcus (2001) and Marcus (2001).

EPA benchmark dose analyses (Geller, 2001c) of these results will also be discussed. The benchmark estimates were generated using the Bench Mark Dose Software version 1.30, and fit a Hill equation constrained such that the exponent on dose was ≥ 1.0 (Geller, 2001c). The BMDL estimates indicate that the thyroid and pituitary hormones are exquisitely sensitive to the effects of perchlorate.

TABLE 5-4. NOAELs AND LOAELs FOR EFFECTS ON THYROID AND PITUITARY HORMONES FROM THE ARGUS 2001 "EFFECTS STUDY" (Crofton, 2001b)

				Effect Level Designation	
Generation	**Hormone**	**Age**	**Sex**	**NOAEL**	**LOAEL**
Dams	T3	GD21	F	1.0	30.0
		PND10	F		
		PND22	F		
	T4	GD21	F	—	0.01
		PND10	F	0.1	1
		PND22	F	1.0	30.0
	TSH	GD21	F	—	0.01
		PND10	F	—	0.01
		PND22	F	0.01	0.1
Fetus and Offspring	T3	GD21	Pooled	—	0.01
		PND5	Pooled		
		PND10	Pooled		
		PND22	F	0.01	1.0
			M		
	T4	GD21	Pooled	0.01	0.1
		PND5	Pooled		
		PND10	Pooled		
		PND22	F	no significant effects	
			M	—	0.01
	TSH	GD21	Pooled	0.1	1.0
		PND5	Pooled	no significant effects	
		PND10	Pooled	—	0.01
		PND22	F	0.01	0.1
			M	—	0.01

[a]Dosages of 0, 0.01, 0.1, 1.0, and 30 mg/kg-day.

5.3.3.3.1 Maternal Hormone Analyses

Exposure to perchlorate produced significant decreases in thyroid hormones and an increase in TSH in the dams at the various ages tested. For effects on maternal T3, there was no age-by-treatment interaction and the NOAEL at all time points was 1.0 mg/kg-day. There was a significant age-by-treatment interaction for effects on maternal T4. Step-down analyses resulted in a LOAEL at 0.01, 1.0 and 30.0 mg/kg-day at GD21, PND9 and PND21. The 0.01 mg/kg-day level is a LOAEL for the dams at GD21. There was also a significant age-by-treatment interaction for the effects on maternal TSH. Step-down analyses resulted in a LOAEL at 0.01, 0.01 and 0.1 mg/kg-day at GD21, PND9 and PND21. As for the effects on T4, there was no NOAEL at GD21 for the effects on TSH. There was no NOAEL for the effects on TSH at PND9 as well. These effects on T4 and TSH at GD21 are consistent with the Argus Laboratories Inc. (2001) analyses. Benchmark dose analyses resulted in BMD estimates of 1.63, 0.006 and 2.38 mg/kg-day for the effects on T3, T4, and TSH at GD21. BMDL estimates were only calculable for T4 in the dams and resulted in an estimate of 0.004 mg/kg-day. Benchmark dose calculations were not performed for the dams on PND9. At PND21, a BMDL estimate was calculable only for TSH in the dams with a resultant estimate of 0.53 mg/kg-day.

5.3.3.3.2 Fetal and Neonatal Hormone Analyses

Maternal exposure to perchlorate resulted in hypothyroidism in the offspring. There were significant dose-related decreases in thyroid hormones and increases in TSH at all time points evaluated.

There were no age-by-treatment interactions for the effects on T3 at any age tested. The LOAEL for GD21, and post-natal days 4 and 9 was 0.01 mg/kg-day. This value is lower than that reported in the Argus Laboratories, Inc. (2001) analyses. The specified benchmark dose analysis were not computable for T3 at PND4 or PND21. There was no significant gender-by-treatment interaction for the effects on T3. The NOAEL for effects on T3 at PND21 was 0.1 mg/kg-day. A BMDL was calculable only for the male pups and resulted in an estimate of 0.13 mg/kg-day.

There were also no age-by-treatment for the effects on T4. The LOAEL was 0.1 mg/kg-day and the NOAEL was 0.01 mg/kg-day for GD21 and PND4 and PND9. At PND21, there was a significant gender-by-treatment interaction for the effects on T4. There was no NOAEL

established for the male pups and 0.01 mg/kg-day was a LOAEL, whereas 0.01 was suggested as a NOAEL in the Argus Laboratories, Inc. (2001) analyses. The females did not show significant effects in either the EPA or Argus Laboratories, Inc. (2001) analyses. BMDL estimates were extremely sensitive for changes in T4 at PND21 in the males with a BMD and BMDL at 0.001 and 2.86 x 10^{-7} mg/kg-day. Benchmark analyses did not converge for the data from the female pups alone or for the combined data.

There was a significant age-by-treatment interaction for the effects on TSH. Step-down analyses revealed a NOAEL at 0.1 mg/kg-day for GD21. There was no significant effect on TSH at PND5, but then no NOAEL on PND9 with a LOAEL at 0.01 mg/kg-day. The LOAEL was also 0.01 mg/kg-day in male pups at PND21. The females were slightly less sensitive as suggested by the significant gender-by-treatment interaction. The NOAEL in female pups on PND21 was 0.01 mg/kg-day. Benchmark analyses on the combined data resulted in a BMD and BMDL of 0.06 and 0.02 mg/kg-day for the effects on TSH.

5.3.3.4 Brain Morphometry Effects

Due to the deficiencies of the remaining tissue blocks from the previous developmental neurotoxicity study (Argus Research Laboratories, Inc. 1998a), it was determined that the recommendation of the external peer review panel to evaluate more sections could not be accomplished unless a new study was performed (Harry, 2001). Thus, one major objective of the Argus Laboratories, Inc. (2001) "Effects Study" was replication of brain morphometric measurements in order to address concerns raised by the US EPA, the NIEHS, and the external peer review panel regarding results observed in the 1998 developmental neurotoxicity study (Argus, Protocol Number 1613-002, 1998a; U.S. Environmental Protection Agency, 1998d). The purpose was to evaluate, under more rigorous experimental conditions and according to the EPA developmental neurotoxicity guidelines (U.S. Environmental Protection Agency, 1998b), whether the effect in the corpus callosum identified by the EPA in the previous assessment (Section 5.3.1) would be replicated.

In addition, another objective was to identify effects that may occur in other brain regions. Details with respect to the rationale motivating the experimental design can be found in Harry (2001). A brief summary of important points will be provided here, but the reader is referred to Harry (2001) for specifics on this protocol and to other review articles (Garman et al., 2001;

Adams et al., 2000; Rice and Barone, 2000; U. S. Environmental Protection Agency, 1998b,g,h) for a fuller appreciation of the state-of-the-science supporting the use of these measures as developmental neurotoxicity indices in risk assessment. The use of the rodent and not a non-human primate was based on the degree of difficulty and the ethical issues involved with conducting such screening studies in addition to the need to replicate previous findings. The work, to document the process of normal development and alterations in the rat cited in these reviews, supports the use of rodent models for determining potential adverse effects on the developing brain.

It should be noted that Argus Laboratories identifies the day of birth as PND1; therefore, the age nomenclature as recommended in the EPA guidelines for PND10 and PND22 actually corresponds to PND9 and PND21 in this study. Likewise, in the previous 1998 Argus Research Laboratories, Inc. Study (Section 5.3.1), the morphometry performed on PND12 was actually done on PND11. While the actual ages were slightly different between the two studies, the concept of capturing an active process of development with brain morphometry remains in effect (Harry, 2001).

The motivation for evaluation of brain morphometry was based on the fact that the formation and maturation of the nervous system is critically dependent upon both a temporal and spatial organization pattern (U.S. EPA, 1998b; Harry, 2001). Within this framework, an interdependency between the various cell types in the brain and a precise spatial relationship of one cell type to one cell type another has been demonstrated. During this time, the developing system is undergoing rapid maturation of organizational and regulatory processes. Thus, the disruption of the developmental profile of one cell type may significantly influence critical events in later development, resulting in an alteration of the normal formation of the brain and its functional connections. Many toxic agents have been shown to interfere with one or more of the developmental processes of the brain (i.e., cell division of neuronal and glia precursor cells, cell interaction with the immediate environment through surface receptors or cell adhesion molecules, regulation of cytoskeletal processes that control proliferation and migration, cell-cell interactions that underlie synaptogenesis, development of the cerebral circulation and the blood-brain barrier, myelination, and programmed cell death). Such perturbations may not be evident by standard histological assessments as often there is little, if any, evidence of cell death. Rather

what is seen is a delay or disruption in the normal development and maturation of specific neural regions (Harry, 2001).

Immersion fixation was the tissue processing method of choice and was both recommended and agreed upon by both the EPA and the PSG for the study. While the tissue fixation method of choice in adult rodents is via cardiac perfusion, even this procedure is not without problems that can compromise tissue integrity. It has been documented that immersion fixation artifacts can influence histological and morphometric evaluations of adult brains; however, a less than optimal cardiac perfusion can also result in morphological artifacts. For the younger animal, there is less of a consensus on the proper manner of fixation. With the decreasing size and blood volume of the younger animal (PND4 and PND9) used in the protocol, the difficulty of ensuring a good fixation via cardiac perfusion is significantly increased over that in the adult. Further, because comparisons were to be made between the 1998 and the 2001 study, consistency in method of fixation was considered to be a critically important variable to maintain as constant across studies.

Following the review of the previous developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a), and in considering design considerations for the subsequent study, the plane of cut for the brain was discussed (Garman, 2001a,b). While sagittal sections for analysis were recommended for some aspects of morphometric analysis, coronal sections were ultimately adopted since comparisons were to be made between the 1998 and the 2001 study. This final design of the study also adhered to the EPA developmental neurotoxicity testing guidelines that call for coronal sections (U.S. Environmental Protection Agency, 1998g, h). It was originally recommended by the NIEHS that measurements of the corpus callosum in coronal sections should not be conducted at the midline due to possible edema artifacts that can occur from the close proximity of the ventricle. Three sites were recommended for measurement that would have been consistent with the evaluation conducted by NIEHS on the sections from the Argus Research Laboratories, Inc. (1998a) study (Section 5.3.1). It was agreed upon in the final design meeting with PSG contractors that, given the time constraints and need for comparison to the 1998 study, one measurement per hemisphere would be recorded at the same site as used in this previous study (Garman, 2001a,b). This was a site just off of the midline of the two hemispheres.

Finally, a question raised in the PSG-contracted review (Toxicology Excellence for Risk Assessment, 2001) with regard to age of sampling as it relates to myelin formation should be addressed. The process of myelination is a "developmental landmark" for the maturation of the brain, that is initiated upon the presence of the axon and continues over an extended period of time. It is a structure that matures over time with the accumulation of protein and structural lamella. One major period of myelin protein and lipid synthesis occurs approximately between PND19 and PND35. Thus, while examination at PND21 would not capture the final accumulation of myelin, it would capture events occurring at a time during which myelin processing and lamella wrapping of the axon is actively occurring. Therefore, this may represent a period of critical development of the myelin sheath. Examination of animals with a mature myelin sheath (e.g., ages greater than PND40) may offer information regarding whether any of the changes seen at earlier time points represent a permanent structural alteration. The majority of studies that have examined myelin development and/or alterations in this developmental process have employed biochemical, molecular, as well as, morphological evaluations to make such determinations regarding delay or hypomyelination. From such studies, the time most appropriate for examination appears to be between the ages of PND15 and PND35. Thus, examination of the corpus callosum at PND9 is probably at the limit of early development for an evaluation of the myelin sheath. However, it should be noted again that this study was intended to determine if the effects seen previously (Argus Research Laboratories, Inc., 1998a) could be repeated. Effects in the corpus callosum in that previous study occurred at the early (PND11) and remained at the late (PND82) time points. Brain weight and the size of the frontal cortex and caudate putamen also were effected at the PND82 sacrifice (Section 5.3.1.1).

In addition, the development of the axonal pathways connecting the two hemispheres via the corpus callosum also continues to develop during this time period. While the study design allowed for the collection of tissue at PND4, it is felt that any measurements recorded at such age would be very limited in their contribution to the interpretation of the currently available data set. In addition, given the variability of the plane of cut and the difficulty in examining brains of young animals, EPA and NIEHS agree that examination of the corpus callosum in younger animals (the remaining materials available for PND4) would present an even greater problem.

Figure 5-14 illustrates where the section levels were taken for the brain morphometry measurements and shows the anatomical landmarks on the ventral and dorsal surfaces of the

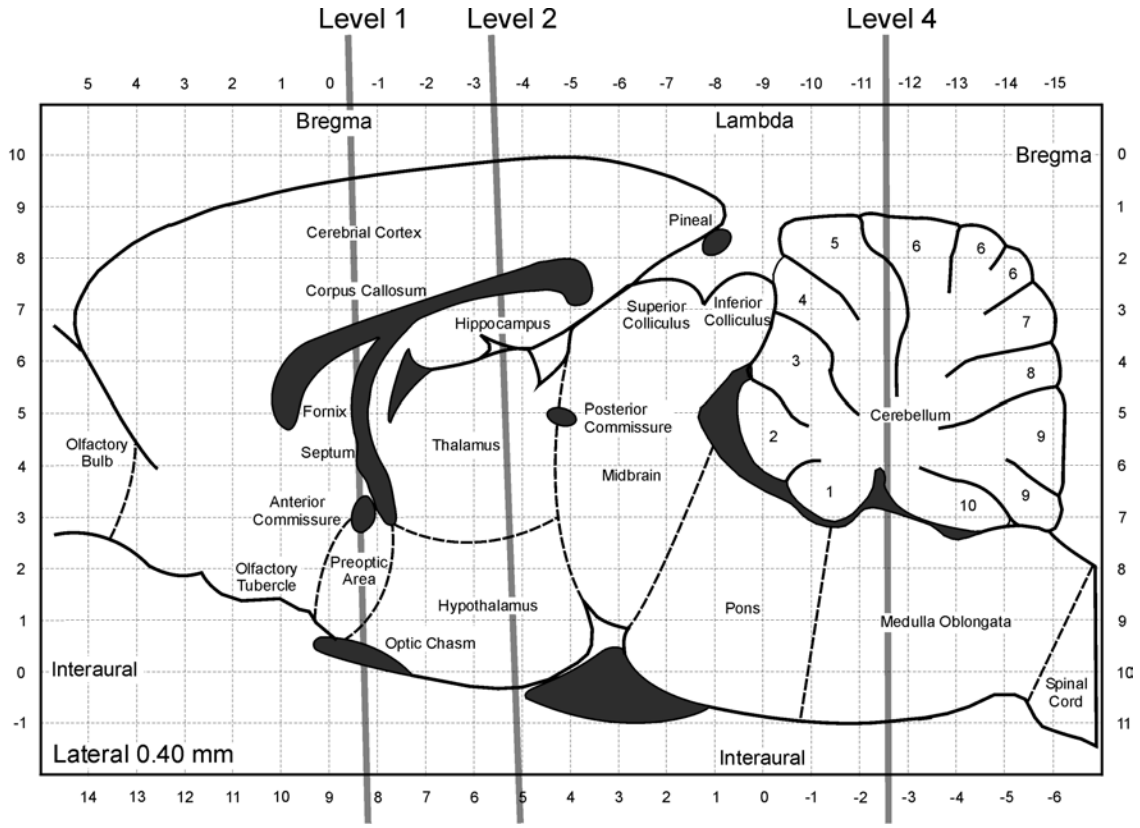

Figure 5-14. Topograph of the approximate anatomical landmarks on the ventral and dorsal surfaces of the brain used for making the morphometry measurements (Garman, 2001c). The topograph provided is for an adult brain, but the same landmarks are used for PND9 and PND21 brains although the sections at these two other ages would differ due to the rapid growth during this period.

brain. The veterinary pathologist who performed the work has noted that while the landmarks were the same for both the PND9 and PND21 brains, it must be appreciated that the sections from one age versus the other would not look precisely similar (Garman, 2001c) due to the fact that the brain is rapidly growing at this time.

Overall, the images of the brain sections from the PND9 and PND21 time points demonstrated that the processing of the brain was adequate for conducting limited morphometric measurements as outlined in the protocol. As mentioned by the PSG-contracted reviewers (Toxicology Excellence for Risk Assessment, 2001) and stated in the study and additional reports (Argus Research Laboratories, Inc., 2001; Consultants in Veterinary Pathology, 2001; Garman,

2001d), there was a greater degree of variation in the PND9 sections than in the PND21 brain sections (Harry, 2001). Many sections in the PND9 brains also showed signs of disruption or damage that may have compromised the measurements. For these reasons the EPA relied upon the PND21 measurements, despite corroborating effects from the materials at PND9.

There were no significant effects of treatment or sex on brain weight, anterior-posterior cerebrum length, or anterior-posterior cerebellar size at either age tested. As discussed in the Argus Research Laboratories, Inc. (2001) report, statistical analyses consisted of Students' t-test comparisons between the control and the corresponding group of each sex at each separate dose level. For example, PND9 male control striatum measurements were compared to measurements for the PND9 male 30 mg/kg-day dose group, then PND9 male control striatum measurements were compared to the PND9 1 mg/kg-day-dose group. These analyses were run separately for both sexes and ages and all brain areas, right and left sides. The Argus Laboratories, Inc. (2001) analyses found a large number of significant effects on brain morphometry at doses of 0.1 and 0.01 mg/kg-day ammonium perchlorate in drinking water.

Guidelines on the assessment of neurotoxicity (U.S. Environmental Protection Agency, 1998b) specify that alterations in brain structure should be considered adverse and relevant to human health risk assessment. Alterations in brain structure are consistent with the mode-of-action for perchlorate, i.e., transient decrements in T4 and T3 during development can result in neurodevelopmental effects. The significant findings reported in the Argus Laboratories, Inc. (2001) report strongly argue, therefore, that adverse effects of ammonium perchlorate are present at the lowest dose tested and that this data set contains only LOAELs, no NOAELs.

While the analysis in the Argus report was provocative, the number of t-tests run increases the risk of introducing Type I error into this analysis. To address this, a more conservative multivariate analysis, profile analysis (Johnson and Wichern, 1988; Tabachnick and Fidell, 2001), was run by the EPA (Geller, 2001d). Profile analysis is more conservative than the analysis described above because a multiple analysis of variance (MANOVA) takes into account any correlations between the independent variables; whereas, the multiple t-tests assume complete independence. This analysis also reduced the number of main effects tests by nesting gender within litter and by constructing a vector composed of all of the morphometric data from each animal, then comparing these vectors. The approach is explained in more detail below.

5.3.3.4.1 Description of EPA Profile Analysis of Brain Morphometry Effects

When a series of measurements are made from a single animal, i.e., within-subjects measurements, they can be used to build a profile or vector of scores across the measurement variables. Profile analysis makes between-groups comparisons using a vector composed of all of the (within-subject) measurements taken from each animal. Its primary test, for parallelism of the vectors, establishes whether the pattern of results between treatment groups is the same or different. It is a much more rigorous and conservative test, requiring that all of the measurements (i.e., all brain regions) show a dependence on dose with the same pattern. This determination also allowed examination of the entire set of data without an *a priori* expectation of effect in one brain region or another or the direction of the effect (i.e., decrease or increase). While there is indication that certain areas of the brain are likely susceptible to the effects on thyroid hormones of perchlorate (e.g., Madeira et al., 1991, 1992, 1993), and the previous study performed by Argus indicated that the corpus callosum was affected (U.S. Environmental Protection Agency, 1998d; Crofton, 1998c), definitive gestational windows for specific brain areas are unknown. Profile analysis determines whether there were dose-related changes in the pattern of brain growth, i.e., brain growth in one region relative to another while precluding prior expectations about specific areas of the brain or the direction and magnitude of these changes.

The profile analysis was run on the data from the PND9 and PND21 animals separately with gender nested within litter (PROC GLM, SAS Institute, Inc, Cary, NC). The data were provided in electronic form from Argus Research Laboratories, Inc. (2001) and in an additional report (Garman, 2001d). Profile analysis requires data from each endpoint for each animal. Data from individual brain regions, both right and left sides, were missing from 8 animals in the PND9 cohort and 3 animals in the PND21 cohort, eliminating these animals from the analysis (Geller, 2001d: Table 1). If a sex by treatment interaction was found, separate analyses were run on males and females. Treatment effects within a brain region were examined with univariate analyses of variance with gender nested within litter. Dunnett's two-tailed t-test was used to compare each dose group to controls at $\alpha = 0.05$ for step-down tests of treatment effects within a brain region as guided by the overall (univariate) treatment or sex by treatment effects.

Right and left side measures of the same brain structures were examined with profile analyses (whole set of data) and repeated measures analyses of variance (univariate analysis on each brain region). While there was no *a priori* reason to expect other than a bilateral effect, the

presence of this kind of bias could reflect either anisometries in brain regions (i.e., lateralization) or sectioning that was not perfectly perpendicular to the anterior-posterior axis of the brain and that would have resulted in sampling brain regions at different depths on right and left side. These analyses, together with examination of the images of the brain sections (Harry, 2001) demonstrated some systematic variability in the sectioning resulting in differences in right versus left measurements in different brain regions. The magnitude of the variability was small and not always in the same direction, even within a brain region (varying with the dose group sampled). The small magnitude of difference relative to the dose-related changes found in this study, the fact that different brain regions varied in their laterality bias in different directions, and that different dose groups varied in different directions all argue for simply averaging the right and left brain region measurements for each animal rather than tailoring different analyses for different brain regions. In addition, averaging could help to reduce variability in the data due to sampling only one histological section/brain region/animal. Therefore, data from right and left sides of the brain were averaged before the analysis of dose effects. Where data were missing from only one side of the brain, the existing measurement was used for the analysis.

Two additional analyses were run with adjustments to the raw morphometry data in response to suggestions made by reviewers hired by the PSG (Toxicology Excellence for Risk Assessment, 2001) designed to subtract variability due to variation in brain size and focus on changes in the sizes of brain areas relative to one another. As suggested by the PSG review, one analysis was run dividing all of the linear dimensions through by the post-fixation brain weight from each brain. However, EPA and NIEHS note that there are little historical data for normalizing data with post-fixation brain weight (Harry, 2001) and that fixation results in the loss of any evidence of hydration-related changes such as edema or other swelling.

The second additional analysis was suggested by the NIEHS and also adjusted for brain size using the anterior-posterior (a-p) measurements of cerebrum and cerebellum and the full width measure of hippocampus to adjust the linear dimensions. In this analysis, frontal, parietal, and corpus collosum dimensions were divided by a-p cerebrum size; dentate, CA1, and CA3 were divided by hippocampal width; and the cerebellar linear measurement was divided by the a-p cerebellum measurement. Hippocampus, a-p cerebrum, and a-p cerebellum were not included in the analysis as separate measures. The striatum and external germinal layer measurements were not adjusted by these other linear dimensions.

An additional two analyses were run on the PND21 data. These analyses omitted (1) the posterior corpus callosum measurement, or (2) the posterior corpus collosum and all hippocampal measures; i.e., all measures that came from the Level II section since there was some indication that there may have been a systematic difference in the plane of sectioning with dose (Harry, 2001).

5.3.3.4.2 Results of EPA Profile Analysis of Brain Morphometry Effects

The brain morphometry profiles were not parallel across treatment groups for PND9 pups (Geller, 2001d: Table 2). The absence of parallel profiles obviates further analysis for equal profiles. This means that the effects of developmental dosing with ammonium perchlorate were different on different brain regions. Planned contrasts show that the 0.01 and 1.0 mg/kg-day doses were significantly different than controls (Geller, 2001d: Table 2A). Adjusting for brain weight had little effect on these results (Geller, 2001d: Table 2B), though the adjustment for the linear size of the different brain regions made the effect at the highest dose (30 mg/kg-day) also significantly different from control (Geller, 2001d: Table 2C).

The brain morphometry profiles were also not parallel across treatment groups for the PND21 pups (Geller, 2001d: Table 2A). Contrasts between each of the dose groups and controls showed that the controls differed from all other dose groups at better than $p < 0.0001$, including at the lowest dose used, 0.01 mg/kg-day ammonium perchlorate in drinking water. The absence of parallel profiles obviates further analysis for equal profiles. The analysis adjusting for brain weight or regional size yielded similar, highly significant effects (Geller, 2001d: Tables 2B, 2C). Sex by dose interactions were significant in the parallel profiles analysis of the raw data and with the data adjusted by brain region size. The parallel profile MANOVA remained significant at $p < 0.0001$ in the overall and contrast tests with the posterior corpus callosum or posterior corpus callosum and all hippocampal measurements (i.e., all measurements taken at section Level II removed from the analysis) decreasing concern for confounding introduced by potential bias in sectioning at this level suggested for the males (Harry, 2001).

The profile analysis was done using the raw (right-left averaged) data values. Because the brain structures measured yield a range of measurements varying 10-fold, it is difficult to plot the raw data vector in a meaningful way in order to see the differences driving the findings of significant differences between dose groups. Figure 5-15 plots the (unadjusted) region-by-region

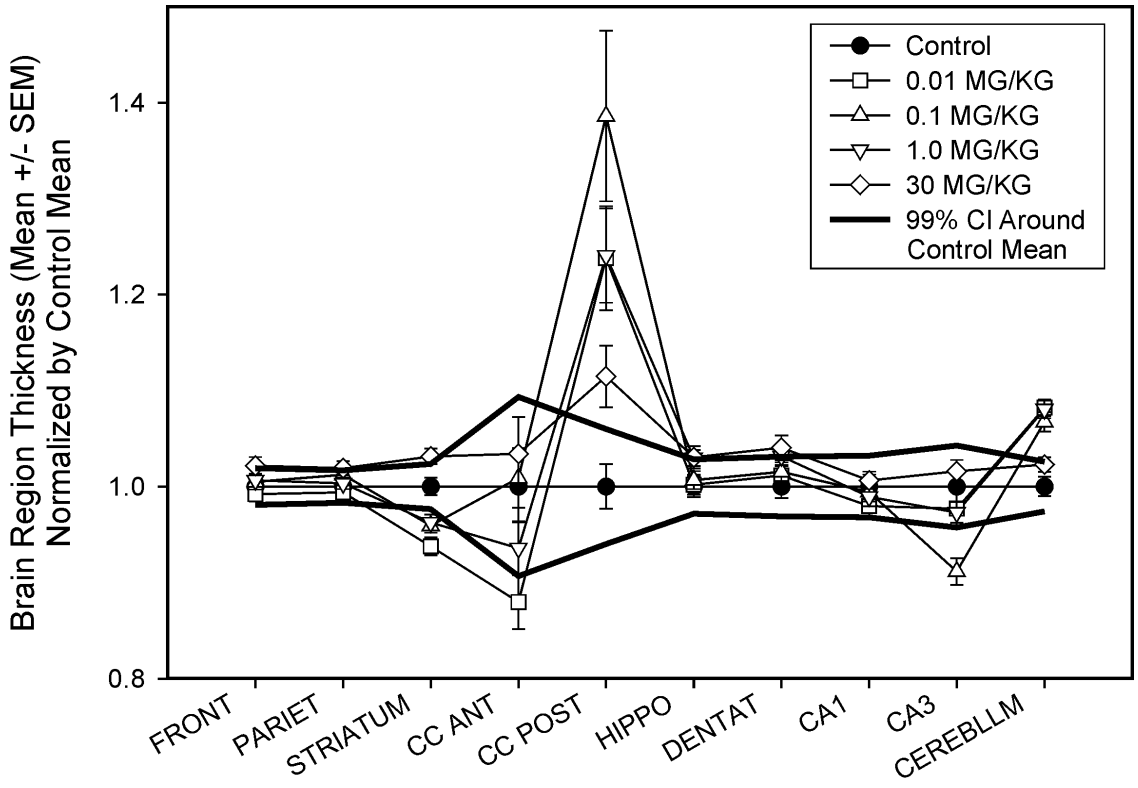

Figure 5-15. Profile analysis of brain morphometry measurements for PND21 rat pup brain regions. The male and female data on linear thickness measurements were combined and normalized by the control mean of each region. The control data are represented by the horizontal line at 1.0. Profile analysis determines whether the vectors of measurements from each treatment group differ from each other and control in a dose-dependent fashion. The heavy line represents the ± 99% confidence interval around the mean control values. Note that while this plot uses the normalized data to more easily illustrate the data vectors, the actual analysis was performed using raw data values (Geller, 2001d). A similar analysis showed effects in PND9 brains (data not shown).

size of each brain structure normalized by the mean size of that brain structure in the controls, male, and female combined for the PND21 pup data. The control group is therefore represented by a horizontal line at 1.0 with associated variability. The other dose groups differ from this horizontal line to different extents, and the parallel profiles analysis tests, in essence, whether these departures make the other dose groups significantly "non-horizontal". Note that the

analysis was not done on the normalized data; the control values were divided through to aid in visualizing the data vectors used in this analysis. The 99% confidence intervals around the control means represent an envelope inside of which comparable values ± standard error of the mean (SEM) are not significantly different from controls.

5.3.3.4.2.1 Univariate analyses of brain morphometry

While the main reason to use profile analysis was to benefit from the power it brings to an analysis by its conservative constraint that requires the entire vector of measurements depend on dose with a consistent pattern, univariate analyses also were evaluated to gain insights into effects on specific regions.

PND9 brains. Univariate tests yielded significant effects of treatment with ammonium perchlorate in the frontal and parietal regions of the cerebral cortex, the striatum, region CA1 of the hippocampus, the corpus callosum, and the external germinal layer of PND9 pup brains (Geller, 2001d: Table 3A). There is an increase in size at the 1.0 mg/kg-day dose in the frontal, parietal, and striatum measurements, and decreases in size in CA1 and the external germinal layer. There were also treatment-by-sex interactions in the corpus callosum and CA1 regions (Geller, 2001d: Table 3A). Both of these brain regions showed a treatment-related decrease in linear extent in females while showing an increase in size in males. While most of the changes in linear extent measured in the sampled brain regions were ±5 to 11%, the male corpus callosum was increased 23% at both the 0.1 and 1.0 mg/kg-day doses.

The adjustment for brain size reduced the significance of treatment effects in the striatum, CA1, and external germinal layer (Geller, 2001d: Table 3A, center). The analysis using adjustment for regional size (Geller, 2001d: Table 3A, right) was nearly identical to the raw data analysis, with the addition of significant effects being noted on cerebellum.

A comparison of the profile analysis and the analysis presented in Argus Research Laboratories, Inc. (2001) shows similar results were obtained on the PND9 brain morphometry with one exception. Both analyses found an increase in linear extent of frontal, parietal, and striatum at 1.0 mg/kg-day ammonium perchlorate and in the corpus callosum at the 0.1 and 1.0 mg/kg-day dose, with the corpus callosum increase limited to males. There was a decrease in the linear extent of the striatum at 0.1 mg/kg-day dose and decreases in the size of region CA1 of

females at the 0.01, 0.1, and 1.0 mg/kg-day doses. The Argus Laboratories, Inc. (2001) analysis did not detect a significant difference in female CA1 at the 0.01 mg/kg-day dose.

A post-hoc analysis of the plane of cut of the PND9 brain sections suggested that the 0.1 and 1.0 mg/kg-day dose groups were sectioned at a different depth than were the other dose groups (Harry, 2001). This likely contributed to the small but significant increase in size of the frontal, parietal, and striatum sections in the 1.0 mg/kg-day dose groups and may have contributed to the large increase in size of the anterior corpus callosum seen in the PND9 males.

PND21 brains. The striatum, cerebellum, and corpus callosum II (posterior sample) all showed significant changes with the lowest administered dose of ammonium perchlorate, 0.01 mg/kg-day (Geller, 2001d: Table 3B, left). The striatum was significantly reduced in size at all but the highest dose. Region CA3 of the hippocampus similarly showed a U-shaped dose response. The cerebellum and the posterior corpus callosum increased in size with dose in an inverted U-shape. There were sex-by-treatment interactions in striatum and frontal cortex such that the female rats showed a stronger dose-related decrease in linear measurement than males. Both males and females show a complex dose response in the anterior corpus callosum measurement. As in the PND9 animals, the changes in linear extent were generally in the ± 5 to 11% range with the exception of the posterior portion of the corpus callosum, which showed an increase in size of 24% in the 0.01 and 1.0 mg/kg dose groups, and a 39% increase in the 0.1 mg/kg dose group.

The adjustments for brain size had little effect on the region by region results at PND21 (Geller, 2001d: Table 3B, center, right). Dividing through by the a-p or hippocampal measurements resulted in additional significant dose effects noted on CA1 and a sex by dose effect on cerebellum.

The Argus Research Laboratories Inc. (2001) and current EPA analyses agreed. Both analyses found a significant decrease in size of the striatum at 0.01, 0.1, and 1.0 mg/kg doses and increases in size of the corpus callosum II (posterior) and cerebellum at the same doses. Both analyses noted the decrease in size of CA3 at the 0.1 mg/kg dose, the decreased anterior corpus callosum in females at 0.01 mg/kg, and the increased size of the frontal region in males at 0.1 and 30 mg/kg.

5.3.3.4.3 Conclusions of EPA Brain Morphometry Analyses of Brain Morphometry Effects

There were significant differences in brain morphometry due to treatment with ammonium perchlorate at both PND9 and PND21 in this study. Tables 2 and 3 in Geller (2001d) enumerate strong effects of developmental exposure to ammonium perchlorate on brain morphometry considered across all regions tested and in the analysis of individual brain regions. These effects were present at PND9 and PND21, with the latter age group showing stronger effects. Many of these effects represent an increase or decrease of ± 10% in the size of a brain region, similar to the range of morphometric alteration noted in a recent study of fetal alcohol syndrome (Bookstein, et al., 2001). The corpus callosum showed a notable increase of 24% or more in linear extent at PND21 in the 0.01, 0.1, and 1.0 mg/kg ammonium perchlorate dosing groups. Adjusting the raw morphometric determinations by either brain weight or measurements of larger brain areas (i.e., cerebrum, cerebellum, and hippocampus) had no strong effect on the results of the analysis.

The significant differences in the parallel-profiles test demonstrate exposure-related changes in relative growth of different brain areas even at the lowest administered dose (Geller, 2001d: Table 2). Univariate analyses to further investigate these effects showed effects on a number of different brain regions at both ages tested. The most sensitive endpoints were the linear dimensions of the striatum, corpus callosum, and cerebellum at the 0.01 mg/kg-day dose when males and females were considered together at PND21. Thus, these analyses ultimately agree with those submitted in Argus Laboratories, Inc. (2001): exposure to 0.01 mg/kg-day ammonium perchlorate during gestational and post-partum (weanling) development resulted in measurable changes in brain structures.

The increase in the size of the corpus callosum in this study replicates that seen in the previous morphometric analysis of rats developmentally exposed to ammonium perchlorate (U.S. Environmental Protection Agency, 1998d, Crofton, 1998c). This is notable given the differences between the two studies. The previous data were obtained from tissues from rats aged PND11 rather than PND9 and PND21, and dose spacing included high doses of 3 and 10 mg/kg rather than 1 and 30 mg/kg as in this study. Fewer animals were used in the previous study (6/dose/sex) than in the current study (approximately 15/dose/sex), and litter identity was considered in the current analysis. It also has been noted by Garman (2001c), a principal investigator with

established experience in performing brain morphometry on a substantial number of studies, that such a treatment-related pattern has not been observed in other studies.

It should be noted that changes in thyroid hormone levels effect different brain regions differently during development. For example, developmental hypothyroidism prolongs the expansion of the external granular layer and increases fissure formation in the cerebellum (Lauder, et. al., 1974). Different brain regions show an inverted U or U-shape dose response; this is not uncommon in biological systems as compensatory or other mechanisms may be triggered at high doses.

Fixation artifacts are not a concern in the study because all brains were fixed and embedded at the same time. In addition, dose-related effects were seen as both increases and decreases in brain region size. EPA concludes from this that whatever artifacts may be present were not large enough to obviate alterations of the magnitude observed. There is some concern over sectioning artifacts because the brains from the different dose groups were sectioned at different intervals after sacrifice (Argus Research Laboratories, Inc., 2001) and post-hoc analysis of the brain sections did reveal some systematic differences in the PND9 animals and in a limited sample of sections examined from the PND21 animals (Harry, 2001). Additional sectioning is being performed by EPA to address whether the anterior to posterior bias selection suggested in the males (Harry, 2001) is a true confounder because normative data for brain measurements at these ages are not available. These new data will be made available to the external peer review panel as soon as possible. Because the analyses conducted without sections from this level still resulted in a significant effect at the 0.01 mg/kg-day dose and the dose-related changes noted in this study have not been noted in other studies with tissue sampler treated similarly (Garman, 2001c), this concern is somewhat mitigated. Certainly to be protective of public health, these effects should be viewed as adverse until additional data either confirm or contradict that conclusion.

In summary, two different analyses of the brain morphometry data from the 2001 “Effects Study” (Argus Research Laboratories, Inc., 2001) yielded significant effects (i.e., alteration of brain structures) of developmental exposure to ammonium perchlorate in drinking water at doses of 0.01 mg/kg-day and higher in a mammalian (rat) model of neurodevelopment. These alterations included a 23-39% increase in the size of the corpus callosum over controls in the progeny of dams dosed with 0.01 to 1.0 mg/kg of ammonium perchlorate in drinking water.

Alteration of brain structures in a laboratory animal model is considered to be an adverse neurotoxic effect (U.S. Environmental Protection Agency, 1998b). One of the analyses used a series of t-tests; the other a more conservative multivariate analysis employing a nested model profile analysis followed by univariate analysis of specific brain regions. The latter method is more likely to be considered a valid analytic method because it better incorporates the design elements of the study and reduces the likelihood of Type I statistical error. These effects on brain morphometry dictate a designation of 0.01 mg/kg-day as a LOAEL.

5.4 DEVELOPMENTAL STUDIES

The 1997 testing strategy included a developmental study in rabbits to evaluate both a potential critical effect and to characterize the toxic effects of perchlorate in a species other than rats. Testing guidance for developmental toxicity typically requires data in two different species. A new study of developmental toxicity in rats was recommended at the 1999 external peer review. This section reviews the historical data on the developmental effects of perchlorate (5.4.1), the 1998 study in rabbits (5.4.2), and the new 2000 study in rats (5.4.3).

5.4.1 Historical Studies

Brown-Grant (1966) examined the effects of perchlorate on implantation and pregnancy outcome in Wistar rats. Potassium perchlorate or potassium chloride (control) was administered at 1.0% (w/v) in drinking water from GD2 through GD8. The daily calculated intake rates were 237 and 371 mg/rat for potassium perchlorate and potassium chloride, respectively. Rats were administered methythiouracil 45 min before injection of 5 μCi sodium radioiodide ($^{131}I^-$) and sacrificed 2 h later. Rats clearly not pregnant were sacrificed on Day 20; whereas, pregnant rats were allowed to deliver prior to sacrifice. Pregnancy was successful in 7/11 control rats and 8/11 perchlorate-treated rats. Among nonpregnant animals, implantation sites were not found. Litter size, number of pups, and pregnancy were not affected.

In the same study, false pregnancy was induced by mating females with vasectomized males. Females were dosed as before on GD2 through GD8 to 0.25 or 1.0% potassium perchlorate or potassium chloride (control). These doses correspond to 63 and 246 mg potassium

perchlorate/rat and 82 and 308 mg potassium chloride per rat, respectively. Deciduoma formation was induced through traumatizing one uterine horn while under anesthesia. Rats exposed to the 0.25% dose were traumatized on GD3 and sacrificed on GD7. Trauma and sacrifice occurred on GD4 and GD8, respectively, in the 1.0%-dose group. Methylthiouracil and sodium radioiodide ($^{131}I^-$) were administered prior to sacrifice as before. Deciduoma formation was not different between dosed and control rats. Thyroid weights were increased significantly in the rats of the 1.0% potassium perchlorate-dose group.

A related study was performed by Brown-Grant and Sherwood (1971). Wistar rats were mated shortly post-partum, and the present litter was culled to nine. The dams were then administered 0.1% potassium iodide or 1.0% potassium chloride, potassium perchlorate, or potassium iodide in the drinking water until sacrifice. The average daily intake of potassium perchlorate and potassium chloride was 615 and 655 mg/rat, respectively; calculated daily doses were approximately 2,440 and 2,660 mg/kg body weight. The litters were sacrificed on GD9 or GD10. The dams then were sacrificed on GD12 or GD13, allowing time for the new blastocysts to implant. Potassium perchlorate again did not affect blastocyst ability to survive prior to implantation or implantation rate after lactation ceased. Relative thyroid weights of the dams and litters were increased significantly compared with potassium-chloride-dosed controls. The high dose of potassium iodide (average daily intake of 234 mg/rat [approximately 1,150 mg/kg]) was maternally toxic.

All dams were sacrificed on Day 12 or 13 and examined for the number of implantation sites. There was 100% incidence of dams with implantation sites for all groups except the perchlorate-treated group in which only 70% of the dams had implantation sites. The number of implantation sites per dam was comparable for all groups. Thyroid weights in the perchlorate-treated dams appeared to be increased compared with the chloride- or iodide-treated dams. Also, thyroid weights of the offspring of perchlorate-treated dams were increased compared with offspring from iodide-treated dams. The authors concluded that treatment with potassium perchlorate had no significant effect on blastocyst survival or the ability to implant under conditions delaying implantation (i.e., concurrent lactation).

Postel (1957) reported administration of 1% potassium perchlorate in drinking water to pregnant guinea pigs (n=16) and a control group (n = 3) receiving a diet of 0.48 μg iodine per gram. Dosing with perchlorate during GD21 through GD48 produced enlarged thyroids in the

fetuses compared to the thyroids of control fetuses. In contrast, perchlorate treatment did not have any effect on the thyroids in dams. Enlarged fetal thyroids also occurred when perchlorate treatment was accompanied by daily subcutaneous treatment with T3 doses as high as 32 μg/kg/day. From water intake and body weight data, the author calculated an average daily dose to the dams of 740 mg/kg-day. The fetuses were not examined for other developmental effects. This study suggested a free-standing LOAEL of 740 mg/kg-day for fetal thyroid enlargement because no other doses were tested. In a separate experiment to test effects on adult guinea pigs, 0 or 1% potassium perchlorate was administered to nonpregnant female guinea pigs for 30, 60, or 90 days. Thyroid enlargement and hyperplasia were apparent in treated animals after 60 or 90 days of treatment.

Similar results in rabbits were described by Lampe et al. (1967). Dams were dosed with 100 mg potassium perchlorate/kg by weight daily, mixed with feed. Dosing occurred from conception through GD21 or GD28. Maternal thyroid weights in treated animals were three times higher than control thyroids; fetal thyroids were nearly four times the control weights. The number of epithelial cells were increased, and the amount of colloid decreased in treated animals. The relative volume of the stroma, the supporting matrix, was increased because of the reduced follicle sizes. Likewise, maternal thyroids showed decreased luminal size and increased epithelial cells. The authors asserted that these results demonstrated that the placenta is permeable to perchlorate. Because fetal thyroids were more enlarged relative to maternal thyroid glands, the fetal thyroid system is independent of the maternal regulatory system and more sensitive to changes in iodine availability.

5.4.2 Segment II Developmental Toxicity Study in Rabbits

A developmental toxicity study was performed in New Zealand White (Hra:[NZW]SPF) rabbits as part of the overall perchlorate testing strategy (Argus Research Laboratories, Inc., 1998c). This study has also appeared in the literature (York et al., 2001a); however, because that manuscript did not use the PWG review of thyroid histopathology and its conclusions on other endpoints are the same as the contract report, the manuscript will not be discussed further in this document. To aid understanding of terminology and the protocol, a schematic of the study design is provided in Figure A-3 of Appendix A to this document. The study design meets the requirements of the 1998 EPA Office of Pollution Prevention and Toxic Substances (OPPTS)

870.3700 guideline. A deviation from the use of double staining was noted in Appendix D of the Argus report, but EPA determined that this should not have had an effect on the overall outcome of this study.

The dose groups tested were 0, 0.1, 1.0, 10, 30, and 100 mg/kg-day of ammonium perchlorate in RO water provided by continual access on presumed GD6 to GD28. Each group was comprised of 25 time-mated does assigned on a randomized basis stratified by weight. Doses were selected on the basis of a dose range-finding study (Argus Research Laboratories, Inc., 1998d) in which thyroid histopathology was evident in the does at 20, 50, and 100 mg/kg-day; thyroid hormone levels (T3, T4, and TSH) in the does were reduced at all doses; and three malformed fetuses from three litters in the 20-mg/kg-day group were observed upon gross external examination. EPA was concerned about these pilot study results, particularly because the original target doses of 0.1 and 10 mg/kg-day were changed on GD13 to 50 and 100 mg/kg-day based on the lack of clinical toxicity at these doses. The fact that these were the doses at which effects were observed, together with the fact that a low number of animals (n = 5) was used in this range-finding study caused EPA to counsel the sponsor (PSG) to examine an expanded range of doses in the definitive study. The dose groups chosen for the definitive developmental study were thus aimed to bracket the dose levels in the range-finding study and to go below the doses causing thyroid hormone perturbations and above those associated with the fetal malformations.

Dosing solutions of ammonium perchlorate were prepared at least weekly from stock solution, and the results of the concentration analyses were within acceptable ranges. Stability of solutions was assumed based on determinations by AFRL/HEST for the 90-day bioassay as discussed in Section 5.2.3. Rabbits were observed for viability at least twice daily, and body weight, food and water consumption, clinical observations, deaths, abortions, and premature deliveries were evaluated daily. On GD29, rabbits were terminated and cesarean sections were performed. Blood samples from the does were taken for evaluation of thyroid and pituitary hormones (T3, T4, and TSH). Gross necropsy was performed on the thoracic, abdominal, and pelvic viscera of each doe. Parameters evaluated in the does included pregnancy status, gravid uterine weight, number of corpora lutea in each ovary, number and distribution of implantations, early and late resorptions, and live and dead fetuses. The thyroids/parathyroids were evaluated histologically. Weight, gross external alterations, sex, in situ brain status (in one-half of the

fetuses in each litter), brain histology (in the other one-half of all fetuses in each litter), cavitated organs, and skeletal and cartilaginous alterations were examined in the fetuses. No measurements of thyroid structure or function were made in the fetuses.

5.4.2.1 Results of Maternal Examinations and Thyroid Histopathology

Two does in the 1.0-mg/kg-day group aborted either dead pups or had late resorptions on GD28. Both of these abortions were considered unrelated to treatment because the incidences were not dose-dependent and were consistent with historical control data for rabbits in that laboratory (Argus Research Laboratories, Inc., 1998c; Appendix J). One doe in the 100-mg/kg-day group delivered prematurely on GD27 (normal delivery in rabbits occurs on GD31), but it was assumed that this rabbit had been identified and shipped incorrectly by the supplier because the pups appeared to be full-term (i.e., they had fur and were nursing). There were no treatment-related effects on maternal clinical signs, body weight, body weight change, gravid uterine weight, or food and water consumption. It is interesting to note that there were decreases (not statistically significant) in several of these endpoints, at the 1.0-mg/kg-day group–the same at which the abortions occurred–as did one adverse necropsy observation of a mottled liver. However, none of these responses showed a dose-response with the current treatment regimen, and none were out of the range of normal occurrence.

The only remarkable histopathology in the does was observed in the thyroids. There was an apparent dose-related but not statistically significant decrease in thyroid weight). The histopathology in the dams as reviewed by the PWG can be found in Wolf (2000; 2001, Table 22). There was a clear dose-response for colloid depletion, hypertrophy, and hyperplasia, indicating that another species has conserved the hypothalamic-pituitary-thyroid feedback regulation. All three indices appeared to be significantly increased at 1.0 mg/kg-day and above. Benchmark dose analyses resulted in BMDL estimates of 0.008 for colloid depletion and 0.42 for hyperplasia. A poor fit prevented BMDL estimation for hypertrophy.

5.4.2.2 Developmental Endpoints

There were no treatment-related effects on gross external endpoints (Argus Research Laboratories, Inc.,1998c, Table 16). With regard to soft tissue anomalies (Argus Research Laboratories, Inc.,1998c, Table 17), there were several occurrences of lung lobe and gallbladder

absence, but their incidence was not treatment related. The statistically significant decrease in folded retina was attributed to an artifact of tissue processing. There were no treatment-related effects in skeletal or ossification alterations (Argus Research Laboratories, Inc.,1998c, Tables 18 and 19), and no indication of an increased incidence of the more apical endpoint (i.e., any skeletal change). The fetal NOAEL thus is identified as greater than 100 mg/kg-day for embryo-fetal developmental toxicity, other than that which may have occurred in the thyroid.

5.4.2.3 Maternal Thyroid and Pituitary Hormone Analyses

The thyroid and pituitary hormone (T3, T4, and TSH) analyses were performed by AniLytics, Inc., for the does in the developmental rabbit study (Argus Research Laboratories, Inc.,1998c). Assays for T3 and T4 were performed using RIA kits according to manufacturer's standard procedures. Assay kits from the same batch number and with the same expiration date were used for the T3 and T4 measurements for each rabbit. The TSH assay was a double-antibody, RIA procedure developed for rabbits and performed by AniLytics, Inc. The analyses discussed in the Argus Research Laboratories, Inc. (1998c) report contain data from both pregnant and nonpregnant rabbits, with both groups combined in the analyses. Because of the known effects of pregnancy on thyroid hormones, EPA decided to reanalyze separately the data from the pregnant and nonpregnant animals. However, EPA determined that the analyses for nonpregnant animals were not useful because of the very limited number of subjects per group (final number of does: n = 3, 1, 0, 1, 1, and 1 nonpregnant does/group, and n = 22, 24, 25, 24, and 23 pregnant does/group for the 0.0, 0.1, 1.0, 10, 30, and 100 mg/kg-day groups, respectively). Therefore, EPA conducted reanalyses for these two groups separately (Crofton, 1998h). All data were taken from Appendix I of the report (Argus Research Laboratories, Inc.,1998c). The analyses used the pregnancy status data subsequently submitted (York, 1998e). Data from dependent measures (T3, T4, and TSH) were subjected to separate one-way ANOVA tests with treatment (dose) as the independent between-subjects variable as calculated in Crofton and Marcus (2001) and Marcus (2001). Mean contrasts were performed using Duncan's Multiple Range Test.

The main effect of treatment was not significant for T3. The T3 data are plotted in Figure 5-16A. There was a main effect of treatment and a significant difference between group means for the control versus 1.0, 10, 30, and 100 mg/kg-day groups on T4. These data are

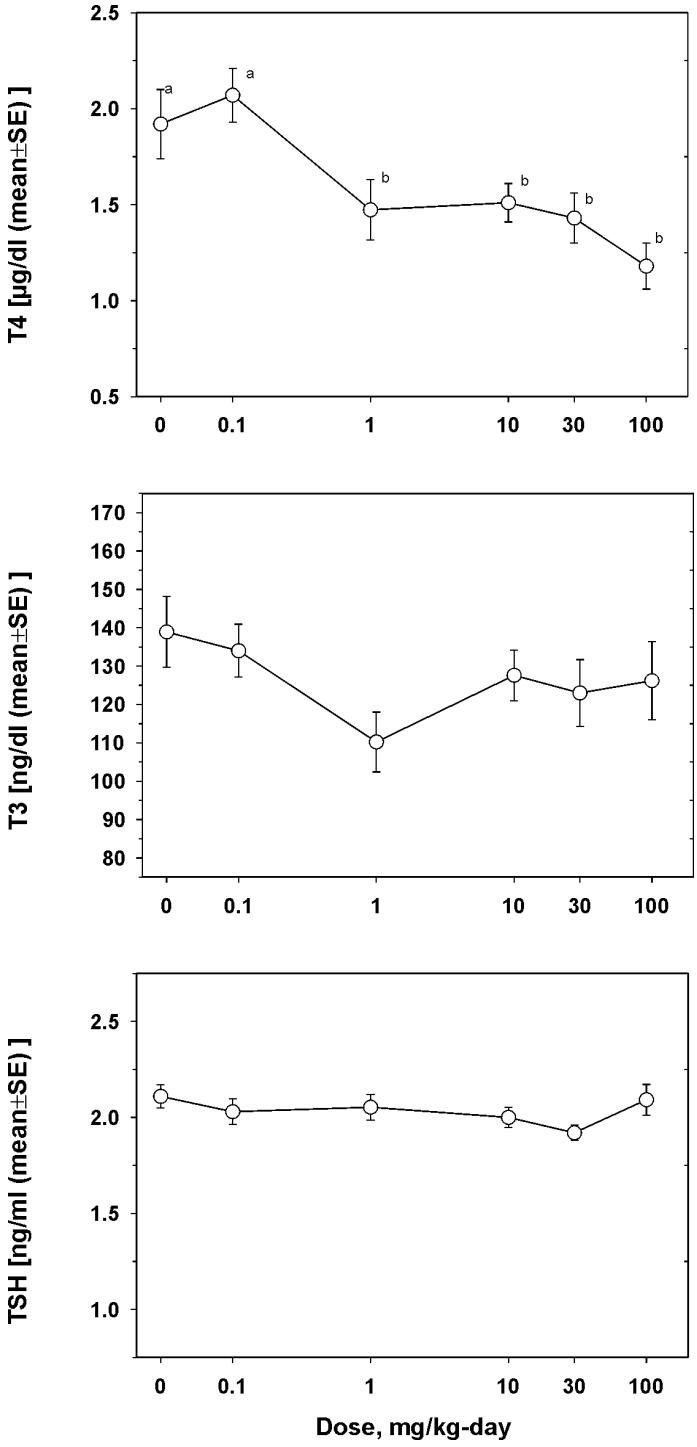

Figure 5-16. Effects from ammonium perchlorate in drinking water administration in pregnant New Zealand rabbits during GD6 to GD28 on T3 (A), T4 (B) and TSH (C) concentrations (ng/dL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Argus Research Laboratories, Inc. (1998c). Means with different letters were significantly different ($p<0.05$). Daily dose was estimated from water consumption data.

plotted in Figure 5-16B. The main effect of treatment was not significant for TSH (Figure 5-16C). Results of these EPA reanalyses are different from those stated in the report. The report (Argus Research Laboratories, Inc., 1998c) states that the NOAEL for T4 was 10 mg/kg-day. The current EPA analyses excluding nonpregnant animals, demonstrate a NOAEL at 0.1 mg/kg-day for T4. There was no statistical significance of any dose on T3 or TSH.

The lack of effect of any dose of perchlorate on T3 and TSH is difficult to explain. One must note that these data are from rabbits (the majority of other data are from rats) and that the data were collected 1 day prior to birth from the maternal compartment (whereas, all other data were collected in adults or from postnatal day time points). In a previous study in guinea pigs (Postel, 1957), enlarged thyroids were found in fetuses; whereas, there was no change in maternal weight or histology. Lampe et al. (1967) demonstrated a larger effect on fetal thyroid weight compared to maternal thyroid weights during late gestational exposure to perchlorate in rabbits. These data warrant caution when comparing effects of perchlorate in the maternal with the fetal/post-natal compartments.

5.4.3 Segment II Developmental Study in Rats

As recommended at the 1999 external peer review, a developmental study in addition to the one in rabbits was performed in rats (Argus Research Laboratories, Inc., 2000). The EPA review (Kimmel, 2000) was first performed on the audited final report (June 2000) and then on clarifications provided by the principal investigator (York, 2000) that do not appear in the final report.

Rats were given continuous access to target dosages of 0.01, 0.1, 1.0, and 30 mg/kg-day ammonium perchlorate in deionized drinking water beginning at least 15 days before cohabitation and continuing through the day of sacrifice. Each dosage group was comprised of 24 females, assigned on a random basis, stratified by weight. There were no maternal deaths. Of these females, 20 were selected for evaluation; of these, 19, 19, 17, 20, and 20 were pregnant in the 0, 0.01, 0.1, 1.0, and 30 mg/kg-day groups. The EPA OPPTS 870.3700 testing guidelines recommend 20 pregnant animals per group at necropsy so that the power of the study to detect an exposure-related response was somewhat lower.

All rats were sacrificed on day 21 of presumed gestation (GD21), and a gross necropsy of the thoracic, abdominal, and pelvic visera was performed. Gravid uterine weights were recorded, and the uterus then excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses, and early and late resorptions. The number of corpora lutea in each ovary was recorded. Placentae were examined for abnormalities (size, color or shape). Each fetus was identified, weighed and examined for sex and gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations. The heads of these fetuses were examined by free-hand sectioning. The remaining fetuses in each litter were examined for skeletal alterations and cartilage development.

5.4.3.1 Results of Maternal Examinations

Three dams in the 30 mg/kg-day group showed an increase in localized alopecia that was statistically significant and was observed over 9-11 days during mid-late gestation. EPA feels that this should be considered biologically significant and exposure-related despite the claim by Argus Research Laboratories, Inc. (2000) and the study director (York, 2000) that such incidence is within the range observed historically at their testing facility.

There were no other maternal parameters that were clearly supportive of exposure-related effects. There was a statistically significant increase in corrected maternal body weight gain over gestation in the 0.1 and 30.0 mg/kg-day groups, and an increase (not statistically significant) in the 1.0 mg/kg-day group. There was also a reduction, again not statistically significant, in gravid uterine weight in three of the four exposure groups. These latter changes may be associated with reduced number of implants in the exposed groups (see below).

5.4.3.2 Developmental Endpoints

The Argus Research Laboratories, Inc. (2000) report (Table B17) did not record preimplantation loss as an endpoint. EPA notes that there is an increase in this parameter over control (12%) at each dose level: 0.01 (18%), 0.1 (20%), 1.0 (16%), and 30.0 (25%) mg/kg-day. Whether this is statistically significant or biologically significant is unclear; although a decrease in live fetuses in three of the four exposure groups that was significant at the highest dosage was reported. Given the reduced power of this study to detect an effect, consideration was paid to this finding. The lack of an effect on live fetuses at the 1.0 mg/kg-day level is not clear, and

these results by themselves are insufficient to establish an effect level below 30 mg/kg-day. EPA recommends that preimplantation loss and embryo/fetal viability should be evaluated in any other study reports on this chemical.

Ossification sites per litter for sternal centers and forelimb phalanges were significantly reduced at 30 mg/kg-day, but Argus Laboratories, Inc. (2000) dismissed them as "reversible developmental delays." EPA disagrees and contends that developmental delays, be they permanent or reversible, are not to be discounted as potential indicators of developmental toxicity. EPA additionally had some concern over the staining technique used for cartilage (Kimmel, 2000) which was not accepted by Argus Research Laboratories, Inc. (York, 2000) as an issue.

5.4.3.3 Conclusions Regarding Developmental Toxicity in Rats

Based on the review of the maternal and fetal data, EPA concludes that there are signs of maternal and developmental toxicity at the 30.0 mg/kg-day level suggesting it as a LOAEL with a NOAEL then at 3.0 mg/kg-day. While none of the results were so clear that a definitive assessment can be made, the suggestive results are important to consider in light of the overall data base and mode of action for the toxicity of perchlorate.

5.5 TWO-GENERATION REPRODUCTIVE TOXICITY STUDY

The 1997 recommendation to characterize the potential perchlorate toxicity on reproductive parameters in a two-generation study was completed in 1999 (Argus Research Laboratories, Inc., 1999). This study has also been reported in the literature (York et al., 2001b), but since that manuscript did not use the PWG review of thyroid histopathology and its conclusions on other endpoints are the same as in the contract report, the manuscript will not be discussed further in this document. A schematic of the study design is provided as Figure A-2 of this document (Appendix A) to aid understanding of terminology and the protocol.

The target doses (30 rats/sex/group) were 0, 0.3, 3.0, and 30 mg/kg-day of ammonium perchlorate in RO water provided by continual access. Concentrations were adjusted based upon actual water consumption and body weights recorded the previous week. Dosing solutions of ammonium perchlorate were prepared weekly, and the results of concentration analyses were

within acceptable ranges (±10%) with one exception in the 3.0-mg/kg-day target group on May 5, 1998 (15.8%). The stock solution was prepared at least once, but the exact number of times was not reported. Stability of solutions was assumed based on determinations by AFRL/HEST for the 90-day bioassay, as discussed in Section 5.2.3.

On arrival, Spraque-Dawley rats were assigned randomly to individual housing, and consecutive order was used to assign the P1 generation rats to cohabitation (one male rat per female rat). The cohabitation period lasted a maximum of 14 days. Females with spermatozoa observed in a vaginal smear or with a copulatory plug observed in situ were considered to be at GD0 and assigned to individual housing. Estrous cycling was evaluated daily by examination of vaginal cytology beginning 21 days before the scheduled cohabitation period and continuing until GD0. The rats were observed for viability at least twice each day of the study and daily for clinical signs. Body weights were recorded weekly during acclimation, on the first day of dosage, weekly thereafter, and at scheduled sacrifice. Feed consumption and water consumption values were recorded at least three times per week. Females were evaluated for duration of gestation (GD0 to the day the first pup was delivered). Day 1 of lactation (LD1, post-partum) was defined as the day of birth and was the first day on which all pups in a litter were weighed individually. Maternal behavior was observed on LD1, 4, 7, 14, and 21. Rats that did not deliver a litter were sacrificed on GD25 and examined for pregnancy status. Each litter was evaluated for litter size (live and dead pups versus live pups only) and pup viability at least twice each day of the 21-day post-partum period, and pups were counted daily. Deviations from expected nursing behavior also were recorded. All F1-generation rats were weaned at the same age based on observed growth and viability at LD21, unless required to be extended to LD28.

At the end of the 21-day post-partum period, all surviving P1 rats were sacrificed. Gross necropsy was performed on all animals, and all gross lesions were examined histologically. Organ weights were obtained for the thyroid, adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, and testes. The thyroids and parathyroids were submitted for histopathological examination. Histopathology of other organs was performed for the control and high-dose groups. Blood was collected for determination of hormone levels (T3, T4, and TSH). Portions of the epididymides were used either for evaluation of sperm count or motility. The left testis was homogenized after weighing for analysis of spermatid concentration (spermatids per gram of tissue).

Pups not selected for continued evaluation in the study also were sacrificed on LD21. Blood was pooled by sex per litter for analysis of T3, T4, and TSH. At least 3 pups/sex/litter were necropsied and examined for gross lesions, including a single cross-section of the head at the level of the frontal-parietal suture and examination of the head for apparent hydrocephaly. Brain, thymus, spleen, and thyroid/parathyroid organ weights were obtained prior to fixation. The adrenal glands, thyroid/parathyroid, kidneys, and liver were retained in formalin.

5.5.1 General Toxicity Results and Evaluation of Reproductive Parameters

There was a statistically significant decrease in water consumption by males, but not by females. The decrease with males and a smaller decrease with females were sufficiently small that they are not considered to be biologically significant (Argus Research Laboratories, Inc., 1999; Tables B5 and B6). There was a significant increase in ovarian weight at the 0.3-mg/kg-day dose level only (Argus Research Laboratories, Inc., 1999; Table C26). There also was slightly increased (not statistically significant) pituitary weight in females at the 0.3- and 3.0-mg/kg-day dose levels.

The fertility results are potentially of concern, but the statistical analyses did not show any significant differences between groups for any of the tested parameters (Argus Research Laboratories, Inc., 1999; Table C21 through C23). However, at 0.3 mg/kg-day, there were four pairs that did not mate compared with one or two pairs in the other groups. Also at 0.3 mg/kg-day, there were three females that showed at least one signal of persistent diestrus and one with persistent estrus (Argus Research Laboratories, Inc., 1999; Table C40). Incidences were lower in all other groups. Only one of those females did not have evidence of mating, but there were also four females that did not have evidence of mating in the 0.3 mg/kg-day group. When mating and conception failures are combined, pregnancy rates were 28/30, 22/30, 26/30, and 24/30 for the 0-, 0.3-, 3.0-, and 30-mg/kg-day groups, respectively. Of the females that were pregnant, litter size was slightly lower at the 3.0- and 30-mg/kg-day dose levels, with values of 15.0, 14.9, 14.1, and 14.0 with increasing dose level. A similar trend was seen in the number of implantation sites (15.8, 15.8, 15.0, and 15.0). None of these results were statistically significant for the P1 generation, and the effect was not seen in the F1 generation. Consequently, this was not considered a significant finding (Clegg, 1999; Rogers, 2000). Note should be made that female intake of perchlorate during the last week of gestation was higher (Argus Research

Laboratories, Inc., 1999; Table C1). Additionally, in many of the perchlorate intake and feed consumption summary data, observations were reported for a low numbers of rats, apparently because of spillage.

In the F1 matings, all three perchlorate-dosed groups had a slightly higher fertility index than did the vehicle controls, but this appears to be due to a control value that was low (Clegg, 1999; Rogers, 2000). These findings, the high dosage level of 30 mg/kg-day is designated as a NOAEL for reproductive parameters (Rogers, 2000), a finding that is consistent with the preliminary evaluation presented by EPA in 1999 (Clegg, 1999).

5.5.2 Evaluation of Thyroid Histology

The histopathology from the completed Argus Laboratories, Inc. (1999) two-generation reproductive study was limited to the thyroid gland and can be found in Wolf (2001; Tables 14 through 21). In addition to the precursor lesion data (colloid depletion, hypertrophy, hyperplasia) discussed in Section 5.5.2.1, Wolf (2000) noted that two animals from the high dose group (30 mg/kg-day) in the F1 generation (second parental generation, P2) in the study had adenomas and one of these animals had two adenomas for a total of three. Although statistically significant decreases in colloid were reported at both the 3.0 and 30.0 mg/kg-day dose levels (Argus, 1999), none of the rats in the other groups (0, 0.3, 3.0 mg/kg-day) developed thyroid follicular cell adenomas (0/30, 0/30, 0/30, respectively). These animals were dosed from conception to 19 weeks of age (adult male F1 rats). The tumors were considered to be treatment related (Wolf, 2000). Compared to the background incidence of thyroid follicular cell adenomas in male F344 rats after 2 years on study at 38/3419 from 67 NTP studies or 1.1% incidence at the 2-year end sacrifice date, this study showed an incidence of 2/30 or 6.7% at 19 weeks. The tumors that occurred in the F1 generation male rat pups at 19 weeks were considered particularly remarkable (Wolf, 2000), and the EPA asked the NIEHS to review this incidence in context with the data from the National Testing Program (NTP). The finding is especially of concern since three of the F1 males in this high dosage group died of unknown causes (Rogers, 2000). This NIEHS analysis of the tumor incidence is described below (Dunson, 2001b) in Section 5.5.2.2.

5.5.2.1 Thyroid Weight, Colloid Depletion, Hypertrophy, and Hyperplasia

Absolute thyroid weight was increased significantly in the P1 males at the 3.0- and 30-mg/kg-day dose levels. An increase was significant in females at 30 mg/kg-day. A significant increase in thyroid weight relative to both body weight and brain weight also occurred at 30 mg in both sexes (Argus Research Laboratories, Inc., 1999; Tables B11 through B13 and C26 through C28). The histopathology for the P1 generation as reported by the PWG can be found in Wolf (2000; 2001, Tables 14 and 15). All three indices (colloid depletion, hypertrophy, and hyperplasia) were present with a clear suggestion of an increase in females for colloid depletion and hypertrophy at 3 and 30 mg/kg-day that supported the thyroid weight changes. Hyperplasia was more prominent at 30 mg/kg-day. Benchmark dose analyses using the male and female data for the P1 generation combined (Table 5-1; Geller, 2001a) result in BMDL estimates of 0.11 mg/kg-day for colloid depletion and 2.44 mg/kg-day for hyperplasia. The data for hypertrophy resulted in inadequate model fit.

The F1-generation (second parental, P2 generation) rats also exhibited all three thyroid histopathological indices in a dose-related fashion with 3 and 30 mg/kg-day as effect levels (Wolf, 2000; 2001, Tables 16 and 17). Benchmark dose analyses (Table 5-1; Geller, 2001a) using the male and female data combined for the P2 generation estimate 0.90, 0.15, and 0.0004 mg/kg-day as the BMDL for colloid depletion, hypertrophy, and hyperplasia. Of note is the dramatic overlap between colloid depletion and hypertrophy in this generation. It was the males in these rats, exposed in utero and then sacrificed at 19 weeks, that showed the 3 adenomas.

The F1-generation weanling rat data are presented in Tables 18 and 19 (Wolf, 2000; 2001) and also exhibit the three thyroid histopathology indices increased at 3 and 30 mg/kg-day. Benchmark dose analyses (Table 5-1; Geller, 2001a) using the male and female data combined result in BMDL estimates of 0.80, 0.057, and 0.66 mg/kg-day for colloid depletion, hypertrophy, and hyperplasia. Again, the overlap among indices is present.

Data for the second weanling generation (F2) rats are presented in Wolf (2000, 2001; Tables 20 and 21). Decreased colloid and hypertrophy remain increased at 3 and 30 mg/kg-day, but hyperplasia was not remarkable. Benchmark dose analyses (Table 5-1; Geller, 2001a) only provided adequate fit to the hypertrophy data and resulted in a BMDL of 0.32 mg/kg-day.

Across the generations, this study results in a range of BMDL estimates (mg/kg-day) for colloid depletion of 0.11 to 0.90, for hypertrophy of 0.057 to 0.32, and for hyperplasia of 0.0004 to 2.44. Of note is the low BMDL value for hyperplasia (0.0004 mg/kg-day) in the P2 generation, the same animals that exhibited tumors.

5.5.2.2 Bayesian Analysis of Tumor Incidence

In order to properly interpret the results from a given toxicological study, it is often necessary to consider the data in light of additional information from outside of the study such as the variability and average level of response for positive and negative controls in past studies that are similar to the current study. It is also necessary to account for confounding effects that an exposure may have on variables that are associated with the outcome of interest. For example, it is important to adjust for animal survival to avoid bias in analyses of animal tumorigenicity (McKnight and Crowley, 1984) and reproductive toxicity (Dunson and Perreault, 2001). Typically, expert knowledge and information from related studies are accounted for only informally in the interpretation of a statistically significant or non-significant result. However, there are clear advantages to formally incorporating such extra information into the statistical analysis because it can be very difficult to interpret statistical significance when some aspect of the data is inconsistent with outside information (e.g., the control response is higher or lower than typically seen in related studies). In addition, the formal incorporation of outside information can improve sensitivity and limit bias when assessing toxicological effects. The advantages of including historical control data, in particular, has been well documented in the toxicological and statistical literature (Dunson and Dinse, 2001; Haseman, Huff, and Boorman, 1984; Ibrahim, Ryan and Chen, 1998; Tarone, 1982).

Although frequentist (i.e., non-Bayesian) hypothesis tests can sometimes incorporate historical control data (see, for example, Tarone, 1982), outside information can be incorporated more naturally and flexibly within a Bayesian analysis. In Bayesian analyses, the unknown parameters in a statistical model are assigned prior probability distributions quantifying uncertainty prior to observing data from a current study. For example, based on experience with an assay system, a toxicologist may be 95% certain that the average level of response among vehicle control animals is between bounds A and B with C being the most likely value. This information can be formally incorporated into a Bayesian analysis through a prior distribution,

for a parameter measuring expected control response, which is centered on *C* and assigns 95% probability to values between *A* and *B*. Alternatively, the prior distribution can be estimated using data or summary statistics for control animals in historical studies if such information is available (Ibrahim, Ryan and Chen, 1998; Dunson and Dinse, 2001). For parameters about which little is known, noninformative or vague prior distributions that assign equal prior probability to a wide range of plausible values can be chosen.

Bayesian inferences about toxicological effects can be based on the posterior distribution for the parameters in the statistical model. The posterior distribution, which quantifies the current state of knowledge about the unknown quantities in the statistical model, is obtained by updating the prior distribution with the information in the data from the current study using Bayes theorem (refer to Gelman et al., 1995 for an overview). One can use the posterior distribution as a basis for conclusions about effects of interest by using posterior means, 95% credible intervals, and posterior probabilities as Bayesian alternatives to the maximum likelihood estimates, 95% confidence intervals, and p-values used in frequentist analyses. For example, as an alternative to a p-value, one could calculate the posterior probability of an increase in the proportion of animals with an adverse response in a treated group relative to the control. Bayesian approaches have been developed for a wide variety of toxicological applications, including risk assessment (e.g., Hill, 1996; Hasselblad and Jarabek, 1996), toxicokinetic modeling (e.g., Bernillon and Bois, 2000), and analysis of skin papilloma data (Dunson et al., 2000).

Without incorporating historical data on spontaneous neoplasms in Sprague-Dawley rats, the difference between 0/30 in the vehicle control and 2/30 in the 30 mg/kg-day group is non-significant by standard tests (e.g., Fisher's exact). However, the reported historical control incidence of thyroid follicular adenomas for male Sprague-Dawley rats in two-year studies is approximately 3-4% (Chandra et al., 1992; McMartin et al., 1992), suggesting that these tumors should be extremely rare among 19-week old animals in the absence of a treatment effect. Without formally incorporating this historical information into the statistical analysis through a prior distribution, it is very difficult to assess the weight of evidence in favor of a treatment-related increase in thyroid follicular adenoma incidence. A Bayesian approach was used to assess the effect of ammonium perchlorate in drinking water on thyroid follicular cell adenoma

incidence in male Sprague-Dawley rats from the two-generation study (Argus Research Laboratories, Inc., 1999).

5.5.2.2.1 Choosing prior distributions based on historical controls

The proportion of control male Sprague-Dawley rats developing thyroid follicular cell adenomas in two-year carcinogenicity studies has been reported in the literature. Chandra et al. (1992) reported a rate of 48/1340 (3.6%), and McMartin et al. (1992) reported a rate of 23/583 (3.9%). In order to incorporate this historical control data into our analysis of the effect of ammonium perchlorate on thyroid incidence at 19 weeks of age, we follow a Bayesian approach. The historical data can be summarized using a Beta (71,1852) prior distribution for the probability of a male Sprague-Dawley rat developing a thyroid follicular cell adenoma (in the absence of treatment with a test agent) by the time of natural death or sacrifice at two years. The Beta prior is the standard choice for a prior distribution on a probability (c.f., Dunson and Tindall, 2000 and Gelman et al., 1996 for further discussion of the Beta prior). The values 71 and 1923 are simply the numbers of control male Sprague-Dawley that did and that did not develop thyroid follicular cell adenomas, respectively, from the Chandra et al. (1992) and McMartin et al. (1992) articles.

To account for the fact that the Argus (1999) study recorded thyroid incidence at 19 weeks and not at the time of natural death or at sacrifice at two years, a prior distribution for the ratio of the probability of thyroid follicular cell adenomas at 19 weeks to the lifetime probability in a two-year study was chosen. Portier, Hedges, and Hoel (1986) suggest that the probability of a control male Fischer 344/N rat developing a thyroid follicular cell adenoma increases approximately in proportion to age$^{4.78}$. Based on this estimate and on the average survival time for male Fischer 344/N rats in the NTP historical control database (95.2 weeks), the prior expectation for the ratio is $(19/95.2)^{4.78}$ = 5e-04. Allowing for a high degree of uncertainty in this prior expectation due to uncertainty in the Portier, Hedges, and Hoel (1986) estimate and in extrapolation from Fischer 344/N rats to Sprague-Dawley rats, a Beta (0.11, 2.6) for the ratio was chosen. This prior has median 5e-04 and 95% interval (0,0.379).

5.5.2.2.2 Results of NIEHS analysis

Using the prior described in the previous subsection and "updating" the prior with control data from the Argus study (i.e., 0 tumors out of 30 control male rats), the NIEHS analysis estimated that a control rat has a 0.15% chance of developing a thyroid follicular cell adenoma by 19 weeks (Dunson, 2001b). In addition, had perchlorate had no effect on the incidence of thyroid follicular cell adenomas, the probability of observing two or more rats with these tumors out of 30 would be approximately 0.005. Thus, the data strongly support the hypothesis that ammonium perchlorate in the drinking water at 30 mg/kg-day causes an increase in the incidence of thyroid follicular cell adenomas.

5.5.2.2.3 Summary of NIEHS analysis of tumor data

Incorporating historical control data in a Bayesian analysis, a significant increase in thyroid follicular cell adenoma incidence at 19 weeks in male Sprague-Dawley rats exposed to 30 mg/kg-day relative to controls was found (Dunson, 2001b). There was no evidence of an increase at low dose levels. This finding raises concern for in utero imprinting (i.e., that pups exposed in utero are subsequently more susceptible to thyroid hormone perturbation during post-natal development and adulthood), a phenomenon that is now appreciated in the endocrine disruption arena (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997).

5.5.3 Thyroid and Pituitary Hormone Analyses

Thyroid and pituitary hormones were assayed in the P1-generation (both sexes), the F1-generation adults, the F1-generation pups (PND21) and the F2-generation pups.

In the P1-generation, there was an unexpected and unexplained increase in T3 levels. Effects on T4 and TSH were as expected, with a significant decrease in T4 and increase in TSH at the 30 mg/kg-day level.

An anomalous increase in T3 was also reported in the F1-generation adults. Significant ($p \leq 0.01$) decreases in T4 of the F1-generation adult males occurred at the high dosage but increases ($p \leq 0.05$) at the mid-doses are unexplained; TSH in the adult males was significantly increased ($p \leq 0.01$) at the 30 mg/kg-day level. Similar results were reported for the F1-generation adult females.

In the F1-generation pups, the only statistically significant effects was an unexpected decrease in TSH at the mid doses in the males and an increase in the females at the lowest. Similarly seemingly spurious results were observed for the F2-generation pups.

Geller (1999b) presented the EPA analysis of thyroid hormones for this study for the P1-and F1-generation using separate repeated-measures ANOVAs with treatment as the independent between-subjects variable and sex as a within-litter repeated-measures variable. Mean contrasts were performed using Tukey's Studentized Range (HSD) test. In order to correct for multiple comparisons, the alpha for significance (for all interaction main effect tests) was adjusted to 0.029 (alpha of 0.05 divided by the square root of the number of ANOVA tests).

In the P1-generation rats, there was a significant dose effect and dose by sex interaction for both T4 and TSH. A NOAEL was identified for males only for T4 and TSH at a dose of 3.0 mg/kg-day.

In the F1-generation (weanling pups) on PND21, the contract laboratories reported a decrease in TSH and an increase in T4. This effect was discounted by Argus Research Laboratories, Inc. (1999) because the decrease was not dosage-dependent and because TSH would be expected to increase and T4 to decrease. EPA found similar results with its analyses, noting that the significant dose effect on female T4 data was due to an elevated level in the 0.3 mg/kg-day group relative to the high dose and also noting that the results were inconsistent with the mode of action for perchlorate (Geller, 1999b).

A significant increase in TSH was found in the adult F1 (P2 generation) rats at 30 mg/kg-day; a finding consistent with the tumors observed at this dosage, but T4 and T3 appeared to have increased in a dose-dependent fashion. Again the reason for this disparity is not clear.

5.6 IMMUNOTOXICITY STUDIES

As discussed in Chapter 3, immunotoxicity studies were included in the perchlorate testing strategy due to indications in humans and laboratory animals that perchlorate may affect immune and hematological function. For example, a study by Weetman et al. (1984) that appeared as a Letter to the Editor in *The Lancet*, investigated the effect of potassium perchlorate on human T- and B-cell responses to mitogens *in vitro*. Perchlorate at concentrations of 0, 0.01, 0.1, and 10 mmol/L (1.17 g/L) were tested in cultures of human peripheral blood lymphocytes. IgG and

IgM in culture superhatants were measured by ELISA after culture for 10 days with pokeweed mitogen (PWM). Perchlorate at 0.1 to 10 mmol/L resulted in inhibition of PWM-induced LgG production at 10 mmol perchlorate/L inhibited IgM production. Lymphocyte proliferation as measured by ^{3}H-thymidine incorporation was reduced by 33 to 35% in cultures from five of six individuals in the presence of the T-lymphocyte mitogen phytohemagglutinin (PHA). Weetman et al. (1984) concluded that perchlorate had significant immunosuppressive activity on lymphocytes at pharmacologically-relevant concentrations in the absence of cytotoxicity, the latter of which was assessed by ethidium bromide/acridine orange fluorescence. Unfortunately, no details were provided as to when viability was determined during the 10 days of lymphocyte culture with perchlorate and PWM. While these and other results were not sufficient to infer that perchlorate was immunosuppressive or had other immunotoxic effects, there was uncertainty with respect to its potential to do so. It was not known whether this could be a direct effect of perchlorate but could plausibly also be due to its anti-thyroid effects.

An array of 14- and 90-day experiments, to evaluate the effects of drinking water administration of ammonium perchlorate on immunotoxicological and hematological parameters were performed using female B6C3F1 (Keil et al., 1998; Kiel et al., 1999; BRT-Burleson Research Technologies, Inc., 2000a,b,c,) or CBA/J Hsd mice (BRT-Burleson Research Technologies, Inc., 2000a,b,c). Parameters also were evaluated 30 days after one 90-day study to assess the reversibility on several observed effects. The mouse was chosen for these studies because it is the typical experimental species for immunotoxicological studies. In addition, data were collected on thyroid and pituitary hormones and thyroid histology to provide additional insight on interspecies variability by comparison with results of the rabbit and rat studies included in the testing strategy. The mice (8 to 10 weeks of age) were acclimated for 1 week prior to initiation of any study. Ammonium perchlorate was obtained from the sponsor (AFRL/HEST), and different lots were used for each of the major study groups (i.e., Keil, et al., 1998; Keil et al., 1999; BRT-Burleson Research Technologies, Inc., 2000a,b,c,). Primary stock solutions were prepared approximately every 1 to 2 months, and dosing solutions were prepared weekly. In the Keil et al. (1998) studies, there was an indication of a trend that mice exposed at the 30 mg/kg-day level consumed slightly less water on a weekly basis (≈3 mL/week less than control). Consequently, differences were noted in the actual exposure for the high-dose group in the 14-day studies. This difference was not as marked in the 90-day studies. Concentration of

dosing solutions was verified by the sponsor (AFRL/HEST; data not shown). The one apparent disparity in dose level (0.1 mg/kg-day; experiment not specified) was rectified after reexamination of calculations (data not shown) (Keil et al., 1998). The mice were exposed to levels of 0, 0.1, 1.0, 3.0, or 30 mg/kg-day in the Keil et al., (1998, 1999) studies; while in the BRT-Burleson Research Technologies, Inc. (2000a,b,c) studies, the mice were exposed to levels of 0, 0.02, 0.06, 0.2, 2.0 or 50 mg/kg-day. The doses were established based on the mean body weight for each treatment group per week. Each dose group generally consisted of 6 to 10 mice, with the exception that some control groups in the BRT-Burleson Research Technologies, Inc. (2000a,b,c) studies had a group size of 20.

A number of 14-day experiments were conducted. In Experiments "C", "G", "I", "J", "T", and "K" (Keil et al., 1998), the mice were sacrificed at Day 14; and body weight, organ weight and cellularity (thymus, spleen, liver, and kidney), a number of immunotoxicology and hematological parameters, thyroid histology, and thyroid and pituitary hormone levels were measured. These data are summarized in Tables 3, 6, 9, 12, 14, 16, 18, and 21 of the "Final Report" (Keil et al., 1999). In Experiments "U" and "V" (Keil et al., 1998), mice were challenged with sublethal amounts (2,300 or 2,700 colony-forming units [CFU]) of *Listeria monocytogenes* on Day 7 and then sacrificed on Day 14. The spleens were removed for a delayed-type hypersensitivity (DTH) assay (Keil et al. 1999: Table 31). In experiments "H", "F", and "M" (Keil et al., 1998), mice were challenged with P815 tumor cells by ip injection. At the 14-day terminal sacrifice, spleens were removed for the cytotoxic T lymphocyte (CTL) activity assay (Keil et al., 1999: Table 23).

A series of 90-day experiments also were conducted. In Experiments "A", "D", and "N" (Keil et al., 1998), mice were sacrificed after 90 days; and body weight, organ weight and cellularity (bone marrow, thymus, spleen, liver, and kidney), a number of immunotoxicology and hematological parameters, thyroid histology, and thyroid and pituitary hormone levels were measured (Keil et al., 1999: Tables 4, 7, 10, 13, 15, 17, 19, 20, and 22). In Experiments "B" and "E" (Keil et al., 1998), these same endpoints were measured after a 30-day recovery period (Keil et al., 1999: Tables 5, 8, 11, and 22,). In Experiment "P" (Keil et al., 1998), mice were challenged with P815 tumor cells by ip injection on Day 76. Spleens were removed at terminal sacrifice for the CTL activity assay (Keil et al., 1999: Table 24).

Two host resistance models, one a bacteria and the other a tumor, were used in 90-day experiments. Mice in Experiment "L" (Keil et al., 1998) were challenged with *Listeria monocytogenes* by iv injection. At terminal (90-day) sacrifice, spleens and livers were removed and cultured for *L. monocytogenes* growth. Unfortunately, the challenge concentration (i.e., 5360 CFU) of bacteria used was excessive, thereby prohibiting enumeration of the bacteria in the spleens of these mice. A second 90-day *L. monocytogenes*-challenge experiment (Keil et al., 1999) was subsequently undertaken using a lower (i.e., 2700 CFU) challenge concentration (see Keil et al., 1999: Table 34). For the tumor model, in Experiments "Q" and "O" (Keil et al., 1998), mice were challenged with B16F10 tumor cells by iv injection on Day 76. At the 90-day sacrifice, the lungs were removed, and the number of tumor nodules in both lungs were enumerated (Keil et al. 1999: Table 33).

The IgM and IgG antibody responses to sheep red blood cells (SRBCs) of mice exposed to ammonium perchlorate for 90 days and the IgM anti-SRBC response of mice exposed for 14 days was determined using an enzyme linked immunosorbent assay (ELISA) (figures on page 59, Keil et al., 1999). Based on EPA comments in 1998 and external peer review recommendation (Research Triangle Institute, 1999), a second contract was let to determine the antibody response to SRBCs using the more traditional antibody plaque-forming cell (PFC) assay (BRT-Burleson Research Technologies, Inc., 2000a,b,c). Unlike the ELISA, which measures SRBC-specific IgM antibody in serum, the PFC assay quantifies the number of plasma cells in the spleen which produce SRBC-specific IgM. The potent immunosuppressant cyclophosphamide (CP) was used as a positive control in these latter studies. In both the 14- and 90-day studies, mice were immunized iv with SRBCs 4 days prior to assay. The positive control mice were injected ip with 15 mg/kg-day CP on the last 4 days of dosing prior to assay.

Concern about potential effects of ammonium perchlorate on contact hypersensitivity, also raised at the 1999 external peer review, were addressed in studies performed by Burleson et al. (2000). Eight-week-old female CBA/J Hsd mice that had been acclimated one week prior to dosing were exposed to 0, 0.02, 0.06, 0.2, 2.0, or 50.0 mg/kg-day for 14 or 90 days. The contact sensitizer, 2,4-dinitrochlorobenzene (DNCB), was applied to the surface of both ears on days 9, 10 and 11 in the 14-day study, and on days 92, 93, and 94 in the 90-day study. Mice were assayed using the local lymph node assay (LLNA) on day 14 and 97 for the 14-day and 90-day

studies respectively. A CP-positive control group was included in each study, with administration of 15 mg/kg-day CP ip for 5 consecutive days prior to assay.

Data from the Keil et al. (1998, 1999) studies were analyzed as follows. Initially, analysis of variance was performed using Tukey's multicomparison ($p < 0.05$) for the various parameters measured. A Fisher's multicomparison test was used in previous interim reports but not in the final one. The previous analyses reported effects. Consequent to criticisms of the analyses performed, as stated in the previous external review Draft Toxicological Review Document on Perchlorate (U.S. Environmental Protection Agency, 1998d), and reinforced by the comments of Dr. Kimber White at the previous external peer review (Research Triangle Institute, 1999), these and new data (i.e., the 14-day antibody response to SRBCs and the 90-day host resistance to *L. monocytogenes*) were analyzed as indicated in the "Final Report" (Keil et al., 1999). That is, data were combined from two or three experiments and evaluated by the Kolmogorv-Smirnov test for normality and Bartlett's test for homogeneity of variance. If data displayed a normal distribution and equal variance, two-way ANOVA, with experiments and treatments as factors, was performed. Tukey's pairwise comparison was performed to determine differences ($p<0.05$) between control and treatments if no interaction was identified due to combining multiple experiments. If a significant interaction was identified in the ANOVA, data from each experiment were analyzed using one-way ANOVA and Tukey's pair wise analysis. The Kruskal-Wallis test was used if data were not normally distributed or variances were not equal; and if significant, the Mann-Whitney test was employed to determine differences ($p<0.05$) between control and treatments.

The results of the BRT-Burleson Research Technologies, Inc. (2000a,b,c) studies were analyzed as follows. Data from each treatment group were compared by first performing a Bartlett's Chi-Square test for variance of homogeneity. If found to be non-significant, ANOVA was employed using dose. If significant, then Dunnett's *t*-test was performed, with $p<0.05$ being significant. On the other hand, if Bartlett's Chi-square was significant, the non-parametric Kruskal-Wallis test was performed, which if significant was followed by a Jonckheere's-Terpera test for dose-dependent trends. The parametric ANOVA and the non-parametric extended Cochran-Mantel-Haenszel test were performed to determine whether the data could be pooled.

Results for the general toxicity and organ weight measures will be discussed in Section 5.6.1. Thyroid histopathology evaluations will be reported in Section 5.6.2, and analyses

of T3, T4, and TSH in Section 5.6.3. Results for the immunotoxicological and hematological parameters are discussed in Sections 5.6.4 and 5.6.5. A summary of the results and their potential significance is presented in Section 5.6.6.

5.6.1 Results for General Toxicity, Organ Weight, and Cellularity Measures

There were no effects observed on body, thymus, spleen, liver, or kidney weights in the 14-, 90-, or 120-day studies (Keil et al., 1999: Tables 6-8). Earlier interim reports indicated considerable variability in the splenic and thymic cellularity of ammonium perchlorate-exposed mice. This variability was due, in large part, to technical errors. Recognizing this, the contractor performed additional studies (i.e., "on at least two or more occasions") in which "no significant changes in cellularity were observed." (Keil et al., 1999). As such, in the "Final Report" no consistent alteration in splenic or thymic cellularity was observed in the 14–, 90-, or 120-day studies (Keil et al., 1999: Tables 9-11), nor in splenic lymphocyte CD4/CD8 subsets (Keil et al., 1999: Tables 14 and 15). With the exception that CD4-CD8+ thymic lymphocytes were increased in mice exposed to 0.1- and 1.0-mg/kg-day doses in the 14-day experiment, there were no other alterations in thymocyte subsets observed in the 14- or 90-day studies (Keil et al., 1999: Table 12). Furthermore, there were no alterations in the number of peritoneal macrophages obtained from mice exposed to any doses of ammonium perchlorate in the 14-, 90-, and 120-day studies (Keil et al., 1999: Tables 9-11), nor in bone marrow cellularity in the 14- and 90-day studies (Keil et al., 1999: Tables 9 and 10). Due to the absence of effects in the latter studies, no 120-day study was performed.

5.6.2 Evaluation of Thyroid Histology

Thyroid histopathology evaluation was performed for two experiments (A and D) in the Keil et al. (1998) study and eventually published in the final report (Keil et al., 1999). These data were transmitted by Warren (1999), and a preliminary review by EPA was presented at the 1999 external peer review (Jarabek, 1999). The materials were provided to the PWG review, and the results are found in Wolf (2000, 2001; Table 23). These results corroborate the preliminary analyses that showed decreased colloid, follicular hypertrophy and hyperplasia to occur at the 30 mg/kg-day dose. Congestion in the intrafollicular capillaries and the nuclear to cytoplasmic

ratio of the follicular cells were not recorded by the PWG but were both noted in the Warren (1999) report at 30 mg/kg-day (Jarabek, 1999). Hypertrophy was additionally observed in the lower doses of experiment "A", and the reason for the disparity between the two studies is unclear. These results support the assertion that the hypothalamic-pituitary-thyroid feedback regulatory mechanism is conserved across species (rats, rabbits, mice and humans) and suggest a NOAEL of 3 mg/kg-day in this strain of mouse.

5.6.3 Thyroid and Pituitary Hormone Analyses

The report (Keil et al., 1998) contains thyroid hormone and thyrotrophin (TSH) data from 14- and 90-day exposures to ammonium perchlorate in B6C3F1 mice. The following is a statistical analysis of the thyroid and pituitary hormone data (T4 and TSH) found in that report. There were no data for T3 reported in the original study submitted to EPA (Keil et al., 1998). The EPA reanalyzed the data that were supplied in Excel® spreadsheets to EPA by Dr. Deborah Keil, and the data are published therein (Crofton, 1998i). Subsequent submission of additional data files also containing data for T3 were included in reanalyses (Crofton, 2001a). Data for dependent measures (T4 and TSH) were subjected to separate analyses. The T4 and TSH data were analyzed with a two-way ANOVA, with duration (14, 90, and 120 days) and treatment (dose) as the independent between-subjects variables as reanalyzed by Crofton and Marcus (2001) and Crofton (2001a) as reported in Table 5-2. Mean contrasts were performed using Duncan's Multiple Range Test.

Results of these EPA reanalyses are different from those stated in the Keil et al. (1998) report. The EPA reanalysis of the T3 data (Crofton, 2001a) found main effects of time and treatment, but no time-by-treatment interaction. Mean contrast testing showed a LOAEL of 0.1 mg/kg-day; however, the dose-related decrease was not linear. The 0.1 and 3.0 mg/kg-day doses differed from controls but the 1.0 and 30.0 mg/kg-day doses did not. There was a significant time-by-treatment interaction for T4. After 14 days of exposure there was no effect with a NOAEL at 30 mg/kg-day; whereas, after 90 days of exposure the LOAEL was 0.1 mg/kg-day. T4 recovered after 30 days postexposure. There was no effect of perchlorate on TSH concentration contrary to the changes in histopathology discussed in Section 5.6.2.

These effects are of interest in that they demonstrate effects in mice comparable in nature to that in rats and indicate that the hypothalamic-pituitary-thyroid feedback system is conserved across species.

5.6.4 Results of Immune Function Assays

No consistent alteration in CTL activity was observed in three 14-day studies ("M", "H", and "F", Keil et al., 1998). No effects were observed on CTL activity in Experiments "M" and "H". However, in Experiment "F", increases in CTL activity were observed at the 0.1-mg/kg-day ammonium perchlorate dose for effector to target cell (E:T) ratios of 100:1, 30:1, and 10:1, and, at the 1- and 3-mg/kg-day doses, for an E:T ratio of 10:1. In a 90-day study ("P", Keil et al., 1998) there were no alterations in CTL activity at any dosages or E:T ratios. The variability and inconsistencies observed in the early interim reports were ascribed to "technical issues" that were consequently "corrected". In fact, the data presented in Tables 23 and 24 (Keil et al., 1999) which includes data for dexamethasone, a potent immunosuppressant and positive control, indicates that there were no effects of ammonium perchlorate AP exposure on CTL activity.

There was also no consistent alteration in the DTH response, as measured by the lymphoproliferation (LP) of splenic lymphocytes from *L. monocytogenes*-challenged mice incubated with soluble *Listeria* antigen (SLA) in two 14-day studies ("U" and "V", Keil et al., 1998). The LP response was increased only in cultured splenic lymphocytes from mice in the 30-mg/kg-day group stimulated with 0.1 μg/mL SLA in Experiment "U" and in cultures of splenic lymphocytes from mice in the 3-mg/kg-day group stimulated with 8 μg/mL SLA in Experiment "V" (Keil et al., 1998). The "Results Summary and Status" page of Keil et al. (1998) indicates that a 90-day DTH study was planned. These 90-day data and a summary of the 14-day data are presented in Tables 32 and 31 respectively, of the "Final Report" (Keil et al., 1999). The data indicated an enhanced LP response in mice dosed at 30-mg/kg-day in both the 14-day and 90-day studies.

No alteration in splenic natural killer (NK) cell activity was observed in two 14-day studies ("G" and "T", Keil et al., 1998). The 14-day Experiment "T" data are presented in a table; however, the raw data and statistics for this study were not found in the submission. Inconsistent results were obtained in two 90-day studies ("D" and "N", Keil et al., 1998) in which NK cell activity was increased at the 30-mg/kg-day ammonium perchlorate in Experiment "N"; however,

no effects were observed at any doses in Experiment "D". A similar increase in NK cell activity at the 30-mg/kg-day dose was observed in the 120-day Experiment "E" (see also the data in Tables 21-22, Keil et al., 1999, in which the positive control dexamethasone was employed). The lack of any change in the number of B16F10 tumor nodules in the lungs of mice from the 90-day "Q" study (Keil et al., 1998; see also Table 33, Keil et al., 1999), particularly at 30 mg/kg-day, suggests that the increased NK-activity does not reflect a significant biological effect (see below). The EPA notes that there is a good deal of variation in NK activity data for the controls in the 14-day "G" study, the 90-day "D" and "N" studies, and the 120-day "E" study, which were 34, 6.4, 13.6, and 18.4 lytic units/10^7 splenic lymphocytes, respectively. Also, the 14-day "G" study was not included in Table 33 (Keil et al., 1999).

Decreased *in vitro* phagocytosis of *L. monocytogenes* was observed at 3 and 30 mg/kg-day of ammonium perchlorate in the 14-day "C" and 90-day "A" studies (Keil et al., 1998). In the 90-day "N" study, macrophage phagocytosis was decreased in all dose groups. However, in the 14-day "G" and 90-day "D" studies and in two 120-day studies ("B" and "E"), no effect on macrophage phagocytosis was observed (Keil et al., 1998). In the "Final Report" (Keil et al., 1999), these alterations were confirmed (i.e., decreased phagocytosis at 1.0 and 30.0 mg/kg-day in the 14-day study (Keil et al., 1999: Table 27) and decreased phagocytosis at 0.1, 1.0, 3.0, and 30.0 mg/kg-day in the 90-day study (Keil et al., 1999: Table 28). However, after a 30 day recovery period (i.e., 120-day study, Keil et al., 1999: Table 29) phagocytic function was comparable across control and treated mice. These data suggest that ammonium perchlorate suppresses the phagocytic capacity of peritoneal macrophages *in vitro*, but that this suppression may be reversed after a 30-day recovery period. Criticism of the use of an *in vitro* rather than an *in vivo* assessment of macrophage function was raised in the 1998 EPA ERD document and at the 1999 external peer review by Dr. Kimber White (Research Triangle Institute, 1999).

No consistent alteration in peritoneal macrophage nitrite production was observed in 14-, 90-, and 120-day studies. Increased nitrite production from macrophages cultured with interferon (IFN) occurred at doses of 3 and 30 mg/kg-day and from macrophages cultured with IFN and lipopolysaccharide for the 30-mg/kg-day dose in the 90-day "D" study (Keil et al., 1998). Also, increased nitrite production from macrophages cultured with IFN was observed at 3 mg/kg-day in the 90-day "N" study (Keil et al., 1998). An increase in nitrite production for macrophages cultured with IFN or LPS alone also occurred for the 30-mg/kg-day group in the 120-day "B"

study (Keil et al., 1998). These data suggest a "trend" toward increased nitrite production at the higher doses of ammonium perchlorate.

A subsequent analysis of these data, as presented in Tables 25 and 26 (Keil et al., 1999), demonstrates "no significant difference in nitrite production of peritoneal macrophages" (Keil et al., 1999).

A 90-day study ("L", Keil et al., 1998) was performed to determine if exposure of mice to ammonium perchlorate results in alterations in resistance to infection with *L. monocytogenes*. A trend toward increased resistance was suggested by the data; however, technical difficulties were encountered. For example, there was variability in the number of *L. monocytogenes* CFU/g liver recovered from control mice. It was not possible to enumerate the number of CFU/g spleen in mice due to the high concentration of bacteria injected and also to an inadequate dilution of spleen cell suspensions. In a subsequent 90-day study, mice were challenged with a lower concentration of bacteria such that both the CFU/g liver and spleen could be determined. These results, presented in Table 34 (Keil et. al., 1999), indicate that ammonium perchlorate exposure does not alter resistance to infectious challenge to *L. monocytogenes*.

No effects were observed in an initial 90-day B6F10 tumor challenge host-resistance model experiment ("Q", Keil et al., 1998). Another 90-day B6F10 tumor challenge experiment (i.e., "O") was performed, and the combined results of these two experiments are presented in Table 33 (Keil et al., 1999). These data indicate that there were no differences in the number of tumors present in the lungs of ammonium perchlorate-exposed mice compared with control mice.

Two separate groups of studies examining the effect that ammonium perchlorate has on the antibody response to SRBCs were performed by independent contractors (Keil et al, 1999; BRT-Burleson Research Technologies, Inc, 2000a,b,c). Initial studies were performed by Keil et al. (1999), in which the IgM and IgG antibody responses were determined using ELISAs. As indicated in the figures on page 59 (Keil et al., 1999), no change in the IgM levels in a 14-day study, nor in the IgM and IgG levels in a 90-day study, was observed between control and any ammonium perchlorate treated mice .

In the second set of studies, the anti-IgM SRBC PFC assay was employed (BRT-Burleson Research Technologies, Inc, 2000a,b,c), using CP as a positive immunosuppressant control. In the 14-day study there were no differences in the PFC response between control and treated mice when expressed either as the number of PFC/spleen or PFC/10^6 spleen cells (BRT-Burleson

Research Technologies, Inc, 2000a,b,c: Figures 3 and 4). On the other hand, in the 90-day study the PFC response was increased in the 2.0 and 50.0 mg/kg-day groups when expressed as the PFC/spleen and increased only in the 50.0 mg/kg-day group expressed as PFC/10^6 spleen cells (BRT-Burleson Research Technologies, Inc., 2000a,b,c: Figures 5 and 6). This disparity was not due to any difference in splenic cellularity between the control and treated mice. In both the 14- and 90-day studies, CP significantly inhibited the PFC response, expressed either as PFC/spleen or PFC/10^6 spleen cells compared to the controls.

The results of the effect that 14- and 90-day exposure to ammonium perchlorate has on the development of a contact hypersensitivity response to DNCB, as determined by the LLNA, indicate that an ammonium perchlorate dose as low as 0.06 mg/kg-day enhances this response. In the 14-day study, the LLNA was increased at doses of 0.06, 0.2, and 50.0 mg/kg-day, but not 2.0 mg/kg-day (BRT-Research Technologies, Inc., 2000a,b,c: Figure 8). The results of the 90-day study were somewhat different in that, while the LLNA was enhanced at 0.06 and 0.2 mg/kg-day, it was suppressed at 50 mg/kg-day (BRT-Research Technologies, Inc., 2000a,b,c: Figure 9). Another disparity between these two studies was that while CP suppressed the LLNA in the 14-day study, it did not suppress this response in the 90-day study.

5.6.5 Results for Evaluations of Hematological Parameters

There were no differences observed between control and dosed mice in 14- or 90-day experiments for erythrocyte cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration; nor in leukocyte differential counts of neutrophils, monocytes, and lymphocytes. Because of the absence of effects in these studies, no 120-day study was performed. No effects were observed in a single 14-day study (Experiment "T", Keil et al., 1998) on platelet counts. An increase in the percentage of reticulocytes was observed in the peripheral blood of mice exposed to 3 mg/kg-day of ammonium perchlorate in a 90-day study ("N", Keil et al., 1998). No other reticulocyte data are available because of "the minimal availability of blood obtained from each mouse" in other studies (Keil et al., 1998). In a subsequent 14-day study, there were no changes in the hematological parameters examined between control and ammonium-perchlorate-treated mice (Keil, et al., 1999: Table 16).

No consistent alteration in the bone marrow stem cell assay was observed. An increase in the number of colony-forming units was observed in bone marrow cell cultures from mice dosed at 30 mg/kg-day in a 14-day study ("K", Keil et al., 1998). However, there was no effect of ammonium perchlorate exposure on the stem cell assay in a 90-day study ("D", Keil et al., 1998). In a subsequent 90-day study, while no alteration in the stem cell assay was observed between control and ammonium perchlorate-treated mice, exposure to the positive control dexamethasone resulted in suppression of the stem cell assay (Keil, et al., 1999: Table 20).

5.6.6 Results Summary

The results of the various studies of immue function are summarized in Table 5-5. Although innate (i.e., macrophage and NK cell function) and cell-mediated (i.e., cytotoxic T lymphocytes [CTL], CD4, and CD8) immune functions were evaluated in the initial studies by Keil et al, (1998), EPA noted that humoral immunity (i.e., B cells and antibody response) was not (Smialowicz, 1999). The EPA suggested strongly that the antibody response to SRBCs is one of the most commonly effected functional parameters in animals exposed to chemical immunosuppressants (Luster et al., 1988). In fact, it is one of the assays required by EPA for test rules. The EPA also requested that an additional 90-day *L. monocytogenes* host-resistance study be undertaken consequent to technical problems associated with the initial 90-day study (Keil et al., 1998). As such, the EPA felt that these data would provide a more comprehensive evaluation of the potential for immunosuppression by ammonium perchlorate. In addition, the EPA requested that thyroid histology and thyroid and pituitary hormone data be obtained in order to provide additional insights on interspecies variability for this effect.

Consequently, the sponsor and investigators, Keil et al. (1998), agreed to perform these assays, the results of which are presented in the "Final Report" (Keil et al., 1999).

Subsequent to receipt of the results of the antibody response to SRBCs (Keil et al., 1999), in which antibody titers were expressed as the O.D. 50 or midpoint titer, rather than the more conventional titer to achieve a 0.5 O.D., a second request to determine the potential effects of ammonium perchlorate on the response to SRBCs was issued. In this same solicitation, the EPA also requested that a sensitization test be performed. The results of these studies are found in BRT-Burleson Research Technologies, Inc. (2000a, b, c).

TABLE 5-5. SUMMARY OF IMMUNOTOXICITY TEST RESULTS

Series/Strain/Sex (Study)	Exposures Period and Doses (mg/kg/d)	Endpoint	NOAEL/LOAEL Designations
Mouse/B6C3F1/Female (Keil et al., 1998;1999)	14-days 0, 0.1, 1.0, 3.0, or 30	Weights: body, thymus, spleen, liver, kidney	None
		Cellularity: spleen, thymus, bone marrow	None
		Splenic CD4CD8 cells	None
		NK cell activity/B16F10 tumor challenge	None/Not Done
		CTL to P815 cells (*in vitro*)	Increased at 0.1, 1.0 and 3.0; no effect in subsequent "corrected" study.
		L. monocytogenes challenge	Not Done
		DTH to *L. monocytogenes* antigen	Increased at 30. NOAEL = 3.0, LOAEL = 30
		Macrophage phagocytosis (*in vitro*)	Decreased at 1.0 and 30. NOAEL = 0.1, LOAEL = 1.0
		Macrophage nitrate (*in vitro* + IFN or LPS)	None
		IgM ELISA to SRBCs	None
	90-days 0, 0.1, 1.0, 3.0, or 30	Weights: body, thymus, spleen, liver, kidney	None
		Cellularity: spleen, thymus, bone marrow	None
		Splenic CD4CD8 cells	None
Mouse/B6C3F1/Female (Kiel et al., 1998; 1999)	90-days 0, 0.1, 1.0, 3.0, or 30	NK cell activity/B16F10 tumor challenge	Increase NK activity at 30 in one experiment and at 30 in 120-day study: NOAEL = 3, LOAEL = 30. No effect on B16F10 tumor challenge.
		CTL to P815 cells (*in vitro*)	None
		L. monocytogenes challenge	None
		DTH to *L. monocytogenes* antigen	Increase at 30. NOAEL = 3.0, LOAEL = 30
		Macrophage phagocytosis (*in vitro*)	Decreased at 0.1, 1.0, 3.0 and 30, LOAEL = 0.1

TABLE 5-5 (cont'd). SUMMARY OF IMMUNOTOXICITY TEST RESULTS

Series/Strain/Sex (Study)	Exposures Period and Doses (mg/kg/d)	Endpoint	NOAEL/LOAEL Designations
		Macrophage nitrate (*in vitro* + IFN or LPS)	None
		IgM ELISA to SRBCs	None
Mouse/CBA/JHsd/Female (BRT-Burleson Research Technologies, Inc., 2000a,b,c)	14-days 0, 0.02, 0.06, 0.2, 2.0, or 50	anti-SRBC PFC/10^6 cells	None
		anti-PFC/spleen	None
		LLNA to DNCB	Increased at 0.06, 0.2, and 50, but not at 2.0. NOAEL = .02, LOAEL = 0.06
	90-days 0, 0.02, 0.06, 0.2, 2.0, or 50	anti-SRBC PFC/10^6 cells	Increased at 50. NOAEL = 2.0, LOAEL = 50
		anti-PFC/spleen	Increased at 2.0 and 50. NOAEL = 0.2, LOAEL = 2.0
		LLNA to DNCB	Increased at 0.06 and 0.2, but not at 2.0; decreased at 50. NOAEL = 0.02, LOAEL = 0.06

NK = natural killer; CTL = cytotoxic lymphocyte; DTH = delayed type hypersensitivity; IFN = interferon; SRBC = sheep red blood cell; PFC = plaque forming colony; LLNA = local lymph node assay; DNCB = 2,4-Dinitrochlorobenzene.

The three immune function parameters altered by ammonium perchlorate exposure were the following: suppression of *in vitro* peritoneal macrophage phagocytosis of *L. monocytogenes*, enhancement of the PFC response to SRBCs, and enhancement of the LLNA to DNCB. These results are summarized and discussed below.

Decreased *in vitro* phagocytosis of *L. monocytogenes* by peritoneal macrophages obtained from mice dosed for 14 days at 1- or 3- and 30-mg ammonium perchlorate/kg-day was observed (Keil et al, 1998, 1999). In mice exposed for 90-days, phagocytosis was decreased in all dosage groups (Keil, 1998, 1999). However, in the 120-day (i.e., 90-day ammonium perchlorate exposure followed by 30-day recovery) studies, no effect on macrophage phagocytosis of *L. monocytogenes* was observed (Keil et al., 1998, 1999). Taken together, these data suggest that

ammonium perchlorate suppresses the *in vitro* phagocytic capacity of peritoneal macrophages, but that this suppression is reversed after a 30 day recovery period.

This decrease in macrophage phagocytic activity could be expected to be reflected in the results of the *L. monocytogenes* infectivity data because, along with other immune system components, macrophages play a pivotal role in resistance to infection by this bacterium. For example, the pathogenesis of *L. monocytogenes* is associated with its ability to grow within mononuclear phagocytes. Complement (C') plays an important role in *L. monocytogenes* infections, as demonstrated by the fact that C'-deficient mice have impaired host resistance to this bacterium. This impairment in C'-deficient mice is caused by the absence of macrophage-associated C'. The T-lymphocytes also play a major role in defense against *L. monocytogenes* because complete elimination of bacteria from infected tissue is accomplished by macrophages activated by T-cell dependent mechanisms.

However, the *L. monocytogenes* host-resistance studies indicate that ammonium perchlorate exposure of mice does not alter the ability to combat this bacterial infection. With the exception that clearance of *L. monocytogenes* from the liver of mice given a 5360 CFU challenge following dosing at 3.0 mg AP/kg/day for 90 days was reduced, no other effect was observed (Keil et a., 1999: Table 43). These data imply that while in vitro phagocytosis by peritoneal macrophages of this bacterium was reduced following ammonium perchlorate exposure, the ability of macrophages from other *in situ* sites (e.g., spleen, liver) to clear *L. monocytogenes* was not altered.

Exposure of mice to 2.0 or 50.0 mg ammonium perchlorate/kg/day for 90, but not 14, days resulted in enhancement of the antibody response to SRBCs as determined by the PFC assay (BRI-Burleson Research Technologies, Inc., 2000a,b,c). In both the 14- and 90-day studies, the PFC response was suppressed by dosing mice with the immunosuppressive positive control CP. The PFC assay is routinely used for identifying chemicals that are immunosuppressive. The reason why the highest dose(s) of ammonium perchlorate, given over 90 days, enhanced this response is not known. It is possible that under these dosing conditions ammonium perchlorate may have an adjuvant-like or enhancing effect on the antibody response to SRBCs. The ELISA data for mice exposed to up to 30.0 mg ammonium perchlorate/kg/day, for 14 or 90 days (Keil et al., 1999), do not corroborate this enhanced response to SRBCs as determined by the PFC

assay. However, taken together, the PFC and ELISA data indicate that ammonium perchlorate does not suppress the immune response to SRBCs.

The LLNA is an accepted approach for identifying chemicals with the potential of causing dermal contact hypersensitivity (CHS) reactions in humans. In this assay the test substance, 2,4-dinitrochlorobenzene (DNCB) was topically applied on three consecutive days to both ears of the mouse. Two days later the mice were injected iv with radioactive uridine (e.g., ^{125}IUDR). Five hours later, the lymph nodes draining the ears, referred to as the "auricular" lymph nodes, were removed and ^{125}IUDR incorporation by the lymph node cells determined. Since the nodes draining the ear (i.e., "auricular" nodes) have no standard anatomical nomenclature, experience in identifying these nodes as well as appropriate and consistent excision of these nodes from control and test mice is critical. The LLNA evaluates the induction phase of the CHS reaction by assessing the influx of lymphoid cells and the differential argumentation of lymphocyte proliferation elicited by exposure to the test chemical relative to that of a vehicle control.

The data from BRT-Burleson Research Technologies, Inc. (2000a,b,c) report that exposure to ammonium perchlorate enhances/exacerbates the LLNA response to DNCB at doses of 0.06, and 0.2 mg/kg/d in both the 14- and 90-day. While a dose of 50.0 mg/kg-day for 14 days also enhanced this response, a dose of 2.0 mg/kg-day did not. Similarly, a dose of 2.0 mg/kg-day in the 90-day study did not enhance the LLNA response to DNCB. In contrast to the 14-day study, exposure of mice to 50.0 mg ammonium perchlorate/kg/day in the 90-day study resulted in suppression of the LLNA response. In the 90-day study, the positive control CP did not suppress the LLNA response to DNCB. The failure of CP to suppress this response in the 90-day vs. 14-day study is disquieting because CP was administered similarly (i.e., 15 mg/kg-day for 5 consecutive days prior to the LLNA) in both studies. The only difference between these two studies was that the mice in the 90-day study were 11 weeks older. This difference in age, however, should not influence the ability of CP to suppress this response. The fact that CP did not suppress the LLNA response in the 90-day study calls into question the performance of this and perhaps the 14-day study.

Application of the LLNA for identification of chemicals that are contact sensitizers routinely involves a demonstration of a dose-related increase in the LLNA using, at a minimum, three increasing concentrations of the test agent. Neither the 14- nor 90-day ammonium perchlorate LLNA data demonstrate a dose-response relationship, which would be expected if

ammonium perchlorate was acting additively or synergistically with DNCB to increase the LLNA response. While higher concentrations of a contact sensitizing agent will increase the LLNA response, there is no information in the literature that indicates such an increase results in a more serious or potentially detrimental effect on the host. Consequently, the physiologic significance of the observed increase in the LLNA response to DNCB in ammonium perchlorate-exposed mice is unknown. This is unlike the situation with immunosuppressive agents where suppression of specific immune function(s) can be linked to a biological detrimental effect (i.e., decreased host resistence to an infectious agent or tumor).

It is interesting to note that there are published reports in which non-sensitizing agents have been employed to improve the sensitivity of the LLNA to detect sensitizers. For example, Vitamin A acetate dietary supplementation enhances the detection of weak sensitizers, and at low concentrations of moderate sensitizers, assessed by the LLNA. The mechanism(s) for this increased detection of contact sensitizers is not known. However, topically applied retinol causes epidermal hyperplasia which may lead to increased numbers of antigen-presenting cells in the epidermis. Retinoids also up regulate the sensitization phase of DTH induction through direct or indirect stimulation of T cells. Non-sensitized mice, fed a diet supplemented with retinol, display somewhat higher LLNA responses compared to control mice on a normal diet. This suggests that dietary retinol itself causes cellular infiltration and/or proliferation in the absence of a contact sensitizer as measured by the LLNA. It may be that ammonium perchlorate, in the absence of DNCB, has the capability of raising the baseline LLNA response compared to water control mice. Unfortunately, there were no negative controls in the Burleson et al. (2000) studies. Appropriate negative controls would have included the following: (1) ammonium perchlorate-dosed non-sensitized mice; (2) ammonium perchlorate-dosed and ammonium perchlorate-challenged mice; and (3) water control mice dermally exposed to ammonium perchlorate on the ear pinna. Another group of appropriate and informative studies would involve ammonium perchlorate-dosed mice that would be challenged with a series of low to moderate concentrations of DNCB, for comparison with the current LLNA “optimal DNCB” response concentration data.

Enhancement of the LLNA to DNCB in mice exposed to 0.06 mg ammonium perchlorate/kg-day for 14 or 90 days represents the Lowest Observed Effect Level (LOEL) for all of the immune function tests performed. While this is the LOEL it is unknown if this is the

Lowest-Observed-Adverse-Effect- Level (LOAEL) because it is not clear that enhancement of the LLNA is a physiologically relevant adverse effect. Studies are needed to determine if ammonium perchlorate itself is a contact sensitizer as determined by the LLNA, as described above, and whether the degree of the LLNA response to ammonium perchlorate itself or to a known contact sensitizer can be linked to a quantifiable adverse outcome.

It is important to note that clinical studies in the 1960s reported that some patients suffering from Graves' disease and treated with potassium perchlorate presented with agranulocytosis and/or skin rashes. While the studies reported by Keil et al. (1998, 1999) indicated that there were no alterations in the proportion of peripheral blood leukocytes of mice dosed with ammonium perchlorate for 14- or 90-days, the work of BRI-Burleson Research Technologies, Inc. (2000a,b,c) suggests that ammonium perchlorate appears to exacerbate the contact sensitizing potential of the known skin sensitizer DNCB. However, due to the uncertainties associated with any attempt to extrapolate from the incomplete database of the mouse LLNA performed by BRI-Burleson Research Technologies, Inc. (2000a,b,c) to the clinical observations of skin rash and agranulocytosis in Graves' disease patients treated with potassium perchlorate, an uncertainty factor based on deficiencies in the database is recommended to be applied to this risk assessment.

CHAPTER 6. CONSTRUCTION OF PBPK MODELS TO ADDRESS PERCHLORATE'S MODE-OF-ACTION

The purpose of this chapter is to describe the progress that has been made in developing physiologically-based pharmacokinetic (PBPK) models to aid interspecies extrapolation of effects observed in the toxicity studies. The models describe perchlorate and iodide kinetics in rats and humans. Because of the complex challenge posed in arriving at a representation of the regulation system for hypothalamic-pituitary-thyroid feedback, the modeling effort was not able to satisfactorily develop models that linked the observed effects of perchlorate inhibition of iodine uptake at the NIS with the resultant hormone perturbations and available toxicological information in the proposed mode-of-action framework.

Because of their potential role in the risk assessment and regulatory applications, the EPA required that all human clinical data utilized in this modeling effort undergo a quality assurance/quality check (QA/QC). The QA/QC report is presented in Merrill (2001a,b). These QA/QC data represent the most contemporary, comprehensive, and consistent set of human pharmacokinetic data available for perchlorate.

The PBPK models discussed herein (Merrill, 2001c,d; Clewell, 2001a,b) were developed by the AFRL/HEST to provide more accurate descriptions of the kinetics of iodide and perchlorate with respect to perchlorate's inhibition of iodide uptake at the NIS and their serum and tissue time courses as well as to aid evaluation of subsequent perturbations in thyroid hormones and TSH. A general discussion of the model development for the various PBPK model structures of perchlorate distribution will be provided in this chapter to aid appreciation of their attributes and applications. Because of the mode of action for perchlorate, an accurate description of iodide kinetics is critical to the description of perchlorate effects on iodide uptake at the NIS so that each of these models also includes iodide-specific parameters and accounts for iodide disposition.

A similar model was developed for each of the various life stages of importance to interspecies extrapolation of the laboratory animal data: adult, pregnant rat and fetus, and the lactating rat and neonate. The adult male rat model was developed using data from the ADME

studies in the perchlorate testing strategy, together with experimental data and parameter values available in the existing literature. The subsequent model structures for the human and various life stages of the rat were similarly developed based largely on the adult male rat structure through scaling and optimization of parameters to available data.

It should be noted that the original motivation for including human studies (as discussed in Chapter 3) in the perchlorate testing strategy was to support such interspecies extrapolation and not to derive NOAEL estimates for thyroid effects in the human population. As discussed in Chapter 4, the EPA feels that both the observational epidemiological and the human clinical studies have significant scientific and technical limitations that preclude their use as the basis for a quantitative dose-response assessment. In addition, some of the clinical studies contained in this database fall in the category of studies not to be considered under EPA's Dec. 14, 2001 interim policy on the use of third-party human studies (U.S. Environmental Protection Agency, 2001c). However, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. Therefore, because of the scientific shortcomings of these studies, they will not be used as "principal studies" in the derivation of an RfD. The clinical study subject attributes (euthyroid adults) and study design issues (sample size, RAIU time points, etc.) made these data less reliable than the laboratory animal toxicological data to ascertain effect levels for the basis of an RfD derivation. Models of perchlorate distribution for human pregnancy and lactation have not been developed.

More detailed discussion can be found for each model structure in the accompanying references provided for each in the sections that follow. The adult male rat and human model (Merrill, 2001c,d) will be discussed in Section 6.2. Section 6.3 discusses the pregnant dam and fetal rat PBPK model (Clewell, 2001a), and the lactating dam and neonate model (Clewell, 2001b) is discussed in Section 6.4. The purpose of providing these model descriptions and a discussion of the data used to develop and validate their structures is to provide the external peer reviewers an opportunity to critically evaluate the model structures, the use of the data in model development or validation exercises, and the model applications.

The simultaneous ordinary differential equations used in the proposed PBPK models to simulate radioiodide and perchlorate distribution were written and solved using advanced continuous simulation language (ACSL) software (AEqis Technologies, Austin, TX).

6.1 MODE-OF-ACTION FRAMEWORK AND UNDERLYING MODELING APPROACH

The mode-of-action model proposal by the EPA for the previous perchlorate assessment and discussed in Chapter 3 served as the conceptual construct for the development of the PBPK models. Shown again in Figure 6-1, the model lays out the biomarkers of exposure and effect in a continuum from ingestion of perchlorate in drinking water and uptake into the blood, the key event of iodide uptake inhibition at the NIS in the thyroid gland, and subsequent effects on thyroid hormone economy leading to neurodevelopmental and neoplastic sequelae.

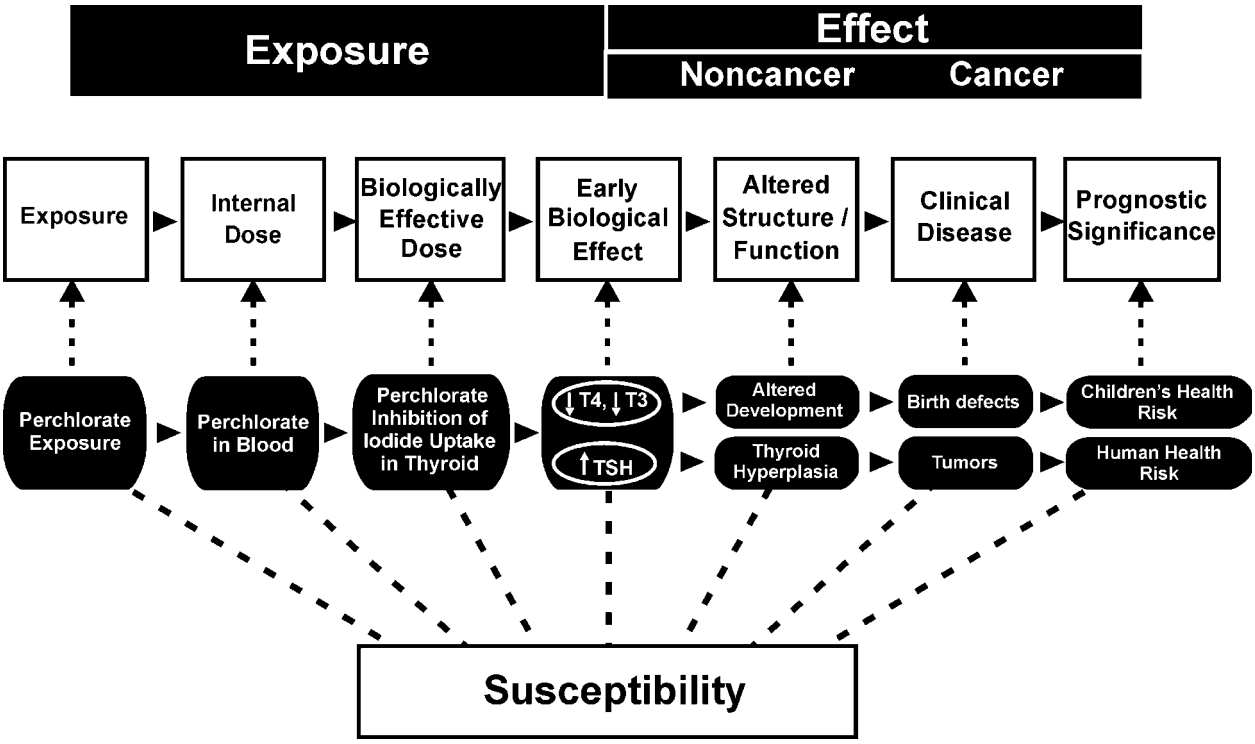

Figure 6-1. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U.S. Environmental Protection Agency, 1994; Schulte, 1989). The model maps the toxicity of perchlorate on this basis by establishing casual linkage or prognostic correlations of precursor lesions.

The temporal pathological and serum hormone changes that accompany exposure to perchlorate corresponding to this continuum are represented in Figure 6-2. The inhibition of iodide uptake at the NIS results in a transient decrease in serum T4 and T3. This transient phase of thyroid hormone deficit is of concern during pregnancy and development due to the critical role that these hormones play in preventing adverse neurodevelopmental sequelae as described in Chapters 3 and 5. The hypothalamic-pituitary-thyroid feedback system is designed to regulate the circulating levels of thyroid hormone and will respond to the thyroid hormone decreases by upregulating TSH production in order to stimulate the thyroid to increase its production of thyroid hormones to compensate. Represented as the "chronic phase" in Figure 6-2, the upregulation of TSH would bring the system back into apparent homeostasis. As depicted in the figure, however, this apparent homeostasis may actually represent subclinical disease in that the system is only maintaining homeostasis by upregulation and can be considered a stressed system with respect to its ability to compensate for additional insults caused by other chemicals or diseases that might impact the thyroid. Further, it should be emphasized that recent epidemiological investigations have indicated concern about decrements in T4, i.e., thyroxinemia without concomitant upregulation of TSH that would constitute hypothyroidism (Morreal de Escobar, 2000; Haddow et al., 1999; Pop et al., 1999).

In order to adequately characterize the transient phase of events, evaluation of the initial effect of perchlorate at the NIS is necessary. This can be accomplished by determining perchlorate inhibition with radioactive iodide uptake (RAIU) studies. The timing and route of administration are important considerations in evaluating these types of studies. Studies of RAIU that occur during the chronic phase, such as longer-term studies of hormones, offer little insight to the critical decrements in T4 that may occur during the transient phase due to iodine inhibition. Likewise, longer-term studies of hormones often represent the upregulated system and may not be especially informative.

6.1.1 Parallelogram Approach to Interspecies Extrapolation

PBPK models have proven to be very useful tools for performing interspecies extrapolation of dose for applications in risk analysis. Interspecies extrapolation is often necessary because, as in this case of perchlorate, critical effects at levels of organization below that of the population (e.g., thyroid histopathology or brain morphometry) can not be evaluated easily or ethically in

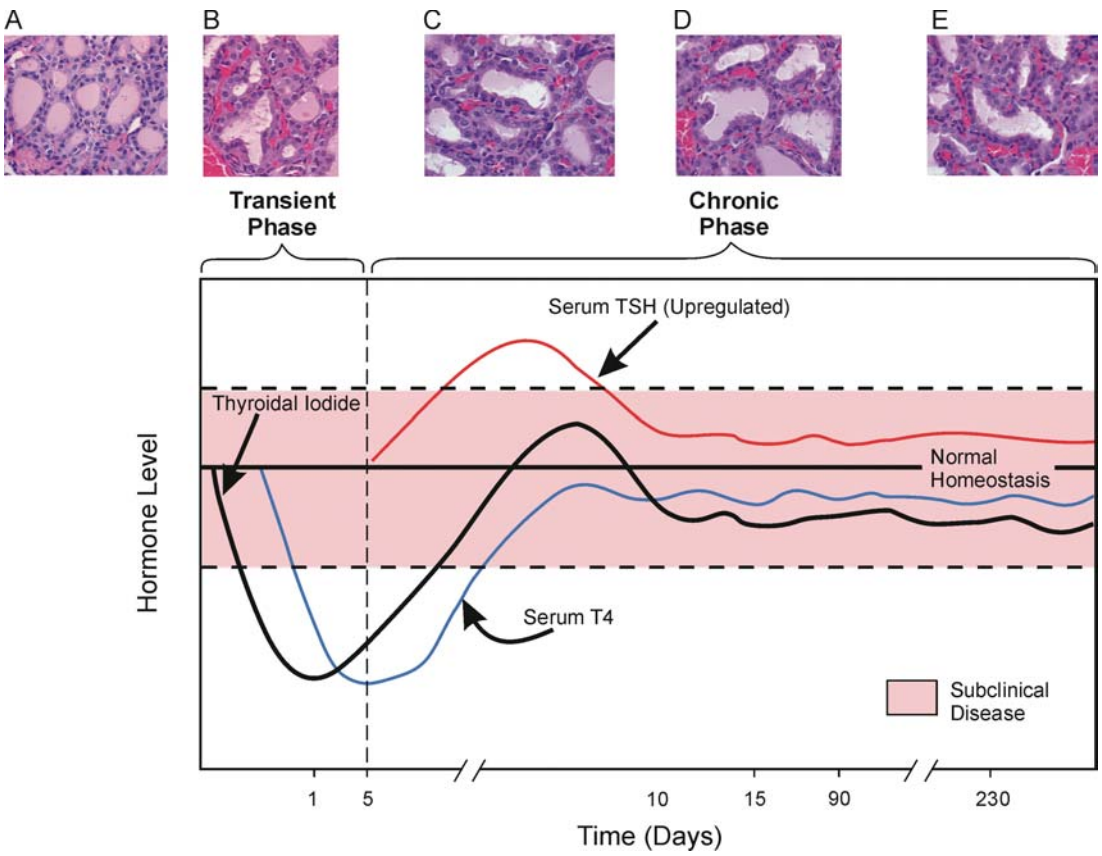

Figure 6-2. Schematic of thyroid and pituitary hormone levels with associated pathology after acute versus chronic dosing with perchlorate. The transient phase is represented by decreases in thyroidal iodide due to the inhibition by perchlorate at the NIS with subsequent drop in T4. The transient drops in T4 can lead to permanent neurodevelopmental sequelae. Once TSH is upregulated via the hypothalamic-pituitary-thyroid feedback, T4 appears to be in normal homeostasis but actually can represent subclinical or undiagnosed disease (hypothyroxinemia). The upregulation of TSH can result in neoplasia. Normal thyroid tissue is represented in Panel A. Panel B shows lace-like colloid depletion which is more pronounced in subsequent panels C, D and E. Panels D and E represent hypertrophy and hyperplasia.

humans. A basic tenet of molecular epidemiology is that these precursor lesions are often more closely related to the exposure than are the traditional outcome measures of morbidity and mortality (U.S. Environmental Protection Agency, 1994).

A parallelogram approach as shown in Figure 6-3 is used to predict the dose-response relationship for humans based on the dose-response in laboratory animals. Because these critical

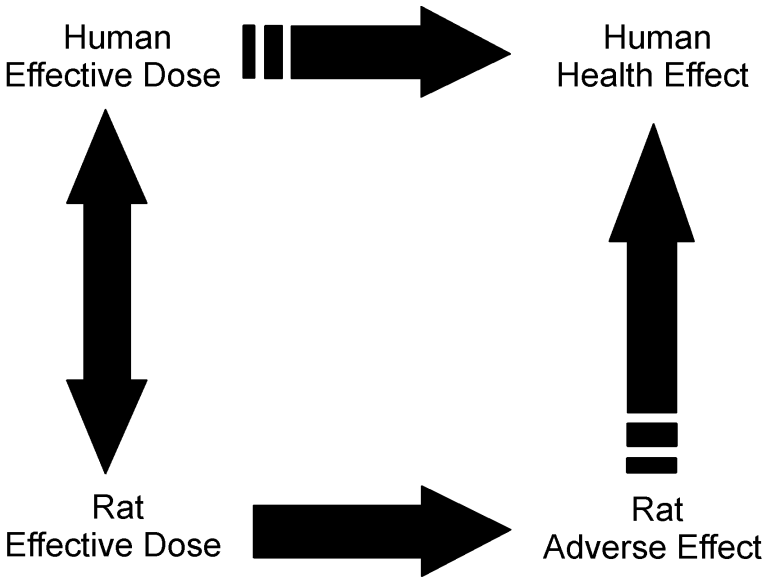

Figure 6-3. Schematic of parallelogram approach used for interspecies extrapolation (U.S. Environmental Protection Agency, 1994). Dose and adverse effect in rat can be used to predict human effective dose and response.

effects cannot be accurately measured in humans, the dose associated with an observed critical effect in the laboratory animal is scaled to the human by adjusting the PBPK model with human physiological parameters and variables. The human model is typically constructed by allometrically scaling some parameters in the laboratory animal model based on body weight, and some parameters such as partition coefficients can be measured *in vitro*. An administered dose associated with the critical effect is determined based on an appropriate internal dose metric. The internal dose is scaled to an equivalent exposure (HEE) in humans by exercising the human model with human parameters and exposure assumptions. Thus, the HEE represents the human exposure that would result in the same amount of internal dose metric in a human as that which caused the effect in the laboratory animal.

The dose-response relationship is considered to be the same as that in the laboratory animal as the default or more biologically-based models may contain additional parameters that also account for species-specific determinants of toxicant-target interaction. Figure 6-4 illustrates the use of the laboratory animal and human PBPK models to arrive at the HEE. Simulations used to

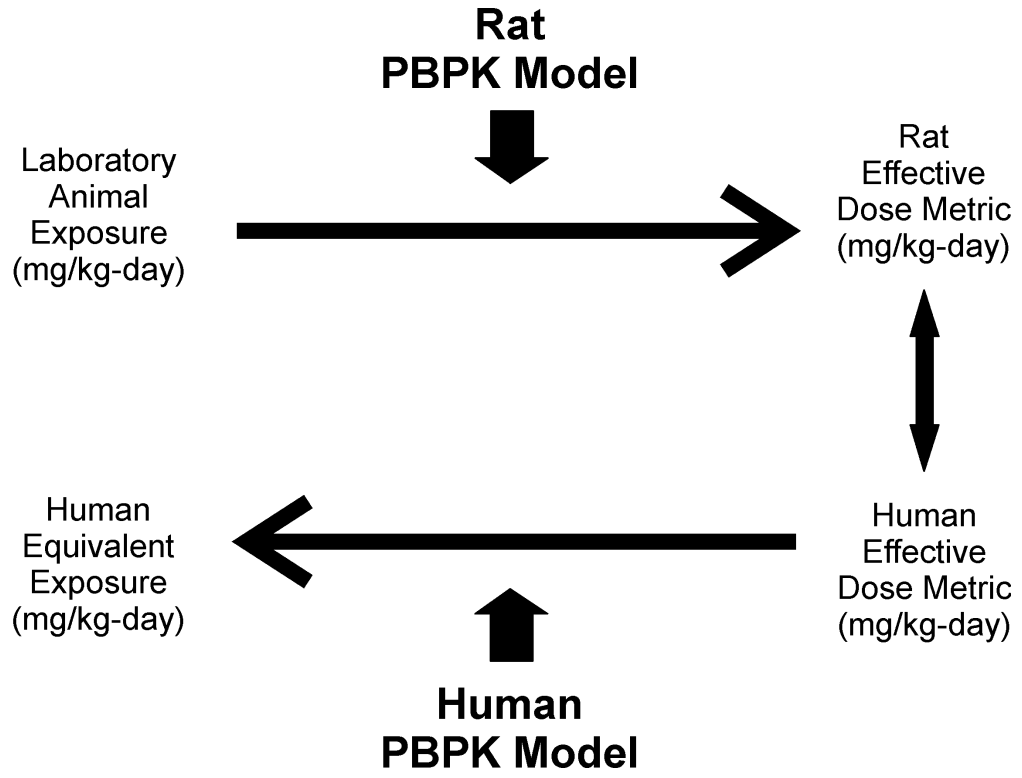

Figure 6-4. Illustration of how human equivalent exposure (HEE) is calculated using PBPK models. An effective internal dose associated with a critical health effect at an administered dose (mg/kg-day) is calculated by simulating the experimental exposure regimen (e.g., 5 days/week) for a relevant metric (e.g., area under the curve in blood, [AUCB]). The human PBPK model is then used to simulate an exposure that achieves the same effective internal dose metric level using human parameters.

arrive at HEE for different internal dose metrics and a sensitivity analysis of the adult model structure will be discussed in Section 6.5.

The parallelogram approach has also been used to predict effective doses for structurally related chemicals (Jarabek et al., 1994). Disposition of one chemical associated with an effect can be predicted for another after appropriate adjustments for chemical structure and activity are made. In the case of these models, it should be appreciated that the accurate modeling of iodide in addition to that of perchlorate represents such a validation.

6.1.2 Extending the Parallelogram Approach to Various Experimental Life Stages

Because effects at various life stages (adult, pregnant dam, fetus, lactating dam, and neonate) were evaluated in the perchlorate laboratory animal studies, the parallelogram approach had to be extended as shown in Figure 6-5. There are no human models of perchlorate disposition for pregnant women, lactating women, fetuses, or children, so the relationships to the adult human HEE had to rely on the relationships determined in the laboratory animal species. This approach assumes that the relationships, expressed as ratios between one life stage and another, will be comparable in humans.

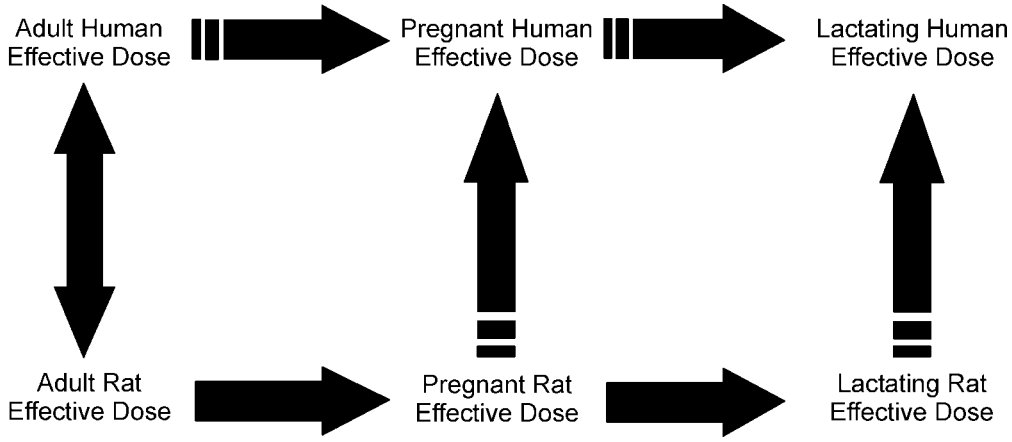

Figure 6-5. Schematic of extended parallelogram approach used for perchlorate due to effects at different life stages. Doses in the pregnant rat and fetus are related back to the adult male rat, likewise, the effects in lactating rats and neonates.

The various PBPK models are used to predict equivalent effective doses at the various administered doses used in the experiments; e.g., 1.0 mg/kg-day ammonium perchlorate given in drinking water to both the adult male rat and the pregnant dam. Each PBPK model is exercised (adult rat and pregnant rat) to predict the amount of internal dose metric achieved at each life stage. The ratio of the effective internal dose metrics of the life stage in question is then used to

adjust the HEE based on the adult male rat. For example, the HEE for the pregnant dam would be found by adjusting the HEE for the adult male rat by the ratio of the male rat:pregnant rat as:

$$\text{Pregnant HEE (mg/kg-day)} = \text{Adult male rat HEE (mg/kg-day)} \times \frac{\text{Adult male rat internal effective dose metric}}{\text{Pregnant rat internal effective dose metric}} \quad (6\text{-}1)$$

This ratio is unitless and accounts for the differences between the two life stages in question in an analogous fashion to the dosimetric adjustment factor (DAF) used in the EPA's inhalation reference concentration methods to extrapolate respiratory tract doses in different regions of the laboratory animal to human equivalent concentrations (U.S. Environmental Protection Agency, 1994). The same ratio approach is used to extend the model predictions to HEE estimates for the fetus, lactating dam, and neonate. Development of the ratios for two internal effective dose metrics, perchlorate area-under-curve (AUC) concentrations in serum and iodide uptake inhibition, will be discussed in Section 6.5.

6.2 ADULT RAT AND HUMAN MODEL STRUCTURES

Because the same model structure is used to describe perchlorate and iodide disposition (absorption, distribution, and elimination) for both the adult male rat and human, this section will describe the development of both of these models together. Data supporting development and validation of the structures will be summarized in this section while additional detail, including some of the governing equations, can be found in the consultative letters from the AFRL/HEST (Merrill, 2001c,d).

As discussed in Chapter 2, the perchlorate anion (ClO_4^-) is very similar in ionic size, shape, and charge to that of iodide (I^-). These shared properties allow perchlorate to interfere with the first stage of thyroid hormone synthesis by competitively inhibiting the active transfer of iodide into the thyroid by the sodium (Na^+)-iodide (I^-) symporter or NIS. The NIS is a protein that resides in the basolateral membrane of thyroid epithelial cells (Spitweg et al., 2000). NIS simultaneously transports both sodium and iodide ions from extracellular plasma into the thyroid epithelial cell via an active process. Energy is provided by the electrochemical gradient across

the cell membrane. The low intracellular concentration of sodium is maintained by sodium-potassium pumps (Ajjan et al., 1998). The kinetics of perchlorate and iodide anions differ mainly in that iodide is organified in the thyroid (thyroid hormone production); whereas, perchlorate is thought to be unreactive and eventually diffuses from the thyroid into systemic circulation.

The proposed PBPK model structure for the adult male rat (Merrill, 2001c) and human (Merrill, 2001d) describes active uptake of iodide and perchlorate in gastric juice, thyroid, and skin, and competitive inhibition of iodide uptake by perchlorate in NIS-containing tissues, as well as venous equilibration with slowly and richly perfused tissues as shown in Figure 6-6. Tissues that exhibited evidence of sodium iodide symporter and were found to concentrate either anion were depicted as compartments with nonlinear uptake (Merrill, 2001c,d). Tissues with active uptake include the thyroid, skin, and gastric mucosa (Wolff, 1998; Chow et al., 1969; Kotani et al., 1998). Although other tissues have been known to sequester iodide and similar anions (e.g., salivary glands, choroid plexus, ovaries, mammary glands, placenta) (Brown-Grant, 1961, Honour et al., 1952; Spitzweg et al., 1998), the iodide and perchlorate pools of these tissues was expected to be too small to significantly affect plasma levels. These tissues were lumped with slowly and richly perfused tissues.

The model also includes separate compartments for plasma, kidney, liver, and fat. These compartments do not maintain concentrations greater than the plasma at steady state, and therefore, were not described with terms for active uptake. The rapid urinary clearance of perchlorate (Yu, 2000) mandated the inclusion of a kidney compartment in the model. A liver compartment was also utilized due to its significant impact on iodide homeostasis. The majority of extrathyroidal deiodination takes place within the liver. Fat was primarily added as an exclusionary compartment. Due to its significant percentage of body weight, the skin represents an important pool for slow iodide turnover.

The modelers at AFRL/HEST found that a separate skin compartment was necessary. Experiments performed with radioiodide in rats resulted in skin:serum iodide ratios of close to one (Yu, 2000). Other researchers have reported higher ratios in rats, but results have not been consistent. Similar observations during dialysis with pertechnate of slow uptake and retention in human skin was observed by Hays and Green (1973) and the skin was therefore maintained as a separate compartment in the model. The skin contains two sub-compartments representing the

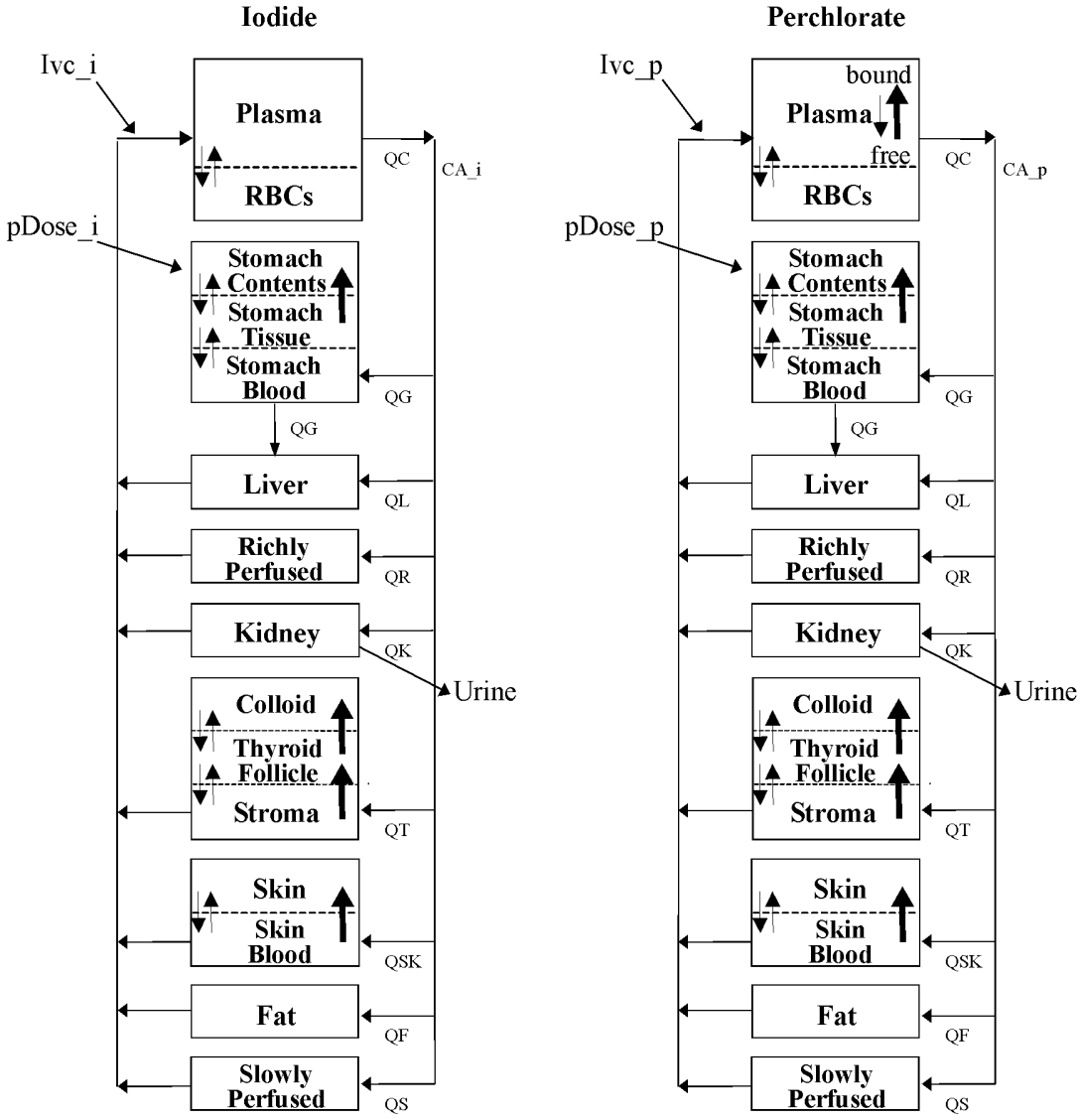

Figure 6-6. Schematic for the adult male rat and human PBPK models of perchlorate and iodide distribution (Merrill, 2001c,d). Bold arrows indicate active uptake (except for plasma binding) at NIS sites in thyroid, gut, and skin. Plasma binding was also described with Michaelis-Menten terms for the association of perchlorate anion to binding sites with first-order clearance rates for dissociation. Small arrows indicate passive diffusion. Boxes represent specific compartments in the model structure. The thyroid consists of the stroma, the follicle, and the colloid; and the stomach consists of the capillary bed, stomach wall, and stomach contents. The skin contains two subcompartments: the capillary bed and skin tissue. Permeability area cross products and partition coefficients were used to describe the first-order movement of the perchlorate (ClO_4^-) and iodide (I^-) anions into deeper subcompartments.

capillary bed and the skin tissue. The thyroid and stomach consist of three sub-compartments: the stroma, the follicle, and the colloid in the thyroid and the capillary bed, stomach wall, and contents in the case of the stomach.

Active uptake into the thyroid colloid, stomach contents, and skin were described using Michaelis-Menten kinetics for nonlinear processes (Figure 6-6, bold arrows). Permeability area cross products and partition coefficients were used to describe the first order movement of the anions (ClO_4^- and I^-) between the capillary bed, tissue, and inner (deep) compartments (Figure 6-6, small arrows) that results from the inherent electrochemical gradient within the tissues. Passive diffusion through the kidney, liver, and fat compartments were described with partitions and blood flows. Plasma binding of perchlorate was described with Michaelis-Menten terms for the association of the perchlorate anions to plasma binding sites and a first order clearance rate for the dissociation. First-order clearance rates from the kidney were also used to describe urinary clearance of the anions.

The blood compartment differs between the perchlorate and iodide models. The perchlorate blood compartment is composed of plasma and plasma proteins to simulate binding. Plasma binding was required to simulate serum perchlorate concentrations at lower doses. Iodinated hormones bind to plasma proteins, but free iodide apparently does not. Therefore, a single compartment for plasma iodide was used. The free anions in plasma are available for diffusion and active uptake into tissues.

The presence of NIS is an indicator of active uptake for iodide. NIS is highly expressed in thyroid epithelial cells. Lower levels of expression have been detected in the mammary gland, salivary gland, skin, stomach, and colon (Ajjan et al., 1998; Spitzweg et al., 1998). However, only the thyroid has been found to organify iodide (Ajjan et al., 1998). The most important regulator of symporter gene and protein expression is thyroid-stimulating hormone (TSH). This is also the case for other important thyroid proteins such as thyroglobulin and thyroid peroxidase (Spitzweg et al., 1998).

The parameters used in the adult male rat and human model for the various compartments are provided in Table 6-1. The parameters were based on literature values or fitted to data using the model as described in the table. It is important to note that the model structure for both species is the same. The difference, per typical for PBPK models, is that there are species- and chemical-specific parameters for each. For example, the volume of the thyroid (as percent of

TABLE 6-1. PHYSIOLOGICAL PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS (Merrill, 2001c,d)

Physiological Parameters				
Tissue Volumes	**Male Rat**	**Source**	**Human**	**Source**
Body Weight BW (kg)	0.3	Measured (rat specific)	~70.0	Subject-specific
Slowly Perfused VSc (%BW)	74.6	Brown et al., 1997	65.1	Brown et al., 1997
Richly Perfused VRc (%BW)	11.0	Brown et al., 1997	12.4	Brown et al., 1997
Fat VFc (%BW)	7.4	Brown et al., 1997	♂ 21.0 ♀ 2.7	Brown et al., 1997
Kidney VKc (%BW)	1.7	Brown et al., 1997	0.44	Brown et al., 1997
Liver VLc (%BW)	5.5	Brown et al., 1997	2.6	Brown et al., 1997
Stomach Tissue VGc (%BW)	0.54	In house male rat ClO_4^- kinetics (Yu et al., 2000)	1.7	Brown et al., 1997
Gastric Juice VGJc (%BW)	1.68	In house male rat ClO_4^- kinetics (Yu et al., 2000)	0.071	Licht and Deen, 1988
Stomach Blood VGBc (%VG)	4.1	Altman & Dittmer, 1971b	4.1	Altman & Dittmer, 1971a
Skin Tissue VSkc (%BW)	19.0	Brown et al., 1997	3.7	Brown et al.,1997
Skin Blood VSkBc (%VSk)	2.0	Brown et al., 1997	8.0	Brown et al.,1997
Thyroid Vttotc (%BW)	0.0077	Malendowicz, 1977	0.03	Yokoyama et al., 1986
Thyroid Follicle VTc (%Vttot)	59.9	Malendowicz, 1977	57.3	Brown et al.,1986
Thyroid Colloid VDTc (%VTtot)	24.4	Malendowicz, 1977	15.0	Brown et al.,1986
Thyroid Blood VTBc (%VTtot)	15.7	Malendowicz, 1977	27.6	Brown et al.,1986
Plasma Vplasc (%BW)	4.1	Brown et al., 1997, Altman & Dittmer, 1971a	4.4	Marieb, 1992; Altman & Dittmer, 1971b
Red Blood Cells VRBCc (%BW)	3.3	Brown et al., 1997, Altman & Dittmer, 1971a	3.5	Marieb, 1992; Altman & Dittmer, 1971b
Adjusted Slowly Perfused VS (L)	0.138	Calculated from model	28.0	Calculated from model
Adjusted Richly Perfused VR (L)	0.01	Calculated from model	5.34	Calculated from model

TABLE 6-1 (cont'd). PHYSIOLOGICAL PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS (Merrill, 2001c,d)

Physiological Parameters				
Tissue Volumes	**Male Rat**	**Source**	**Human**	**Source**
Blood Flows				
Cardiac Output QCc (L/hr-kg)	14.0	Brown et al., 1997, Hanwell & Linzell, 1973	16.5	Brown et al., 1997; Hanwell & Linzell, 1973
Slowly Perfused QSc (%QC)	24.0	Brown et al., 1997	5.2	Brown et al., 1997
Richly Perfused QRc (%QC)	76.0	Brown et al., 1997	17.5	Brown et al., 1997
Fat QFc (%QC)	6.9	Brown et al., 1997	22.0	Brown et al., 1997
Kidney QKc (%QC)	14.0	Brown et al., 1997	1.0	Leggett & Williams, 1995; Malik et al., 1976
Liver QLc (%QC)	17.0	Brown et al., 1997	1.6	Brown et al., 1997
Stomach QGc (%QC)	1.61	Malik et al., 1976	13.0	Calculated, using 24% QC as flow to all slowly perfused tissues (Brown et al., 1997)
Skin QSkc (%QC)	5.8	Brown et al., 1997	33.0	Calculated, using 76% QC as flow to all richly perfused tissues (Brown et al., 1997)
Thyroid QTc (%QC)	1.6	Brown et al., 1997		
Adjusted Slowly Perfused QS (%QC)	11.3	Calculated from model		
Adjusted Richly Perfused QR (%QC)	41.8	Calculated from model		

body weight), the maximum capacity of thyroid iodide or perchlorate uptake, and plasma binding of perchlorate. The chemical-specific parameter for each model for both perchlorate and iodide are provided in Table 6-2.

In order to simulate the daily dosing regimen of the drinking water experiment, the rats were assumed to drink at constant rate for 12 of the 24 hours per day (1800 to 0600 hours). A pulse function in ACSL was used to introduce drinking water to the gastrointestinal (GI) compartment of the rat for the first 12 hours of each 24-hour period and to stop dosing while the rat was presumably sleeping. Intravenous (iv) dosing was introduced into the venous blood compartment of the model. Intraperitoneal (ip) injection was introduced into the model in the same manner as the iv dosing.

6.2.1 Data and Methods

This section summarizes the AFRL/HEST data and data available in the literature that were used for model development. Details on experimental methods, including protocol design, exposure regimen, chemical source and purity, animals (housing, feeding, surgical procedures, etc), and the analytical methods for measurement of RAIU; of perchlorate in plasma, urine and tissues; and of thyroid hormones and TSH can be found in the associated consultative letters from AFRL/HEST (Merrill, 2001c,d; Yu, 2000, 2001, 2002; Yu et al., 2000).

6.2.1.1 Studies in Laboratory Rats

The studies performed at AFRL/HEST included both "acute" iv experiments to measure radiolabled iodide or perchlorate as well as measurements of the same after drinking water administration. These two different regimens provided a better characterization of the transient ("acute") and chronic behavior necessary for an accurate description of the disposition of the anions. Adult male Sprague-Dawley rats (330 ± 35 g; n = 6 rats per group) that were purchased from Charles River Laboratory (Raleigh, NC) were used in the experiments.

In these experiments, the term total iodine includes bound iodine plus fee inorganic iodide. Carrier doses included tracer doses of carrier free radiolabled iodide ($^{125}I^-$) along with non-radiolabeled iodide. Free $^{125}I^-$ radioactivity was determined by subtracting the bound from total measurements (Merrill, 2001c; Yu, 2000, 2001, 2002; Yu et al., 2000).

TABLE 6-2. CHEMICAL-SPECIFIC PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS (Merrill, 2001c,d)[a]

Partition Coefficients (unitless)	Male Rat			Human		
	Perchlorate	Iodide	Source	Perchlorate	Iodide	Source
Slowly Perfused/Plasma PS_	0.31	0.21	Yu et al., 2000; Halmi et al., 1956	0.31	0.21	Halmi et al., 1956;Yu et al., 2000
Richly Perfused/Plasma PR_	0.56	0.40	Yu *et al.*, 2000; Halmi *et al.*, 1956	0.56	0.40	Halmi *et al.*, 1956;Yu *et al.*, 2000
Fat/ Plasma PF_	0.05	0.05	Pena et al., 1976	0.05	0.05	Pena et al., 1976
Kidney/Plasma PK_	0.99	1.09	Perlman et al., 1941	0.99	1.09	Perlman et al., 1941; Yu et al., 2000
Liver/Plasma PL_	0.56	0.44	Perlman et al., 1941	0.56	0.44	Perlman et al., 1941; Yu et al., 2000
Gastric Tissue/Gastric Blood PG_	1.80	1.40	Yu et al., 2000; Yu, 2000	1.80	0.50	Yu et al., 2000;Yu, 2000
Gastric Juice/Gastric Tissue PGJ_	2.30	3.00	Yu et al., 2000; Yu, 2000	2.30	3.50	Yu et al., 2000; Yu, 2000
Skin Tissue/Skin Blood PSk_	1.15	0.70	Yu, 2000, Perlman et al., 1941	1.15	0.70	Perlman et al., 1941; Yu, 2000
Thyroid Tissue/Thyroid Blood PT_	0.13	0.15	Chow & Woodbury (1970)	0.13	0.15	Chow & Woodbury (1970)
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	Chow & Woodbury (1970)	7.00	7.00	Chow & Woodbury (1970)
Red Blood Cells/Plasma	0.80	1.00	Yu et al., 2000; Rall et al., 1950	0.80	1.00	Rall et al., 1950; Yu et al., 2000
Max Capacity, Vmaxc (ng/hr-kg)						
Thyroid Colloid Vmaxc_DT	1.0E+04	4.0E+07	Fitted	2.5E+5	1.0E+8	Fitted
Thyroid Follicle Vmaxc_T	2.2E+03	5.5E+04	Fitted	5.0E+4	~1.5E+5	Fitted
Skin Vmaxc_S	6.2E+05	5.0E+05	Fitted	1.0E+6	7.0E+5	Fitted
Gut Vmaxc_G	3.0E+05	1.0E+06	Fitted	1.0E+5	9.0E+5	Fitted
Plasma Binding Vmaxc_Bp	9.5E+03	—	Fitted	5.0E+2	—	Fitted

TABLE 6-2 (cont'd). CHEMICAL-SPECIFIC PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS (Merrill, 2001c,d)[a]

Partition Coefficients (unitless)	Male Rat			Human		
	Perchlorate	Iodide	Source	Perchlorate	Iodide	Source
Affinity Constants, Km (ng/L)						
Thyroid Lumen Km_DT	1.0E+08	1.0E+09	Golstein et al., 1992	1.0E+8	1.0E+9	Golstein et al., 1992
Thyroid Km_T	2.5E+05	4.0E+06	Gluzman & Niepomniszcze, 1983; Wolff, 1998	1.8E+5	4.0E+6	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Skin Km_S	2.0E+05	4.0E+06	Gluzman & Niepomniszcze, 1983; Wolff, 1998	2.0E+5	4.0E+6	Gluzman & Niepomniszcze, 1983; Wolff 1998
Gut Km_G	2.0E+05	4.0E+06	Gluzman & Niepomniszcze, 1983; Wolff, 1998	2.0E+5	4.0E+6	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Plasma binding Km_B	1.1E+04	—	Fitted	1.8E+4	—	Fitted
Permeability Area Cross Products (L/hr-kg)						
Gastric Blood to Gastric Tissue PAGc_	0.80	0.10	Fitted	0.6	0.2	Fitted
Gastric Tissue to Gastric Juice PAGJc_	0.80	0.10	Fitted	0.8	2.0	Fitted
Skin Blood to Skin Tissue PASkc_	1.0	0.10	Fitted	1.0	0.06	Fitted
Plasma to Red Blood Cells PARBCc_	0.10	1.00	Fitted	1.0	1.0	Fitted
Follicle to thyroid blood PATc_	4.0E-05	1.0E-04	Fitted	1.0E-4	1.0E-4	Fitted
Lumen to Thyroid Follicle PADTc_	0.01	1.0E-04	Fitted	0.01	1.0E-4	Fitted
Clearance Values (L/hr-kg)						
Urinary excretion CLUc_	0.07	0.05	Fitted	0.126	0.1	Fitted
Plasma unbinding Clunbc__	0.1	—	Fitted	0.025	—	Fitted

[a]All parameters listed are notated in the model by either an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR_*i*, PR_*p*, Vmaxc_T*i*, Vmaxc_T*p*, etc.).

6.2.1.1.1 Acute iv Experiments in Rats

Radiolabeled iodide ($^{125}I^-$) kinetics. Male rats were administered a single iv tail-vein injection with physiological saline (control group) or 33 mg/kg $^{125}I^-$ (with carrier) in physiological saline. Rats were euthanized by CO_2 asphyxiation at 5, 15, and 30 minutes (min), 1, 2, 6, 9, 24, 32, 48, and 96 hours (hr) post dosing to collect thyroid and blood from the vena cava. Rats for the 24 hour time point were placed individually in metabolism cages to collect urine.

In an additional study, male rats were intravenously dosed with 33 mg/kg $^{125}I^-$ (with carrier) and euthanized at 0.5, 2 and 6 hours post dosing. Total, bound, and free $^{125}I^-$ were analyzed in thyroid and serum, and total $^{125}I^-$ was measured in skin and gastric contents (Yu, 2001).

Radiolabeled $^{36}ClO_4^-$ kinetics. Naïve adult male rats (300 ± 20 g) were dosed once by iv tail-vein injection with 3.3 mg/kg radiolabeled perchlorate. Due to the low specific activity, a smaller dosing level could not be achieved. Each rat received less than 6 μCi. Rats were euthanized by CO_2 asphyxiation at 0.5, 6, 12, 24, 32, and 48 hours after dosing. The thyroid, intestinal tract, intestinal tract contents, muscle, skin, liver, kidney, spleen, bladder, plasma, and red blood cells were harvested from the rats and stored at -20°C until analysis of $^{36}ClO_4^-$. Rats for 12, 24, 32, and 48 hours time points were placed individually in metabolism cages for urine collection. Metabolism cages were washed with 500 mL de-ionized water. Urine and cage wash samples were stored under the same conditions until analysis.

$^{125}I^-$ Kinetics and Inhibition from Acute iv Dosing with ClO_4^-. Rats were injected with one of five doses of perchlorate (0.0, 0.01, 0.1, 1.0, and 3.0 mg/kg). At 2 hours post dosing, they were challenged with $^{125}I^-$ with carrier (33 mg/kg) by intravenous injection and euthanized at 5, 15, and 30 min, 1, 2, 6, 9, and 24 hours post dosing of iodide. This corresponds to 2.08, 2.25, 2.5, 3, 4, 8, 11, and 26 hours, respectively, after dosing with perchlorate. Blood and thyroid were harvested from all time point groups; urine was collected from rats in the 24 hours dose group. Perchlorate and iodide levels were determined in the thyroid, serum and urine.

In an additional study, three rats were intravenously dosed with 0.0, 0.1, and 1.0 mg/kg perchlorate and challenged two hours later with 33 mg/kg $^{125}I^-$. Rats were euthanized at 15 min, 1, 2, and 4 hours after they were dosed with iodide. Levels of perchlorate and $^{125}I^-$ were determined in thyroid, serum, skin and gastric contents (Yu, 2001).

6.2.1.1.2 Drinking Water Studies in Rats

Three drinking water studies (1, 5, and 14 days) were performed with target perchlorate concentrations of 0.0, 1.0, 3.0, 10.0, and 30.0 mg/kg-day with adult male rats continually exposed via drinking water. At the end of day 1, 5, or 14, rats (n=6 per group) were challenged once with 33 mg/kg $^{125}I^-$ with carrier and euthanized at 2 hours post iodide dosing. Blood and thyroid gland were collected for ClO_4^- and $^{125}I^-$ analyses in serum. For the 10 and 30 mg/kg dose groups, perchlorate was measured in serum and thyroid on day 5; however, the iodide inhibition study for these dose groups was conducted on Day 14.

6.2.1.2 Human Studies

The data used in development of the Merrill (2001d) human model were obtained from Hays and Solomon (1965) or recent data, both published and unpublished, that underwent the QA/QC check described in the introduction of this chapter (Merrill, 2001a,b). These data included the published and unpublished data from a human study of drinking water exposure to perchlorate that measured RAIU in the thyroid (Greer et al., 2000).

Data supporting model validation were obtained from another unpublished drinking water study conducted under contract to AFRL/HEST by Drs. Holger Leitolf and Georg Brabant of the Medizinische Hoschschule, Hanover, Germany. Urinary perchlorate clearance data by Eichler (1929), Kamm and Drescher (1973), and Durand (1938) were also used to validate model predictions.

6.2.1.2.1 Human Iodide Kinetic Data (Hays and Solomon, 1965)

A comprehensive human kinetic study on early iodide distribution was reported in 1965 by Hays and Solomon. The authors studied the effect of gastrointestinal cycling on iodide kinetics in nine healthy males after an iv dose of 10 μCi radiolabeled iodide ($^{131}I^-$), approximately 3.44×10^{-3} ng $^{131}I^-$/kg body weight. Frequent measurements of radioiodide uptake in the thyroid, gastric secretions, plasma, and cumulative urine samples were taken during the three hours following injection. Gastric secretions were collected using a nasogastric tube with constant suction while the subjects remained in a resting position (only standing to urinate). Saliva was not collected separately and therefore pooled, to some extent, with gastric juices. To account for the removal of gastric iodide from circulation and to determine its impact on free iodide

distribution, the authors ran a control session on the same subjects without aspirating gastric secretions. Aspirated gastric secretions accounted for 23% of the $^{131}I^-$ administered.

6.2.1.2.2 Perchlorate Kinetics and Inhibition of Thyroid Iodide Uptake (Greer et al., 2000)

Perchlorate data. As described in Chapter 4, Greer et al. (2000) recently studied the effects of repeated low level exposure to perchlorate on humans. Subjects received 0.5, 0.1, 0.02, or 0.007 mg/kg-day perchlorate in drinking water over a two week period. Each dose group consisted of eight healthy volunteers (four males and four females) with no signs or symptoms of thyroid disorders (euthyroid). The daily dose was dissolved in 400 mL water and divided into four 100 mL servings that were ingested at approximately 0800, 1200, 1600, and 2000 hours.

Baseline serum and urine samples were collected before the first perchlorate treatment. During perchlorate exposure, serum samples were collected at the following approximate times: day 1 at 1200 and 1600, day 2 at 0800, 1200, and 1700, day 3 at 0900, day 4 at 0800 and 1200, day 8 between 0800 and 0900 and day 14 at 0800 and 1700. Serum samples were also collected on post-exposure days 1, 2, 3, and 14. Twenty-four hour urine collections were taken on exposure days 1, 2, 14 and post-exposure days 1 through 3. Serum and 24-hour urine samples from the study were provided to AFRL/HEST compliments of Dr. Monte Greer of Oregon Health Science University (OHSU), Portland, OR, and Dr. Gay Goodman of Intertox, Seattle, WA. The samples were analyzed for perchlorate at the Operational Toxicology Branch, Human Effectiveness Directorate at the Air Force Research Laboratory (AFRL/HEST), Wright Patterson Air Force Base (WPAFB), OH, using the analytical methods described in Merrill (2001d).

Iodide Inhibition Data. Eight and 24 hour thyroid $^{123}I^-$ uptakes (radioiodine uptake or RAIU) were measured one to two days prior to perchlorate treatment (baseline) on days 2 and 14 of perchlorate exposure and 14 days after perchlorate exposure was discontinued. A gelatin capsule containing 100 mCi of $^{123}I^-$ was administered orally at 0800, before the first perchlorate solution for that day was drunk. Thyroid scans were then taken 8 and 24 hours later.

Thyroid and Pituitary Hormone Data. The serum samples were also analyzed for TSH, T4, T3, and free T4 at OHSU. However, these hormone data were not used in the PBPK model described below. Statistical analysis of the data is described in Attachment 2 of Merrill (2001d).

In summary, there was little effect of perchlorate on levels of T4, free T4, or T3. TSH decreased significantly from baseline by Exposure Day 3. On Post-Exposure Day 1, the TSH

levels of the subjects in the 0.5 mg/kg-day group had decreased by an average of 35% from baseline (ranging from 17% to 52%). Therefore, it appears that TSH was dropping while inhibition remained the same. It is possible that there is an increase in thyroid sensitivity to TSH as an early response to inhibition (Brabant et al., 1992). This increased sensitivity (possibly an increase affinity of the TSH receptor) could possibly decrease circulating TSH levels while T4 has not decreased sufficiently yet to stimulate the hypothalamus to increased TRH secretions. After perchlorate was discontinued, between Post-Exposure Days 1 and 15, the mean TSH level increased significantly over baseline (23% greater than baseline), with TSH of one subject remaining below baseline. The drop in TSH during perchlorate exposure and the rise above baseline measurements after perchlorate seem counter-intuitive to the TSH regulation expected but may be part of a rebound phenomenon as the NIS begins to upregulate.

In addition, the data by Greer et al. (2000) showed an increase in radioiodide uptake in excess of baseline measurements 14 days after perchlorate exposure. An increase in radioiodide uptake is expected due to the rise in TSH mentioned above. This rebound effect has been noted in other human inhibition studies (using both iodide and perchlorate as inhibitors). Saxena et al. (1962) evaluated the prophylactic doses of iodide required to suppress thyroid uptake of $^{131}I^-$ in euthyroid mentally defective children. They found a minimal effective oral dose of 1500 to 2000 μg iodide per square meter of body surface per day was required to completely suppress $^{131}I^-$ uptake. Within a week after iodide administration was stopped, a rebound of uptake was noted. In some instances these uptakes were even higher in subsequent weeks.

6.2.1.2.3 Supporting Kinetic Studies

Both urine and serum perchlorate concentrations for a validation exercise were provided from a recent unpublished study by Drs. Brabant and Leitolf of Medizinische Hochschule, Hanover, Germany. In their study, seven healthy males ingested 12.0 mg/kg perchlorate dissolved in 1 liter of drinking water every day for two weeks. The daily perchlorate dose was divided equally in three portions and ingested three times per day (approximately between 0600 and 0800, 1100 and 1300 and 1800, and 2000 hours). Blood specimens were collected on days 1, 7, and 14 of perchlorate treatment and on the two mornings after perchlorate administration was discontinued. Samples were analyzed for perchlorate at AFRL/HEST.

Three published studies reported cumulative urine concentrations collected from healthy males after receiving a high oral dose of perchlorate (Durand, 1938; Kamm and Drescher, 1973; Eichler, 1929). Oral doses administered in these studies were 784 mg $NaClO_4$ (635 mg ClO_4^-; Durand, 1938); 1000 mg $NaClO_4$ (765 mg ClO_4^-; Kamm and Drescher, 1973), and 2000 mg $KClO_4$ (1400 mg ClO_4^-; Eichler, 1929). The studies did not report serum perchlorate levels but could be used to validate the model.

Stanbury and Wyngaarden (1952) measured radioiodide uptake in a patient with Grave's disease. The patient received a tracer dose of $^{131}I^-$ as a control before perchlorate dosing and again one hour after administration of 100 mg $KClO_4$. Thyroid scans of radioiodide uptake were performed both after the control and perchlorate sessions to determine the level of inhibition.

6.2.2 Adult Male Rat Model Development

This section summarizes some key features necessary to the development of the adult male rat model structure and shows results of predictions made with simulations against experimental data used to parameterize and validate the model.

6.2.2.1 Physiologic Parameters and Tissue Partition Coefficients

The adult male rat volumes and blood flows were obtained from the literature or the AFRL/HEST studies as described in Table 6-1. Allometric scaling was used to account for parameter differences due differences in body weights between rats and humans. Because no steady-state values from infusion studies were available, the partition coefficients for iodide and perchlorate were estimated from the various studies listed in Table 6-2. The liver:serum and muscle:serum ratios of 0.56 and 0.31 were obtained in the AFRL/HEST radiolabled perchlorate ($^{36}ClO_4^-$) iv study described above. The liver:serum partition value was used to represent partitioning to the liver and richly perfused compartments and the muscle:serum value to represent the slowly perfused compartment.

For compartments with nonlinear uptake of the anions, effective partition coefficients were used that represented either approximate tissue:serum concentration ratios or electrical potential gradients. Chow and Woodbury (1970) measured electrochemical potentials within the thyroid stroma, follicular membrane, and colloid at three different doses of perchlorate. The measured difference in electrical potential between the thyroid stroma and follicle was interpreted by

Merrill (2001c) as an effective partition coefficient for the perchlorate and iodide anions, hindering the entry of negatively charged ions into the follicle. The equal and opposite potential from the follicle to the colloid enhances passage of negatively charged species into the colloid and indicates an effective partition coefficient of greater than one. The equivalence between electrical potential differences $\phi_i - \phi_f$ and effective partition coefficients for the thyroid subcompartments (stroma:follicle and follicle:colloid) were estimated in the manner of Kotyk and Janacek (1977) based on the Chow and Woodbury (1970) data as described in Merrill (2001c).

6.2.2.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities, and clearance rates (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the adult male rat model were derived. The values can be found in Table 6-2 and details on derivation in Merrill (2001c).

6.2.2.2.1 Affinity Constants and Maximum Velocities for Active Transport Processes

Kinetic values for the saturable (Michaelis-Menten) active uptake process of perchlorate, the affinity constant and maximum velocity capacity (Km_*p* and Vmaxc_*p*), were not available in the literature nor were they determined experimentally at AFRL/HEST. Only the affinity of iodide for NIS was available in the literature. The Merrill (2001c) adult rat model uses a Michaelis-Menten affinity constant (Km) value of 4.0×10^6 ng/L to describe the affinity of iodide (Km_*i*) across compartments involving active transport by NIS (e.g., in the thyroid and gastric juices). This was based on the mean value of 3.96×10^6 ng/L for iodide derived by Gluzman and Niepomniszcze (1983) from thyroid slices of 5 normal individuals. The thyroid slices were incubated with several medium iodide concentrations. The experimentally determined Km values for iodide are similar across species (Gluzman and Niepomniszcze, 1983) and across different tissues (Wolff, 1998). This average literature value was therefore used for iodide in tissues described with active uptake.

The values for perchlorate affinity were originally assumed to be the same as those for the Km of iodide, due to the similar mechanism in which the two anions are transported into the tissues. Thus, the iodide values were adjusted for the difference in mass to give an estimated

value for the affinity of perchlorate. The molar equivalent of iodide's Km for perchlorate is 3.1×10^6 ng/L. However, these values were not adequate for use in the models. Several literature sources suggest that perchlorate may have a significantly higher affinity for NIS than iodide. In his 1963 paper (Wolff and Maurey, 1963) and his 1998 review, Wolff concluded that perchlorate has a greater affinity than iodide for the NIS. This assumption was based upon his work with iodide, perchlorate, and several other anions actively sequestered in the thyroid. Wolff measured the Km of a few of the anions and inhibition constants (Ki's) for several ions, including perchlorate. As noted in Chapter 2, Wolff found that the relative potency of inhibition by the various anions could be described with the following series: TcO_4^->ClO_4^->ReO_4^->SCN^- >BF_4^-> I^->NO_3^->Br^->Cl^-. Wolff reported that the measured Km values for several of these inhibiting anions were not the same as those measured for iodide. In fact, measured values for Km and Ki for several of the inhibiting anions revealed that affinity increased with increased inhibitory potency.

Several studies suggest perchlorate is a more potent inhibitor than iodide. In the rat thyroid, Wyngaarden et al. (1952) have shown that perchlorate was a more powerful inhibitor of the iodide trap than thiocyanate. Halmi and Stuelke (1959) showed that perchlorate was ten times as effective as iodide in depressing tissue to blood ratios in the rat thyroid and gut. Similarly, Harden et al. (1968) compared human saliva to plasma radioiodide concentration ratios after equimolar doses of perchlorate and iodide. The saliva:plasma iodide ratios during resting conditions were approximately seven times lower after a molar equivalent dose of perchlorate versus iodide. Lazarus et al. (1974) also demonstrated that perchlorate was taken up to greater extent in mice salivary glands than iodide. These studies, in addition to the work of Chow et al. (1969), support the use of a lower Km for perchlorate uptake in the tissues with sodium iodide symporter. Based on this information, a value of 2.5×10^5 ng/L for the thyroid (Km_T*p*) and 2.0×10^5 ng/L for skin (Km_S*p*) or gut (Km_G*p*), approximately 10 times lower than that of iodide, was estimated by Merrill (2001c,d) to represent perchlorate's affinity for transport by the NIS.

The apical follicular membrane (between the thyroid follicle and colloid) also exhibits a selective iodide uptake mechanism. Golstein et al. (1992) measured a Km value of approximately 4.0×10^9 ng/L for the transport of iodide between the thyroid follicle and colloid (Km_DT*p*) in bovine thyroid. This iodide channel also appears to be very sensitive to

perchlorate inhibition and shares a similar permeability to perchlorate as to iodide. The ability of perchlorate to inhibit iodide uptake at the apical follicular membrane suggests that the Km of perchlorate at the apical follicular membrane (Km_Dt*p*) is also lower than that of iodide. Model simulations of thyroid inhibition supported a value of 1.0×10^8 ng/L, approximately ten times less than that of iodide.

Whereas the Km is similar across tissues containing NIS, the maximum velocity term (Vmaxc) does vary between tissues and species (Wolff, 1998), being lower in humans than other species (Gluzman and Niepomniszcze, 1983; Wolff and Maurey, 1961). Maximum velocities or capacities (Vmaxc) were not found in the literature and were estimated for a given compartment by fitting the simulation to the data at varying doses.

6.2.2.2.1 Effective Partitions, Permeability Area Cross Products and Clearance Values

Permeability area cross products and partition coefficients were used to describe diffusion limited uptake in tissues requiring subcompartments. The permeability area values in the Merrill (2001c) model were fitted by setting the partition coefficients to the literature values in Table 6-2. Fitted clearance values were used to describe first-order urinary excretion rates and reversible plasma binding to serum. Equations for these representations are provided in Merrill (2001c).

6.2.2.3 Adult Male Rat Model Simulation Results and Validation

The simulations shown in this section result from exercising the model with the physiological and chemical-specific parameters provided in Tables 6-1 and 6-2. Figure 6-7 illustrates the model predictions versus data time course for the iv radiolabeled perchlorate study described in Section 6.2.1.1.1. The model produced good simulations for the trend of the data but slightly over predicts the thyroid concentrations at later time points (Panel A). Model predictions fit the data well for perchlorate concentrations in the serum (Panel B) and kidney (Panel C), as well as the amount excreted in the urine (Panel D). Other tissue concentrations not shown herein also were predicted well by the model (Merrill, 2001c).

Figure 6-8 shows that plasma binding of perchlorate was necessary to provide adequate model predictions. Thyroid, serum, and urine were collected from the iv studies described in Section 6.2.1.1.1 using cold (i.e., not radiolabeled) perchlorate at 0.01, 0.1, 1.0, and 3.0 mg/kg.

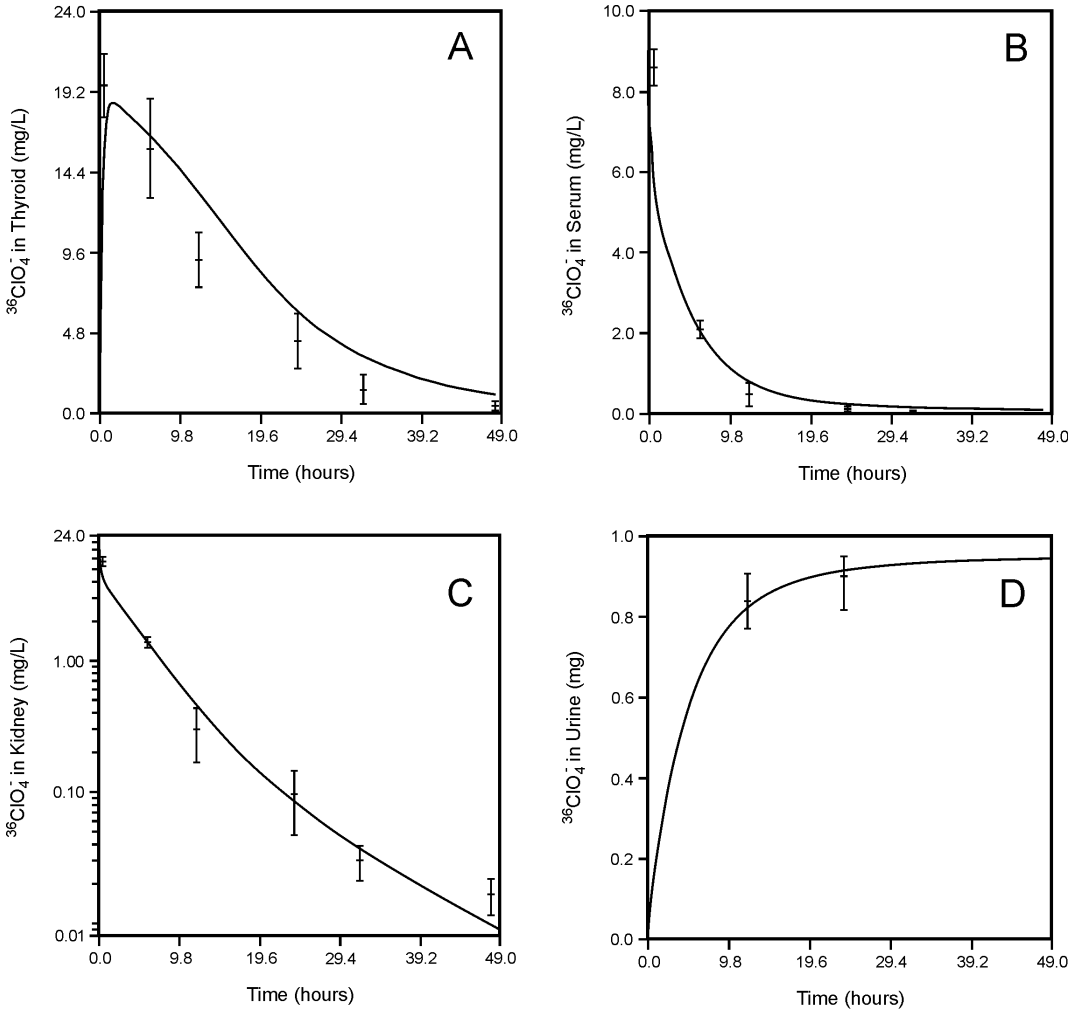

Figure 6-7. Adult male rat PBPK model predictions after an acute iv dosing with radiolabeled perchlorate ($^{36}ClO_4^-$). Panels A and B show model predictions (lines) versus data time course (mean ± SD) of labeled perchlorate (mg/L) in the thyroid and serum. Panel C shows model predictions versus data time course of labeled perchlorate (mg/L) in the kidney. Panel D shows cumulative excretion (mg) of labeled perchlorate in the urine (Merrill, 2001c).

Model predictions without plasma binding (Panel A, left) resulted in an underestimation of serum perchlorate concentrations at the 1 mg/kg-day dosage level and below. Low serum predictions suggested either greater uptake into other tissues or protein binding. To provide better estimates of perchlorate serum concentrations at the 0.01 and 0.1 mg/kg doses, Merrill (2001c) added protein binding to the venous blood compartment of the model. An affinity

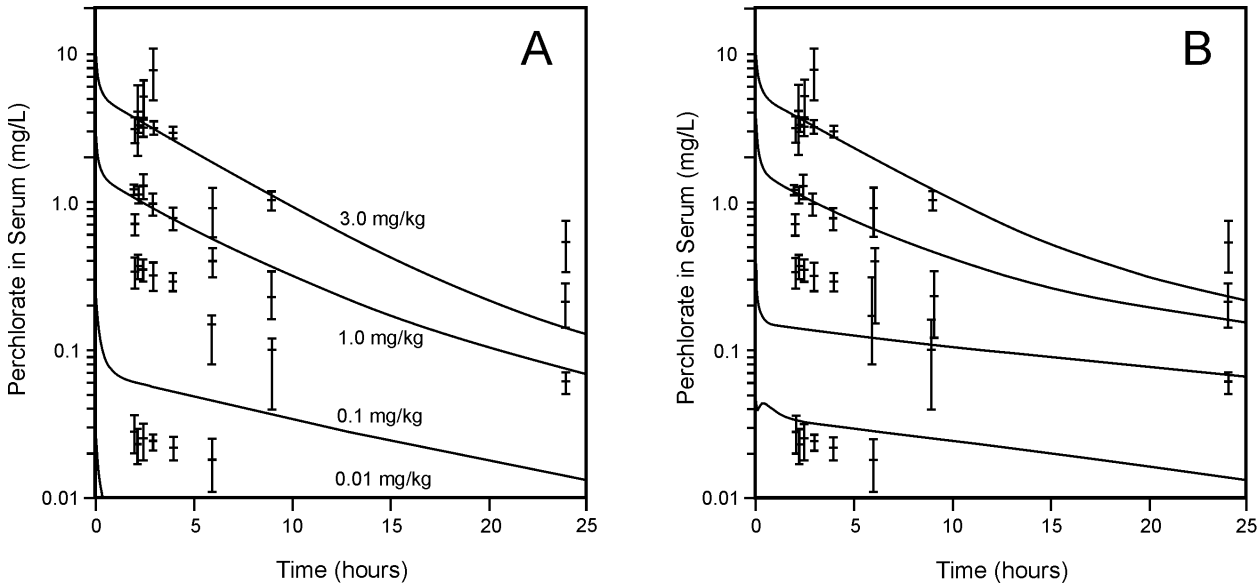

Figure 6-8. Simulations illustrating the necessity of including plasma binding in the adult male rat PBPK model structure (Merrill, 2001c). Model predictions (lines) versus data time course (mean ± SD) of perchlorate concentration (mg/L) in serum after doses of 3.0, 1.0, 0.1 and 0.01 mg/kg-day are shown in Panel A without and in Panel B with plasma binding. Only part of the simulation for the 0.01 dose in Panel A can be seen in the lower left corner. Data of Yu (2000).

constant for this binding of perchlorate in the blood (Km_B*p*) of 1.1E6 ng/L and a maximum velocity capacity for this blood binding (Vmaxc_B*p*) of 9.3E3 ng/h/kg was fitted to serum levels from doses ranging 0.01 to 3.0 mg/kg (Panel B, right). The model underpredicts serum perchlorate from the 0.1 mg/kg dose group; but it fits serum at 0.01 mg/kg and cumulative urine across the doses. Interestingly, the urinary excretion at 0.01 mg/kg was lower than the other doses, accounting for elevated serum concentrations. Mean 24 hour urinary excretions (± SD) of perchlorate were approximately 97% (± 2), 72% (± 1), 87% (± 17), and 91% (± 13) of the administered iv dose for the 0.01, 0.1, 1.0, and 3.0 mg/kg dose groups, respectively.

The literature discussed in Chapter 3 and in Merrill (2001c) suggests that serum albumin is the major binding protein; however, it does not confirm that albumin is the only binding site. Merrill (2001c) notes that no studies were found that evaluated whether perchlorate or similar anions bind to thyroglobulin. However, Yamada (1967) studied the effects of perchlorate and

other anions on T4 metabolism and noted a significant decrease in serum protein-bound iodide (PBI) in thyroidectomized T4-maintained perchlorate-fed rats. In a 1968 in vitro study, Yamada and Jones reported that T4 was displaced from plasma protein as indicated by an uptake of T4 by muscle in the presence of plasma taken from perchlorate-fed rats. This suggested, but did not demonstrate directly, that perchlorate interferes with T4 binding with plasma proteins.

Pertechnetate is known to bind to plasma proteins. Hays and Green (1973) studied the blocking of pertechnetate binding with human serum proteins by other anions. Perchlorate was found to be one of the most effective, while iodide was ineffective. In dialysis studies, inorganic iodide did not bind to plasma proteins. The pertechnetate binding appeared to be reversible in serum.

Simulations of thyroid perchlorate concentrations and of the amount of perchlorate excreted in the urine from the four dose groups are shown in Figure 6-9. It was noted that the thyroid concentrations resulting from the 3.0 mg/kg cold perchlorate study were slightly higher than those from the radiolabeled perchlorate ($^{36}ClO_4^-$) study at 3.3 mg/kg (Figures 6-9A and 6-7A, respectively). This may reflect the analytical differences in measuring cold versus radiolabeled perchlorate. The model slightly underpredicts the thyroid concentrations at 3.0 mg/kg, based on the cold perchlorate data (Figure 6-9A), and slightly overpredicts the $^{36}ClO_4^-$ thyroid concentration at 3.3 mg/kg (Figure 6-7A).

The model is able to adequately predict data from studies that were not used in the development process. Figure 6-10 shows the model predictions versus the data of Chow and Woodbury (1970) and Eichler (1929). Model predictions fit the data well for radiolabeled perchlorate concentration in the thyroid (A); whereas, the serum (B) is underpredicted. Merrill (2001c) notes the difference and provides some plausible explanations. The rats in the Chow and Woodbury (1970) study were functionally nephrectomized by ligating the renal pedicle of both kidneys and given the radiolabeled perchlorate ip. Analytical differences between AFRL/HEST and Chow and Woodbury could exist, and it is also possible that the nephrectomization creates physiological changes that can not be accounted for sufficiently by "turning off" urinary excretion in the model simulations. One hypothesis is that saturation in NIS-containing tissues occurs to a lesser extent as a result of increased extracellular sodium cation (Na^+) and possibly other competitive anions when renal clearance is blocked, thereby increasing the arterial radiolabeled perchlorate. While the underprediction in serum would suggest the need for an

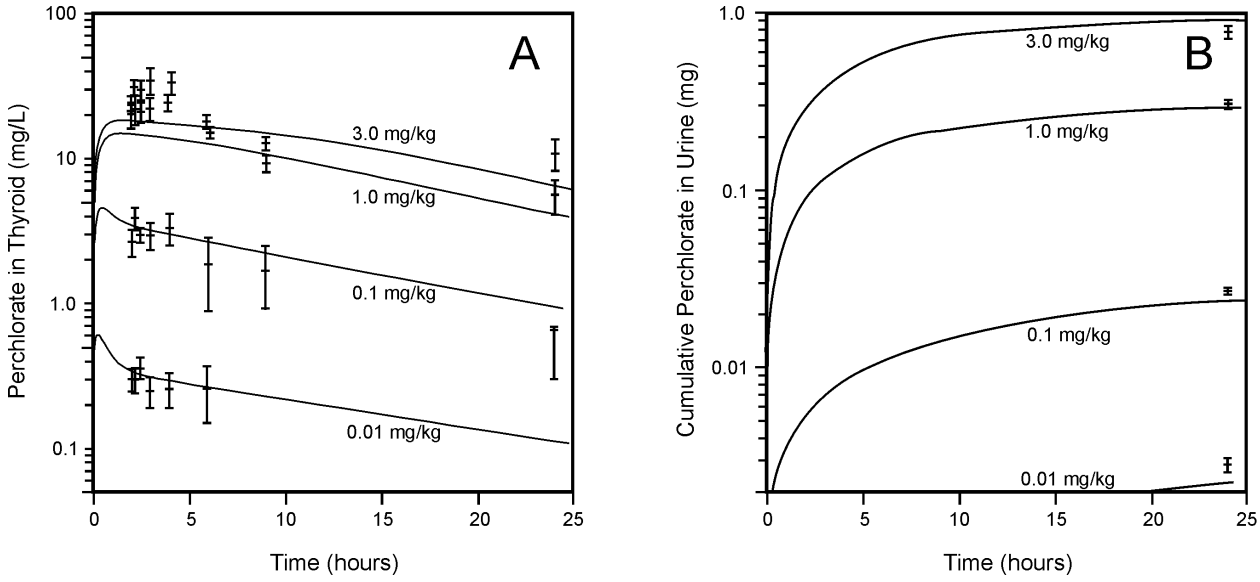

Figure 6-9. Adult male rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations in the thyroid (mg/L) in Panel A or cumulative excreted perchlorate in the urine (mg) in Panel B (Merrill, 2001c). Male rats were dosed iv with 3.0, 1.0, 0.1 or 0.01 mg/kg-day perchlorate (Yu, 2000).

increased binding constant for perchlorate, this was not consistent with the data from AFRL/HEST for studies at lower doses (Merrill, 2001c). Panel C in Figure 6-10 shows the model predictions versus the data of Eichler (1929) for cumulative perchlorate excreted in the urine. These rats were given perchlorate subcutaneously (sc) at doses of 1.6, 8.0, and 49 mg/kg.

The adult male rat model (Merrill, 2001c) is also able to predict iodide distribution. Figure 6-11 shows the model predictions versus a time course for radiolabeled iodide data from the AFRL/HEST experiments outlined in Section 6.2.1.1.1. Adequate fit is demonstrated for both the thyroid and serum concentrations at doses of radiolabeled iodide differing by an order of magnitude (0.033 and 0.33 mg/kg).

Figure 6-12 demonstrates the fit of the model simulations of perchlorate thyroid concentration (mg/L) after drinking water exposures to perchlorate. The model was coded to simulate oral dosing for 12 hours per day, assuming that rats drink fairly continuously during their waking hours. The same perchlorate parameters used to describe the "acute" (iv) kinetics also adequately described serum concentrations from these "chronic" drinking water exposures

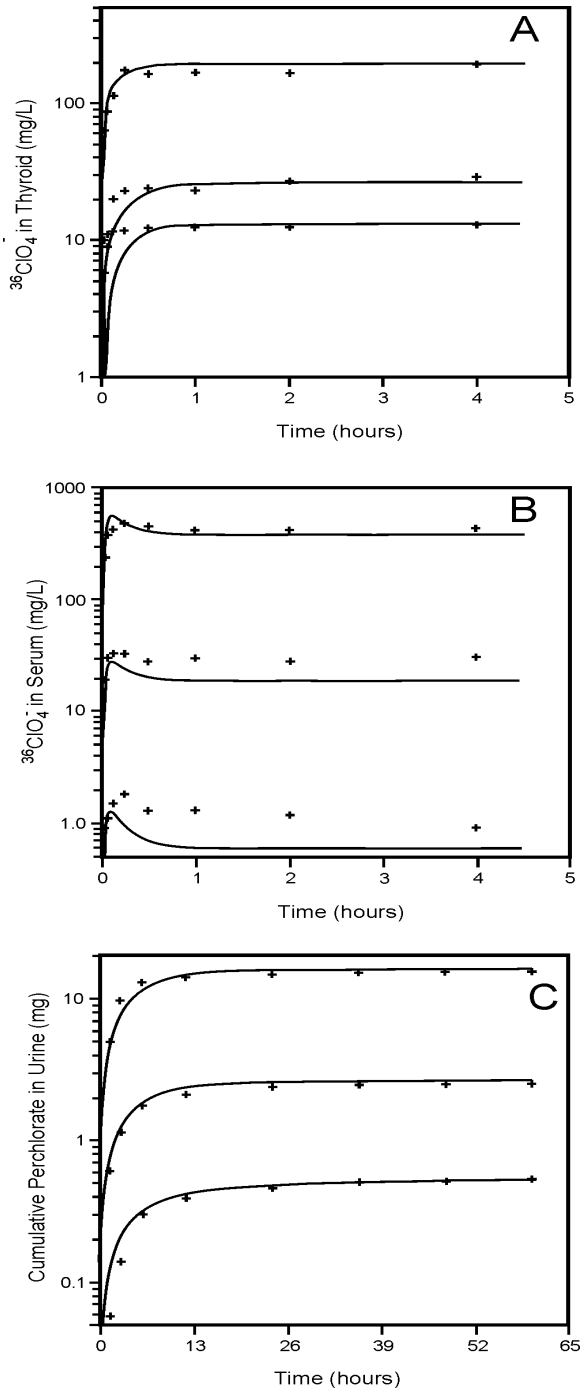

Figure 6-10. Validation for male rat PBPK model of perchlorate disposition (Merrill, 2001c). Model predictions (lines) versus data time course for concentrations (mg/L) in the thyroid (A) and serum (B) for ip administration in rats of 200, 10, and 0.5 mg/kg $^{36}ClO_4^-$ (data of Chow and Woodbury, 1970). Panel C shows model predictions (lines) and data time course for cumulative perchlorate in the urine (mg) of male rats after subcutaneous doses of 1.6, 8.0, and 49 mg/kg (data of Eichler, 1929).

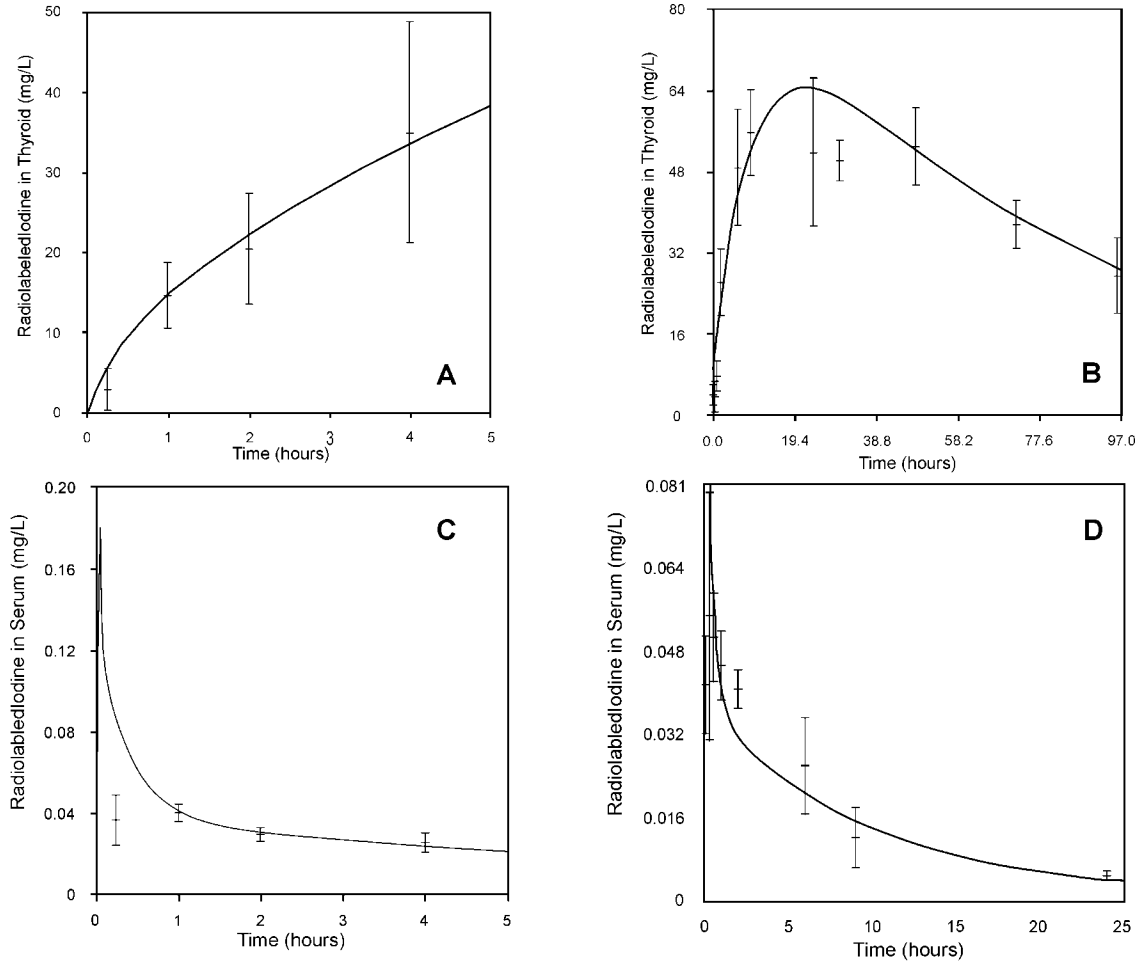

Figure 6-11. Male rat PBPK model (Merrill, 2001c) predictions (lines) versus data time course (mean ± SD) of iodide concentrations (mg/L) at two doses of $^{125}I^-$ with carrier, 0.033 mg/kg or 0.33 mg/kg, in the thyroid (A) or (B) and in the serum (C) or (D). Data of Yu (2001).

(data shown in Merrill, 2001c) but failed to predict thyroid concentrations from the 3.0 mg/kg-day dose and higher. TSH in these same studies was increased during drinking water exposure across all doses so that Merrill (2001c) accounted for the TSH-induced upregulation in the NIS by fitting an increased effective thyroid follicle:stroma partition coefficient (PT_*p*) at these higher doses. Merrill (2001c) noted that TSH is not expected to increase NIS in tissues other than the thyroid (Brown-Grant, 1961**)** and that these simulations agree. Given the small size of the thyroid, its upregulation would not decrease serum concentrations significantly. This explains

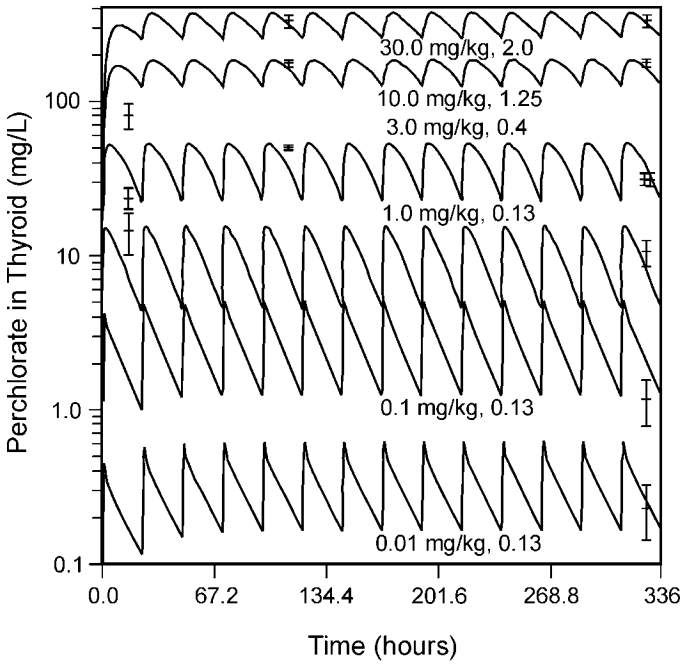

Figure 6-12. Male rat PBPK model predictions (lines) versus data time course (mean ± SD) of thyroid perchlorate concentrations (mg/L) in male rats during ingestion of 30, 10, 3.0, 1.0, 0.1, or 0.01 mg/kg-day in drinking water for 14 days (Merrill, 2001c). Data across the doses were fit by increasing the thyroid follicle:stroma effective partitioning for perchlorate (PT_*p*) from 0.13 to 0.4, 1.25, and 2.0 at the 3, 10, and 30 mg/kg-day doses.

why the model successfully predicted serum perchlorate concentrations across drinking water doses with the same parameters used to describe acute exposures and why it could not predict thyroid concentrations above 3 mg/kg-day.

It could be expected that other parameters (e.g., follicle size and follicular Vmaxc) would also increase with TSH stimulation. There is an increase in percent of thyroid volume attributed to the follicle cells (Conde et al., 1991; Ginda et al., 2000), total protein, RNA and DNA content, and the incorporation of labeled amino acids into protein (Pisarev and Kleiman de Pisarev, 1980). However, Merrill (2001c) notes that adequate predictions could be achieved by adjusting additional parameters; although, without incorporation of regulation by the hypothalamic-pituitary-thyroid axis, such adjustments provide little additional insight.

The ability of the adult male rat model to predict iodide uptake inhibition in the thyroid is demonstrated in Figure 6-13 for a single iv dose of perchlorate (right) or for a 14-day drinking

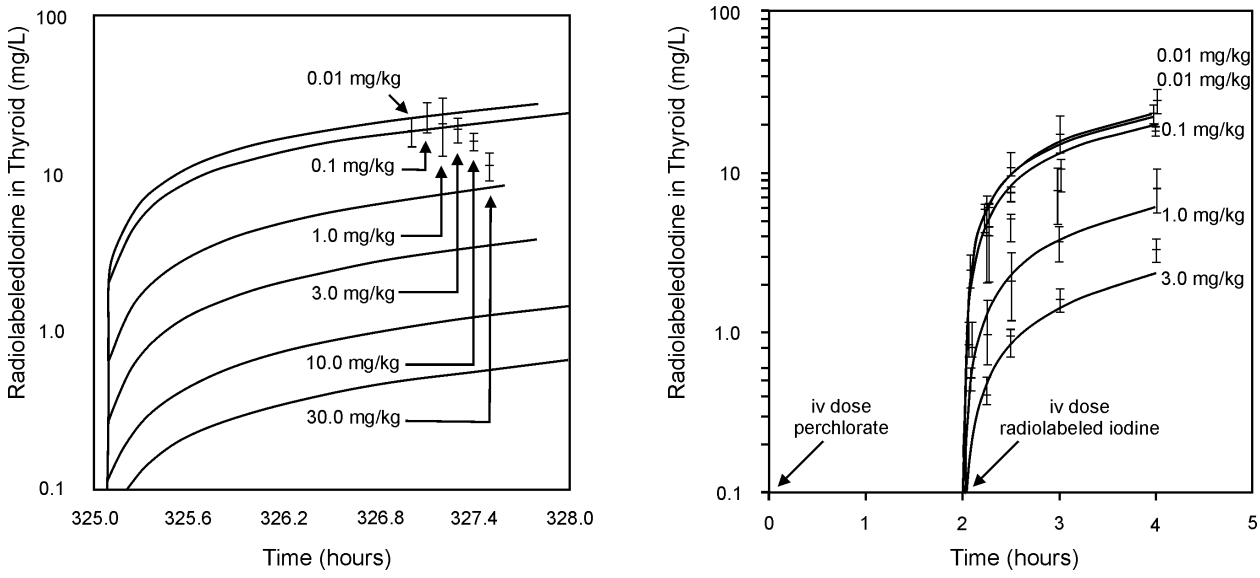

Figure 6-13. Male rat PBPK model predictions (lines) versus data time course (mean ± SD) of iodide uptake inhibition in male rats administered perchlorate either by a single iv dose (right) or in drinking water for 14 days (left), followed by an iv dose of 33 ug/kg $^{125}I^-$ with carrier (Merrill, 2001c). Perchlorate doses were 3.0, 1.0, 0.1, and 0.01 mg/kg-day. Inhibition at the 0.01 and 0.1 mg/kg-day doses overlaps for the iv dose (right).

water exposure (left). Perchlorate-induced inhibition of $^{125}I^-$ uptake in the thyroid was 13, 24, 70, and 88% at 2 hours and 11, 29, 55, and 82% at 9 hours after iv dosing with $^{125}I^-$ with carrier for the 0.01, 0.1, 1.0, and 3.0 mg/kg dose groups. Good simulations were achieved across doses. However, at 3.0 mg/kg, the model slightly overpredicts inhibition 6 hrs after the perchlorate dose (4 hours after $^{125}I^-$ administration). TSH was measured from the highest dose level (3.0 mg/kg) between 8 and 48 hours post dosing and was found to increase between 8 and 12 hrs. It is possible that TSH was already elevated at 6 hrs, allowing upregulation of the thyroid to compensate for inhibition at that time point, which the model would not predict. Yu (2000) provides greater details on hormone fluctuations resulting from the AFRL/HEST experiments.

With respect to iodide inhibition after 14 days of drinking water exposure to perchlorate at 0.01, 0.1, 1.0, 3.0, 10.0, and 30.0 mg/kg-day (Figure 6-13, left), the model overpredicts inhibition at the 1.0 mg/kg-day dosage and greater. TSH-induced upregulation of the thyroid compensates for competitive inhibition, resulting in little or no inhibition of radioiodide uptake on Day 14 of

exposure in all dose groups except 30 mg/kg-day. In all treated groups, TSH levels were already increased after the first day. Serum T4 initially decreased in all dose groups except the 0.01 mg/kg-day group. By day 14, T4 levels had increased to control values in the 0.1 and 1.0 mg/kg-day dose groups. FT4 increased in all dose groups on day 1, returned to normal values by day 5, and were significantly elevated across all dose groups by day 14 (except the 0.1 mg/kg-day group).

6.2.3 Human Model Development

The adult human PBPK model (Merrill, 2001d) was developed concurrently with that for the adult male rate (Merrill, 2001c) and updates the preliminary structure provided to EPA (Merrill, 2000). Much of the early development was based upon generalizations from previous AFRL/HEST work on perchlorate (Fisher, 1998a; 2000) and the work of Hays and Wegner (1965) describing iodide kinetics. As discussed above and shown in Figure 6-1, a nearly identical model structure to that of the adult male rat was used for the adult human. The human physiological parameters will of course be different as these should be species-specific. This section will only highlight notable differences in parameter sources between the two models.

6.2.3.1 Physiologic Parameters and Tissue Partition Coefficients

Human tissue volumes and blood flows were obtained from the literature as shown in Table 6-1. Merrill (2001d) notes that considerable variability was reported for some parameters. For example, blood flow to the gastrointestinal (GI) tract can increase ten-fold in response to enhanced functional activity (secretion and digestion) (Granger et al., 1985). Blood flows used in the model represent estimates of resting values. Human data on the volume of the gut capillary bed (VGBc) were not found in the published literature. Therefore, Merrill (2001d) used a value derived from rat stomach data (Altman and Dittmer, 1971a) for the volume of the gastrointestinal blood (VGBc) in the human model.

Thyroid volume was obtained from ultrasound measurements on 57 healthy volunteers with no thyroid disorders (37 to 74 years of age) in a study conducted by Yokoyama et al. (1986). The mean thyroid volume was 13.4 ± 4.1 mL and mean thyroid volume to body weight ratio was 0.251 ± 0.074 mL/kg (mean ± SD), approximately 0.03% of body weight. Yokoyama et al. (1986) found a positive correlation between thyroid volume and both body weight and age, with

weight having the most pronounced influence. The percent of total thyroid volume attributed to the thyroid follicular epithelium, colloid, and stroma were estimated from histometric measurements of patients at necropsy by Brown et al. (1986). Their findings on the histological features of thyroids of men and women showed overlapping distributions without evidence of a significant difference between sexes. However, a significant sex difference in total fat mass is reported in humans, with women having approximately 10% more fat than men (Brown et al., 1997). Based on these data, Merrill (2001d) used a gender-specific value for this parameter.

6.2.3.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities, and clearance rates (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the human model were derived. The values can be found in Table 6-2, and the details on derivation are in Merrill (2001d).

6.2.3.2.1 Affinity Constants and Maximum Velocities

The Michaelis-Menten affinity constant (Km) estimates for perchlorate and iodide in the various tissues with active transport were developed in the human in an analogous fashion to that in the rat, as described above in Section 6.2.2.2., based on Golstein et al. (1992), Gluzman and Niepomniszcze (1983), and Wolff (1998). The maximum velocity capacity (Vmaxc) values were estimated for the various compartments by fitting the simulations to available data at various doses (Merrill, 2001d).

6.2.3.2.2 Effective Partitions, Permeability Area Cross Products, and Clearance Values

Permeability area cross products and clearance values for perchlorate and iodide were developed by fitting to literature values in an analogous fashion to that for the rat described in Section 6.2.2.3 (Merrill, 2001d).

6.2.3.3 Adult Human Model Parameterization and Validation

The human PBPK model for iodide was developed based on the data of Hays and Solomon (1965) described in Section 6.2.1.2.1. Model predictions versus the data are shown in Figure 6-14 for iodide concentrations (ng/L) in the serum (A), thyroid (B), and gastric juice (C);

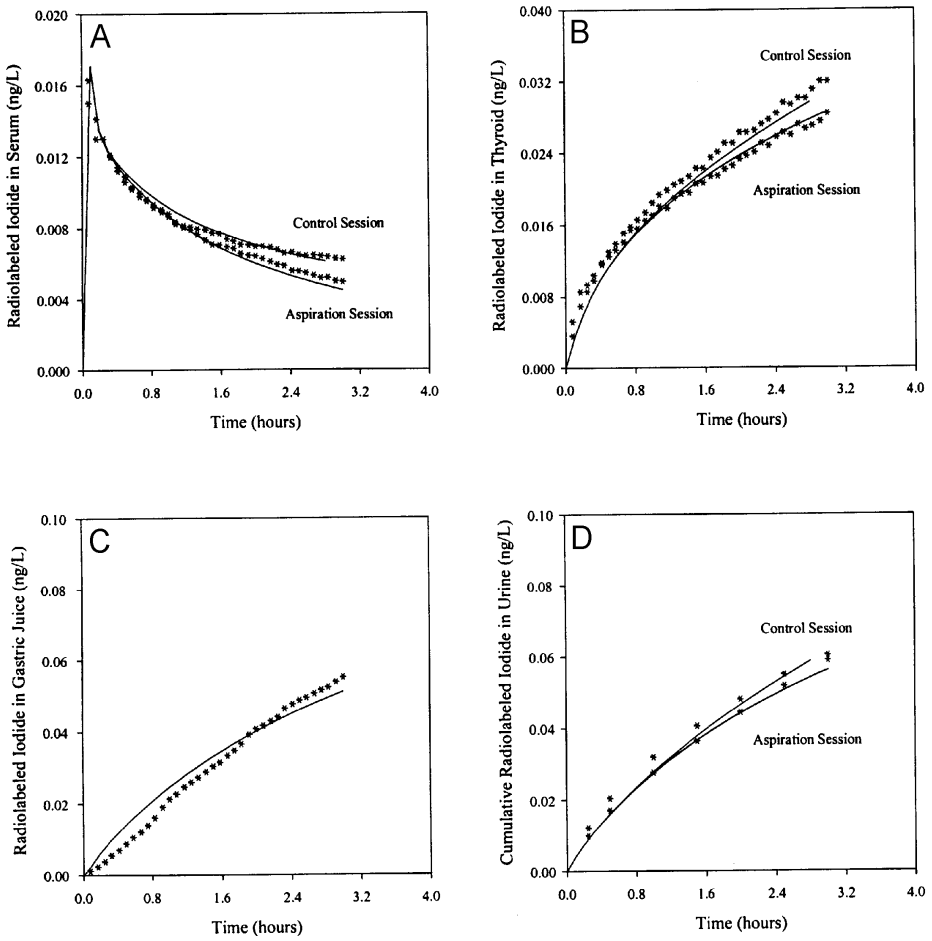

Figure 6-14. Human PBPK model (Merrill, 2001d) predictions (lines) versus mean $^{131}I^-$ concentration (mg/L) time course (asterisks) in serum (A), thyroid (B), gastric juice (C), and urine (D). Data of Hays and Solomon (1965) are for nine healthy males dosed with 10 μCi $^{131}I^-$ (approximately 3.44 ng/kg).

cumulative iodide excreted in the urine (ng) is shown in D. In this study, aspirated gastric juice accounted for an average of 23% of the iv dose within 3 hours after iv injection with radiolabeled iodide ($^{131}I^-$) (Merrill, 2001d). Simulation of the gastric juice removed during the aspiration session (Figure 6-14, C) required mathematically removing the amount of $^{131}I^-$ reabsorbed by the stomach wall. This was accomplished by adjusting the rate of reabsorption of $^{131}I^-$ from gastric juice to gastric tissue during the aspiration session as described in Merrill (2001d). The Vmaxc values for the gut and thyroid were then obtained by fitting values of $^{131}I^-$ uptake into gastric juice

from the aspiration session (lower lines in Figures 6-14; B and C). The urinary clearance value was fitted to simulate both cumulative urine content and serum iodide concentration from the aspiration session data (lower lines in Figures 6-14; A and D). Once parameters were established using the aspiration session, the rate of change in the gastric juice and partitioning back into the gastric juice from the systemic circulation was fitted to predict the corresponding increase in $^{131}I^-$ in plasma, thyroid, and urine seen in the control session versus the aspiration session (upper lines in Figures 6-14; A, B and D).

Figure 6-15 illustrates that, as for the adult male rat model, plasma binding of perchlorate was necessary to fit the serum concentration data of the 14-day study by Greer et al. (2000). The model indicates that humans have a lower binding capacity for perchlorate than rats.

For example, the Vmaxc value for perchlorate is 9.310^3 ng/hr-kg in the male rat versus 5.0×10^2 ng/hr-kg in the human. Merrill (2001d) noted that while the effect of the plasma binding is subtle at 0.5 mg/kg-day dose, including the plasma binding improved the fit for uptake and clearance at the 0.1 and 0.02 mg/kg-day dosage levels.

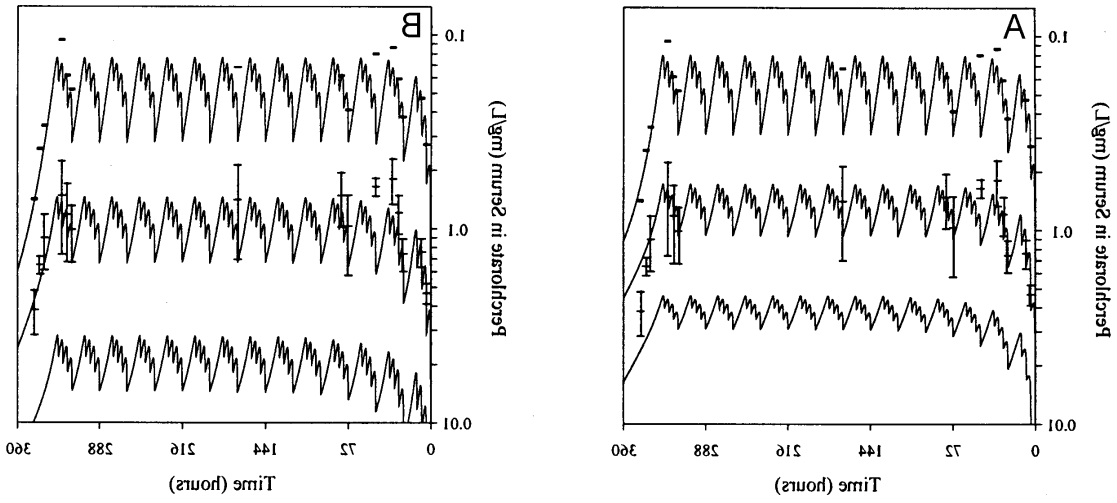

Figure 6-15. Simulations illustrating the necessity of including plasma binding in the human PBPK model structure (Merrill, 2001d). Model predictions (lines) versus data time course (mean ± SD) are shown with (A) and without (B) plasma binding for serum concentrations (mg/L) from 4 male subjects dosed with perchlorate at 0.5, 0.1, or 0.02 mg/kg-day for 14 days (data of Greer et al., 2000).

Cumulative urinary perchlorate excretion (mg) predictions versus the data (mean ± SD) at each dosage level are shown in Figure 6-16. Merrill (2001d) also simulated serum concentration (mg/L) and cumulative urinary perchlorate levels (mg) for each individual in the 0.5, 0.1, and 0.02 mg/kg-day dose groups of the Greer et al. (2000) study. An average value for urinary clearance of perchlorate (ClUc_*p*) of 0.126 L/hr-kg (± 0.050) was calculated from the individually fitted values. Figures 6-17 and 6-18 show a representative plot of model prediction versus individual subject data at the 0.5 and 0.1 mg/kg-day dosage. Additional plots provided in Merrill (2001d) provide an appreciation for the high degree of variability in the data.

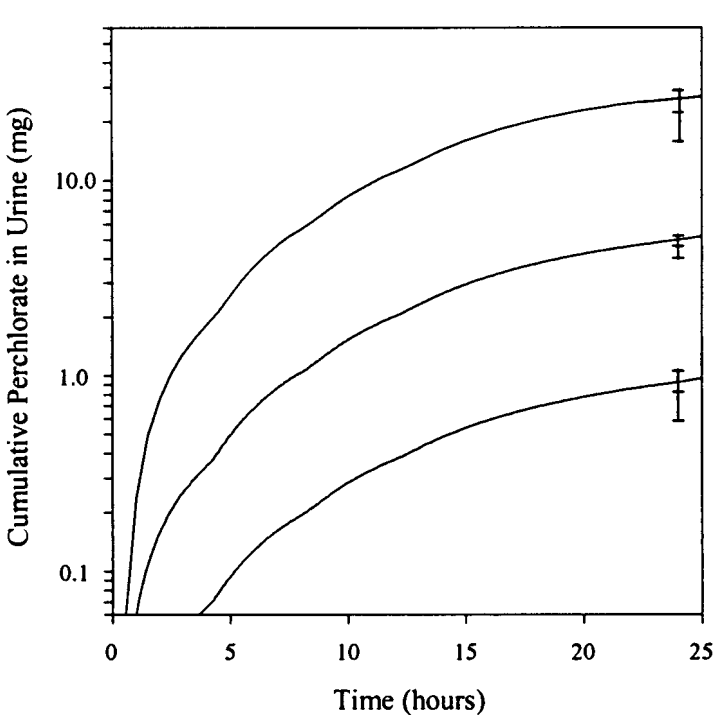

Figure 6-16. Human PBPK model predictions (lines) versus data (mean ± SD) of the observed cumulative urine excretion (mg) in male subjects dosed with perchlorate 0.5, 0.1, or 0.02 mg/kg-day for 14 days. Model of Merrill (2001d) and data of Greer et al. (2000).

Serum perchlorate levels were not available for the 0.02 mg/kg-day dose group, but cumulative urinary excretion amounts (mg) for this group were fitted using the average

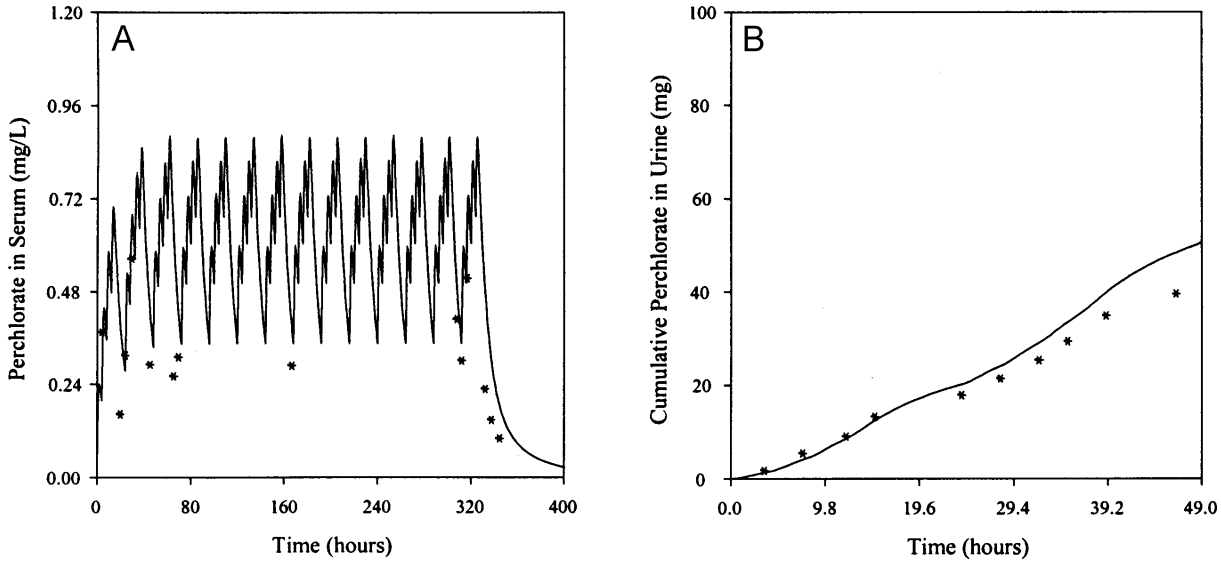

Figure 6-17. Human PBPK model predictions (lines) versus data of one subject's serum perchlorate concentration (mg/L) shown in (A) and corresponding 48-hour cumulative urine perchlorate (mg) shown in (B). Subject consumed 0.5 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (ClUc_*p*). Model of Merrill (2001d) and data of Greer et al. (2000).

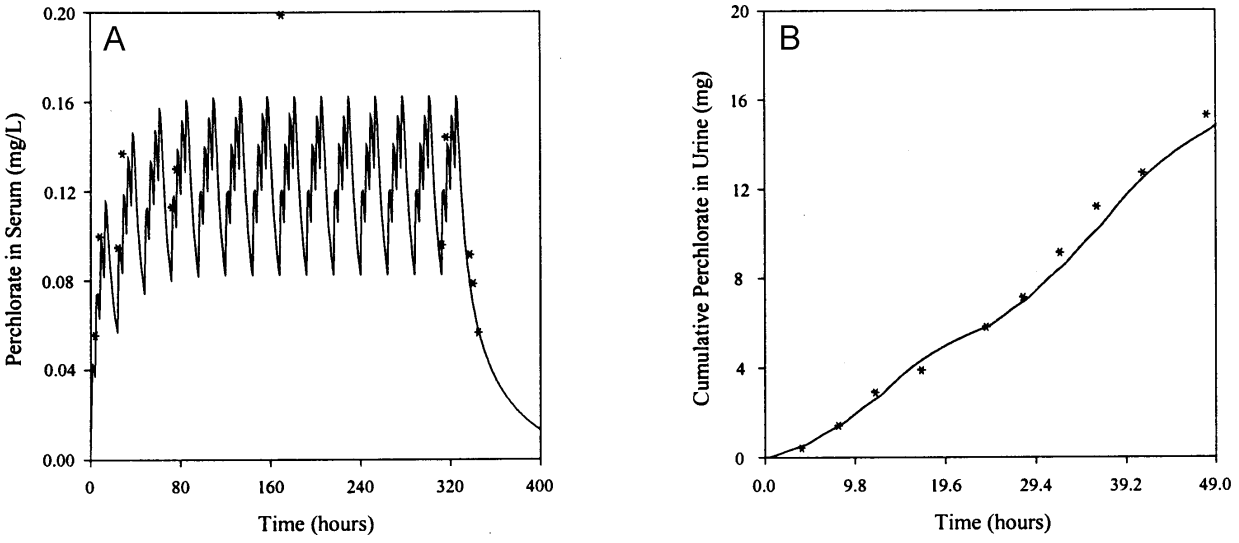

Figure 6-18. Human PBPK model predictions (lines) versus data of one subject's serum perchlorate concentration (mg/L) shown in (A) and corresponding 48-hour cumulative urine perchlorate (mg) shown in (B). Subject consumed 0.1 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (ClUc_*p*). Model of Merrill (2001d) and data of Greer et al. (2000).

perchlorate urinary clearance (ClUC_*p*) value of 0.126 L/hr-kg calculated from the individual fits for the 0.1 and 0.5 mg/kg-day groups. Figure 6-19 shows the model predictions versus 48-hour cumulative urine perchlorate (mg) for two different subjects.

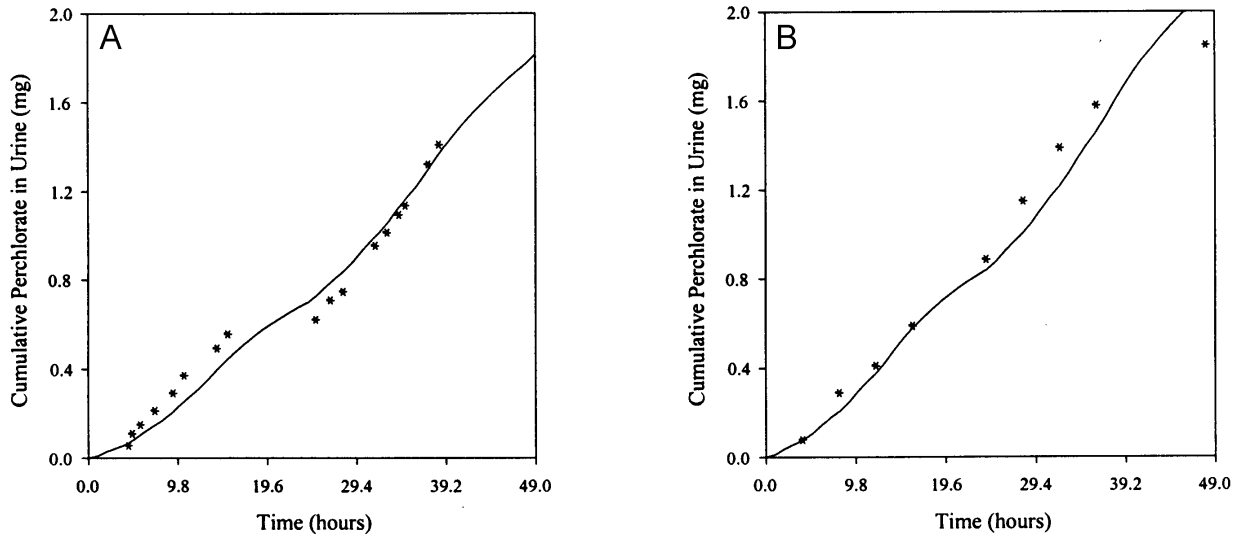

Figure 6-19. Human PBPK model predictions (lines) versus data of 48-hour cumulative urine perchlorate (mg) shown for two different subjects. Subject consumed 0.02 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (ClUc_*p*). Model of Merrill (2001d) and data of Greer et al. (2000).

Due to its small size, variations in the thyroid parameters have little effect on serum concentrations of both iodide and perchlorate. As described for Figure 6-14, Merrill (2001d) estimated parameters for iodide disposition, including those of the thyroid, from fits to the data of Hays and Solomon (1965). Using these same iodide parameters, baseline thyroid RAIU measurements performed by Greer et al. (2000) were fit with the model by adjusting the Vmaxc for the thyroid follicular epithelium (Vmaxc_T*i*). Figures 6-20, 6-21, 6-22, and 6-23 illustrate the model predictions of thyroid RAIU versus data for subjects in the 0.5, 0.1, 0.02, and 0.007 mg/kg-day dosage groups, using either the individual's Vmaxc_T*i* (left) or an average value (right). The average Vmaxc_T*i* (1.5×10^5 ng/hr-kg) was obtained from fitting baseline

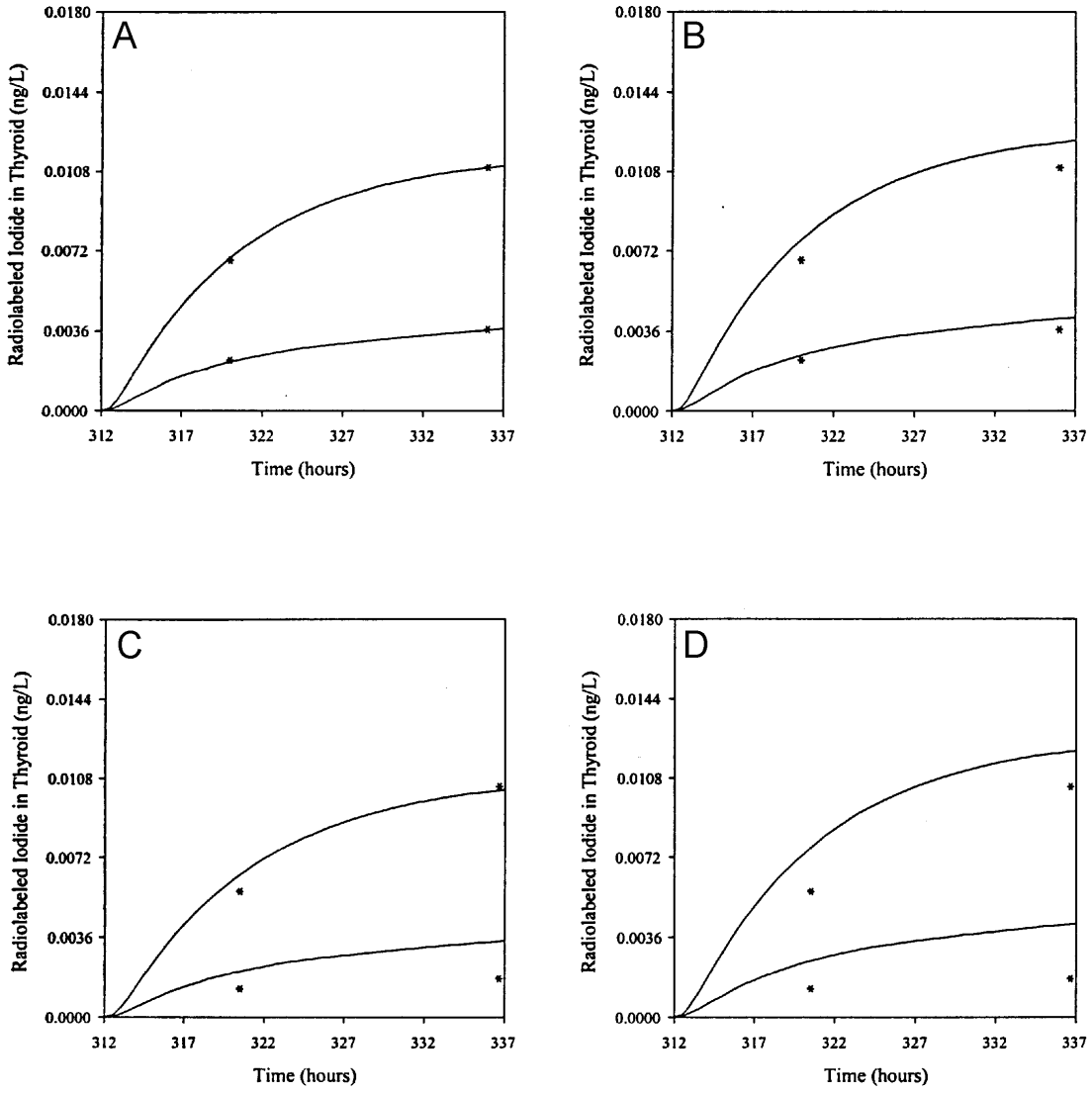

Figure 6-20. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.5 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 1.3×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.24×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.5×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

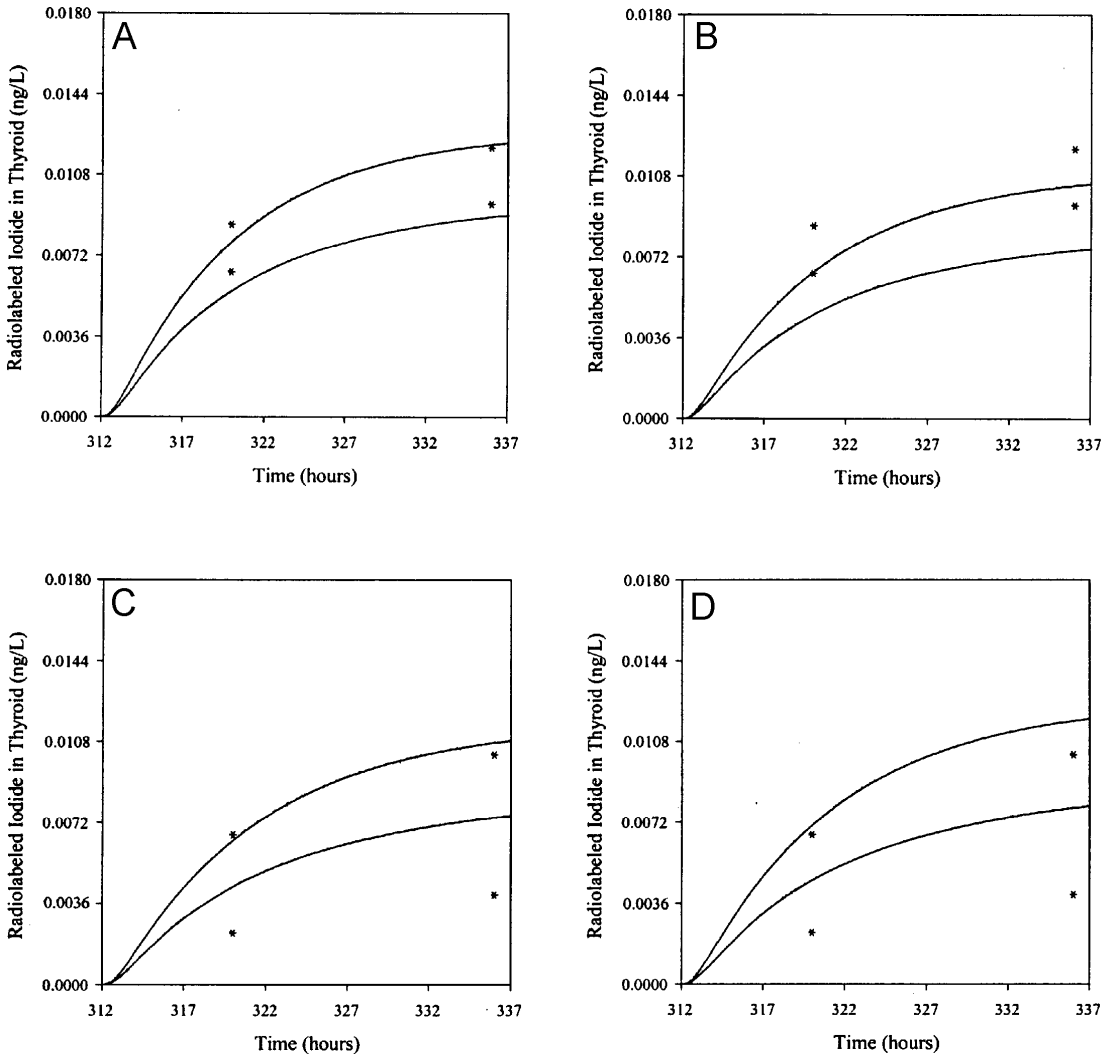

Figure 6-21. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.1 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 1.65×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.2×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.5×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

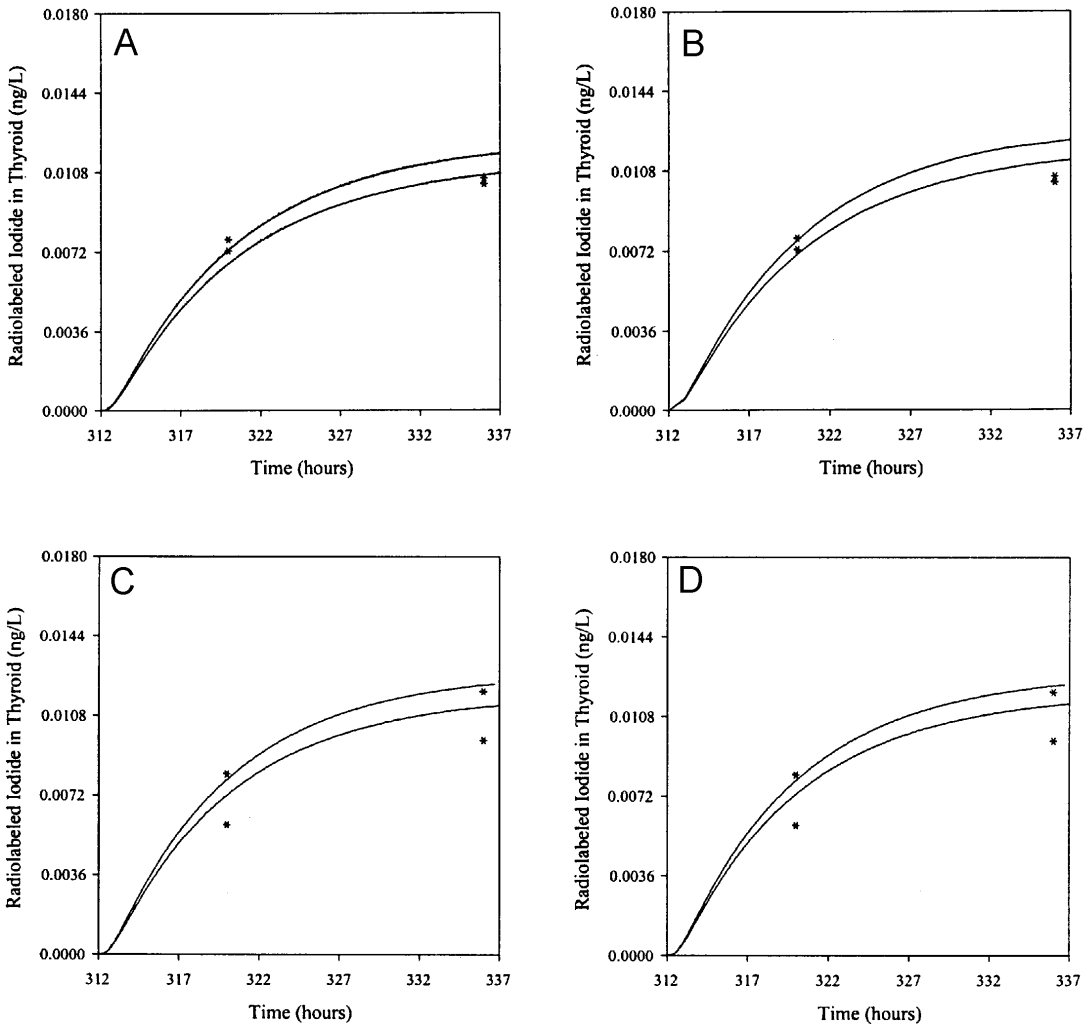

Figure 6-22. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.02 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 1.4×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.5×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.5×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

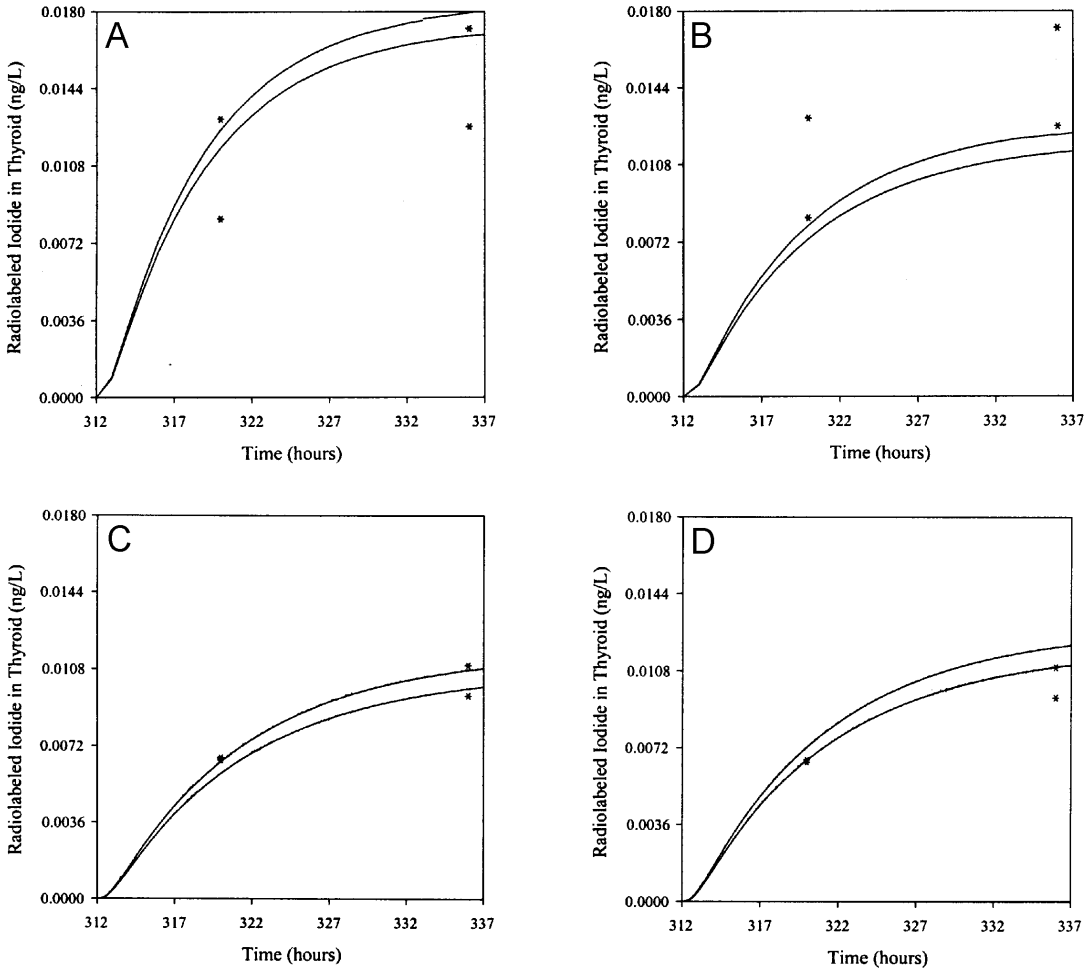

Figure 6-23. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.007 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 2.8×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.24×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.35×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

radioiodide uptake measurements provided by Greer et al. (2000) across doses (see Merrill, 2001d; Table 3). Merrill (2001d) hypothesized that the large variability in Vmaxc_T*i*, ranging from 5.0×10^4 to 5.0×10^5 ng/hr-kg, may be attributed to variability in endogenous iodide levels, as dietary iodide was not controlled. Merrill (2001d) estimated these values from best visual fits of baseline 8- and 24-hour thyroid RAIU data. Inhibition data restricted to each time point (i.e., 8- versus 24-hour time points) and from higher dose groups would be useful to test the robustness of the model to predict inhibition of uptake of iodide in the thyroid.

The ability of the human model to predict data from other independent experiments not used to develop the model is illustrated in Figure 6-24. The model adequately predicts cumulative perchlorate in urine (mg) reported in three published studies using therapeutic perchlorate dose levels (Merrill, 2001d). Oral doses administered in these studies were approximately 9.07 mg/kg (Durand, 1938), 9.56 mg/kg (Kamm and Drescher, 1973), and 20 mg/kg (Eichler, 1929). It is worth noting that the previously determined urinary clearance value (ClUc_*p*) of 0.126 L/hr-kg was used with all validation data and that an adequate fit was observed.

The ability of the model to predict cumulative perchlorate in urine from three different studies at three different doses with the same set of parameters, established from the studies by Hays and Solomon (1965) and Greer et al. (2000), demonstrates the usefulness of the model and provides validation for the model structure and the physiological and chemical parameters used.

The model also predicts serum perchlorate concentrations at 12 mg/kg-day from an unpublished study performed by Dr. Georg Brabant at the Medizinische Hochschule, Hanover, Germany (Figure 6-25). Subjects received 12 mg/kg-day perchlorate in drinking water near meal times. Variability in the observed serum measurements is believed to reflect variability in the dosing regimen, as the experimental protocol was less fixed than that used in Greer et al. (2000). Again the usefulness of the model is demonstrated by its ability to successfully predict serum concentrations from a dose 24 times higher than the high dose used to establish perchlorate parameters (0.5 mg/kg-day).

The model is also able to successfully predict the thyroidal iodide uptake in a subject from the Stanbury and Wyngaarden (1952) study with patients with Grave's disease. The maximum velocity capacity in the follicular epithelium (Vmaxc_T*i*) had to be increased to 5.0E6 ng/hr-kg, a factor of ten times higher than in normal subjects, in order to achieve this fit (upper line in

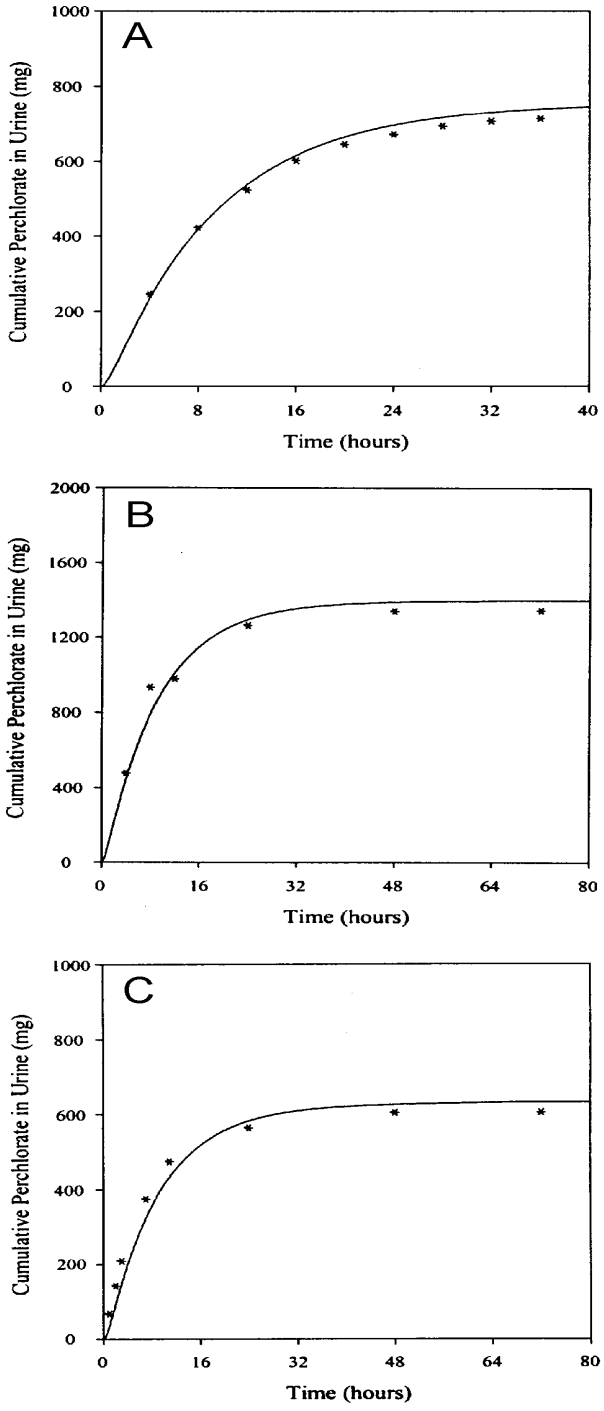

Figure 6-24. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for cumulative perchlorate excretion in urine (mg) in a healthy male after an oral dose of 9.56 mg (A), 20 mg (B) or 9.07 mg (C). Data are from three different studies. Data of Kamm and Drescher (1973) for (A), Eichler (1929) for (B) and Durand (1938) for (C).

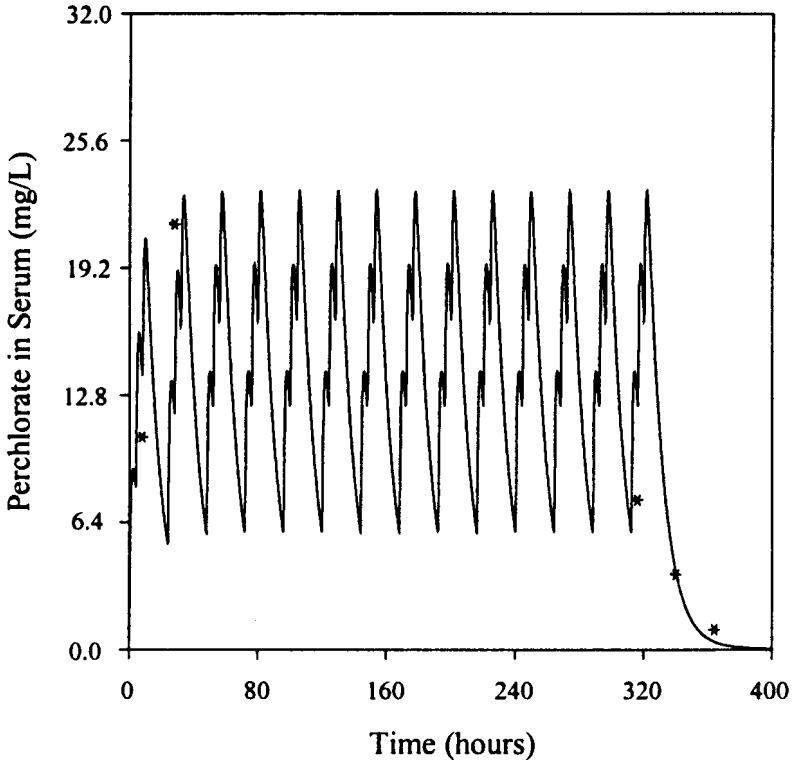

Figure 6-25. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for serum perchlorate concentrations (mg/L) in 5 subjects received 12 mg/kg-day in drinking water (data of Brabant and Letiolf, 2000 as cited in Merrill, 2001d). Subjects were instructed to ingest the solution 3 times/day for 14 days. Serum samples were collected 2 hours after the first dose, after 12 pm on day two, the morning of day 14 and post exposure days 1 and 2. Usefulness of the model is demonstrated by its ability to successfully predict serum concentrations at a dose 24 times higher than the dose used to develop parameters in the model.

Figure 6-26). This increase in Vmaxc_T*i* is supported in the literature, as Gluzman and Niepomniszcze (1983) measured elevated Vmaxc(s) in thyroid specimens from subjects with Grave's disease. However, the model underpredicts the degree of inhibition caused by perchlorate in this subject (Figure 6-26, lower line). It would appear that the increased inhibition could be attributed to a lower Km value. However, Gluzman and Niepomniszcze (1983) noted that the Km did not differ greatly between thyroid specimens from hyperthyroid subjects and

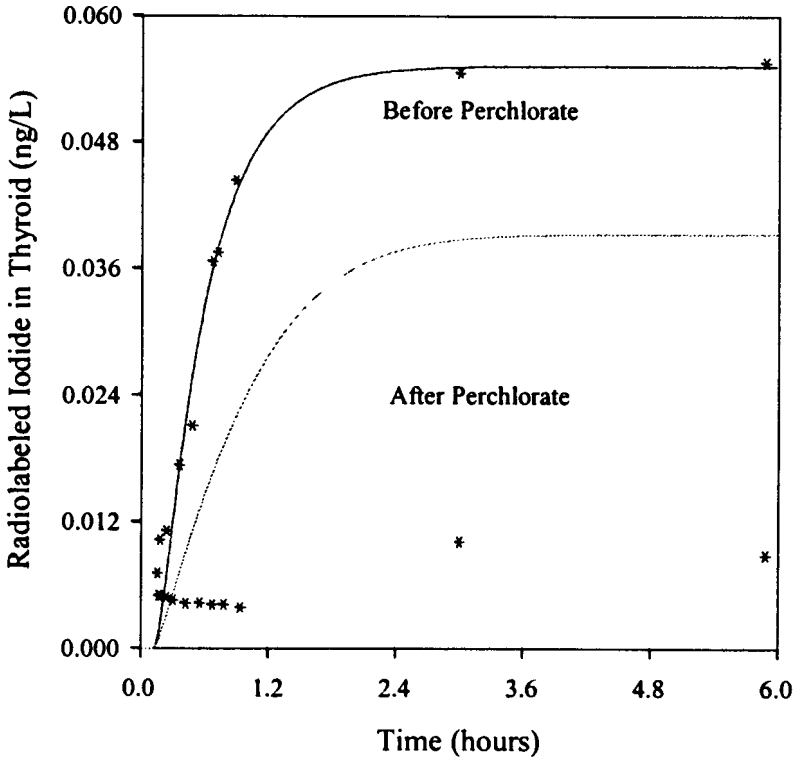

Figure 6-26. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for RAIU in the thyroid ($^{131}I^-$ ng/L) of a male with Graves' disease after an iv dose of 10 μCi $^{131}I^-$ before and after a 100 mg dose of potassium perchlorate. Data of Stanbury and Wyngaarden (1952).

normal subject. This suggests that the increased inhibition by perchlorate seen in Grave's disease may be attributed to a mechanism other than NIS affinity (Merrill, 2001d).

6.2.4 Summary

The proposed model structures for the adult male rat (Merrill, 2001c) and adult human (Merrill, 2001d) have been shown to adequately describe both perchlorate and iodide disposition by demonstrating good correspondence between predicted tissue compartment concentrations and measured values in the thyroid, serum, red blood cells, urine, liver, muscle, skin, and stomach in the rat and by adequately predicting serum concentrations and cumulative urine after drinking water exposure to perchlorate spanning four orders of magnitude (0.02 to 12.0 mg/kg-day) in the human. Serum perchlorate levels for human subjects were not available at

0.02 mg/kg-day; however, the model did predict the cumulative urine from that dose group (Figure 6-19).

The model structure of the thyroid requires three compartments (stroma, follicle, and colloid) to quantify rapid organification in the gland. Differences in model parameters between iodide and perchlorate indicate that iodide kinetics are very similar to perchlorate kinetics, but cannot be applied directly. The main differences involve the saturable kinetics of the thyroid, skin, and stomach, with perchlorate exhibiting higher Vmaxc's except in the skin. Because organification of iodide occurs in both the thyroid follicle and colloid, their respective Vmaxc's are over 1,000 and 10 times higher than those for perchlorate, which is discharged unchanged. Perchlorate affinity for the symporters into the follicle and colloid were approximately an order of magnitude greater (lower Km) than those of iodide.

The thyroid perchlorate concentrations from high drinking water exposures in the rat were fitted by increasing the effective follicle:stroma partition coefficient (PT_*p*) to account for TSH stimulation and upregulation of NIS. Since these values were not supported by additional data, thyroid concentrations may not be as reliable. Further, the toxic effects of perchlorate are most likely due to secondary effects on thyroid hormones due to its action at the NIS.

The model, however, could simulate serum concentrations from drinking water exposures using parameters established from the acute data. The thyroid, given it's small size, would not be expected to significantly alter serum concentrations, even during hyperstimulation. Although TSH has not been shown to increase the NIS in other tissues, NIS-containing tissues were not obtained from the AFRL/HEST studies to support this.

The models support plasma protein binding of perchlorate in both species; a saturable term is required to simulate serum concentrations at lower doses. It is possible that perchlorate competes with thyroxine for the same binding sites of plasma proteins, as the work of Yamada and Jones (1968) suggests. Urinary clearance values of 0.05 L/hr for iodide and 0.07 L/hr for perchlorate were used across data sets in the rats, and average urinary clearance values were found to be 0.1 L/hr-kg for iodide and 0.126 L/hr-kg in humans. Excretion constants were highest among the 0.1 mg/kg-day group. With the urinary excretion rates fitted to cumulative urine data, the model tends to slightly underestimate serum perchlorate levels at repeated low doses. Elevated serum concentrations may indicate plasma binding of perchlorate. Yamada and Jones (1967) studied effects of different anions on plasma binding to thyroxine and noted that

some of the thyroxine had been displaced after perchlorate was introduced. Thus, it is possible that perchlorate competes with thyroxine for the same binding sites of plasma proteins (Merrill, 2001c,d; Clewell, 2001a).

While there are limited data suggesting iodide and perchlorate uptake through the skin, the models and the kinetic studies required this assumption in the models for both rats and humans. Without the skin compartment, the models overestimated circulating plasma inorganic iodide and perchlorate in both species. Due to its large size, skin appears to be an important pool for slow turnover of these anions. Brown-Grant (1961) noted that the uptake of iodide was higher in the male rat and pup than in the female. The findings of Merrill (2001c) agree, with the rat model requiring a higher Vmaxc in the skin for the male rat than that reported for the pregnant rat (Clewell, 2001a) discussed in the next section. Cutaneous uptake of iodide and perchlorate in mice and rats has been reported (Brown-Grant and Pethes, 1959; Zeghal et al., 1995). The lack of reported iodide in human skin from clinical radioiodide scans may be due to the difficulty in differentiating it from background radioactivity.

Merrill (2001d) notes that GI clearance of iodide is rapid and plays an important role in radioiodide conservation. Further, Merrill (2001d) suggests that the appearance of time-course radioiodine in stomach contents of any species is complicated by the fact that it reflects more than sequestration of radioiodide by NIS. Its appearance also reflects radioiodide contributed through the gradual accumulation of iodide in saliva that is swallowed involuntarily throughout the study. Several studies that examined sequestration of these anions in digestive juices have all shown high variability in the concentrations measured over time (Honour et al., 1952; Hays and Solomon, 1965; Merrill, 2001d). There is a tendency for the gastric juice to plasma ratio to be low when the rate of secretion of juice is high (Honour et al., 1952). Fluctuations in the secretion rate are probably the most important factor in determining the pattern of the concentration ratios in individuals. Therefore, variability in stomach or GI tract parameters between models is expected. However, the early rise in the gastric juice:plasma ratio mentioned earlier is a constant feature across these data sets, whether or not an attempt was made to eliminate contamination of gastric juices by dietary contents or saliva. The human model successfully predicted this same trend.

Merrill (2001d) also noted dietary iodine and endogenous inorganic iodide levels to be clearly important in modeling iodide and perchlorate kinetics, because excessive iodide levels

cause the ion to inhibit its own uptake. Plasma inorganic iodide (PII) is rarely reported in the literature due to analytical difficulties, and it was not available in any of the studies presented in this paper. While measurements of tracer radioiodide can be fitted to predict transfer rates, its use is limited when attempting to predict the saturation of nonlinear compartments, such as the thyroid that are dependent upon the existing amount of iodide already present. Subsequent modeling efforts on predicting subsequent effects of iodide inhibition on thyroid hormone synthesis and regulation in humans will require the capability of the model to predict PII.

6.3 PREGNANT RAT AND FETAL MODEL STRUCTURE

This section describes the model developed by AFRL/HEST in response to concerns about interspecies extrapolation of effects due to perchlorate exposure during gestation (Clewell, 2001a). The model predicts the distribution of perchlorate within the pregnant and fetal rat through gestation and at birth and predicts the short-term effect of acute perchlorate exposure on iodide kinetics, including iodide uptake into the maternal thyroid. The general model structure relied on the adult male rat model (Merrill, 2001c) described in Section 6.2 and approaches to gestational growth of the dam and fetus were based on the work of O'Flaherty et al. (1992) and Fisher et al. (1989) with weak acids.

The model structure is shown in Figure 6-27. Table 6-3 provides the physiological parameters for the pregnant rat and fetus PBPK models. Table 6-4 provides the perchlorate-specific parameters, and Table 6-5 provides the iodide-specific parameters for each.

The compartments shared with the adult male rat were developed as described in Section 6.2. The pregnant rat model also includes a mammary gland and placenta compartment. The mammary gland consists of two subcompartments that represent the capillary bed and the tissue. The mammary gland has been shown to concentrate both perchlorate and iodide during lactation. However, the mammary NIS is regulated by hormones produced during lactation and has been found to increase at the onset of lactation (Tazebay et al., 2000). This concentrating mechanism does not appear to be as established during pregnancy. Studies reported by Yu (2000) showed mammary gland:plasma ratios of less than one for perchlorate. However, mammary gland perchlorate levels are slowly built up and remain high well into the clearance phase of the serum. This behavior suggested a very slow diffusion between the mammary gland

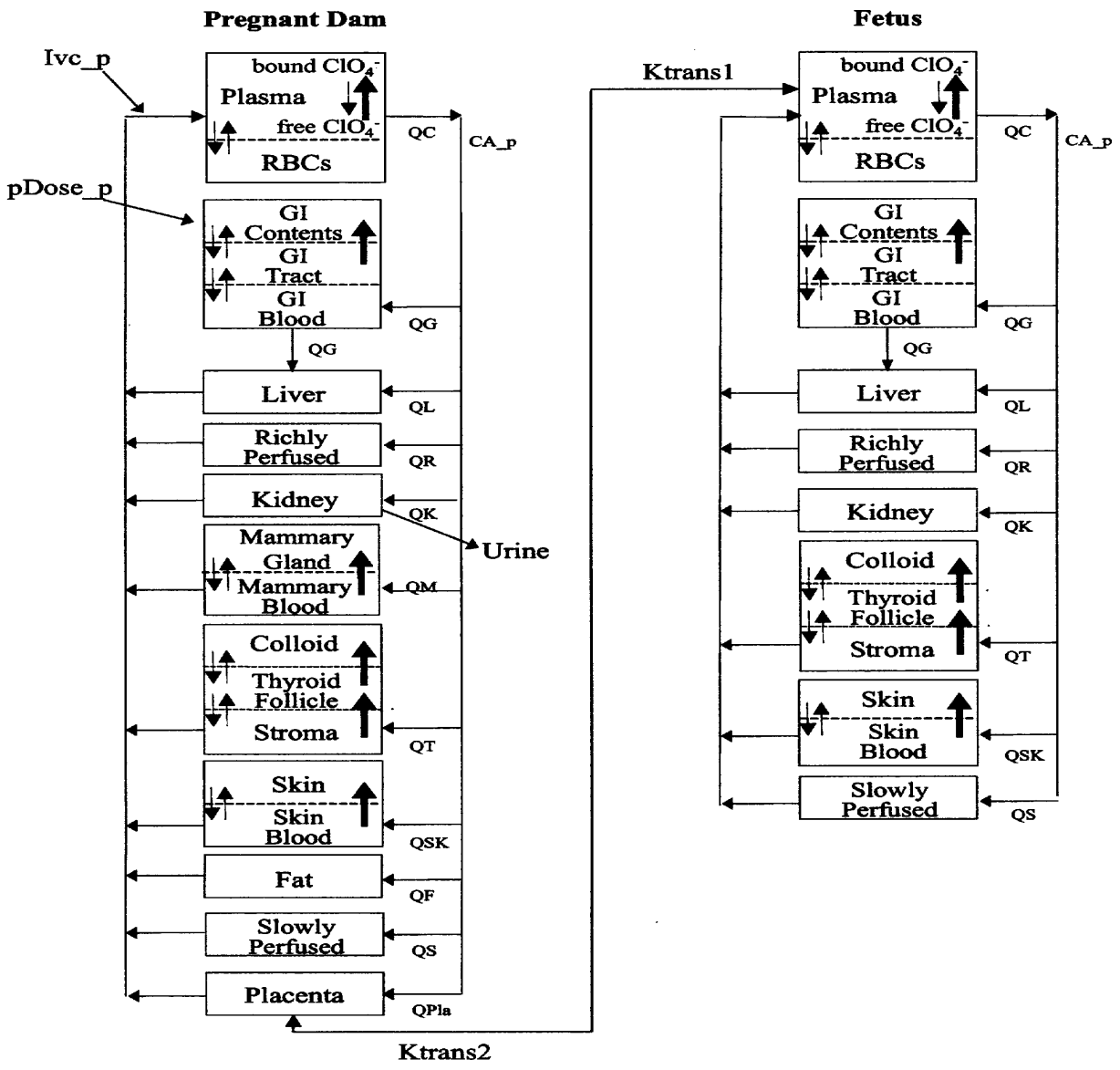

Figure 6-27. Schematic for the pregnant dam and fetal rat PBPK model of perchlorate and iodide distribution (Clewell, 2001a). Bold arrows indicate (except for plasma binding) active uptake at NIS sites into the thyroid, GI contents, and skin. Plasma binding was also described with Michaelis-Menten terms for the association of perchlorate anion to binding sites with first-order clearance rates for dissociation. Small arrows indicate passive diffusion. Boxes represent specific compartments in the model structure. The thyroid consists of the stroma, the follicle, and the colloid; and the stomach consists of the capillary bed, GI wall, and contents. The skin and mammary gland each contain two subcompartments representing the capillary bed and tissue. Permeability area cross products and partition coefficients were used to describe the first-order movement of the perchlorate (ClO_4^-) and iodide (I^-) anions into deeper subcompartments. Placental-fetal transfer and urinary clearance were represented by first order clearance rates.

TABLE 6-3. PHYSIOLOGICAL PARAMETERS FOR THE PREGNANT RAT AND FETUS PBPK MODEL (Clewell, 2001a)

Physiological Parameters	Pregnancy		
Tissue Volumes (%BW)	**Dam**	**Fetus**	**Source**
Body Weight *BW* and *V1fet* (kg)	0.280 - 0.361	0.0 - .0045	O'Flaherty et al., 1992
Slowly Perfused VSc (%BW)	74.6	74.6	Brown et al., 1997
Richly Perfused VRc (%BW)	11	11	Brown et al., 1997
Fat *VFc* (%BW)	10.0 - 11.0	0.0	Naismith et al., 1982
Kidney *VKc* (%BW)	1.7	1.7	Brown et al., 1997
Liver *VLc* (%BW)	3.4	3.4	Brown et al., 1997
GI Tract *VGc* (%BW)	3.60	3.60	Brown et al., 1997
GI Contents *VGJc* (%BW)	7.20	7.20	Yu et al., 2000
GI Blood *VGBc* (%VG)	2.9	2.9	Altman and Dittmer, 1971
Skin Tissue *VSkc* (%BW)	19.0	19.0	Brown et al., 1997
Skin Blood *VSkBc* (%VSk)	2.0	2.0	Brown et al., 1997
Thyroid Total *VTtotc* (%BW)	0.0105	0.0234	Malendowicz and Bednarek, 1986; Florsheim et al., 1966
Thyroid Follicle *VTc* (%BW)	45.9	61.4	Malendowicz and Bednarek, 1986; Conde et al., 1991
Thyroid Colloid *VDTc* (%BW)	45	18.3	Malendowicz and Bednarek, 1986; Conde et al., 1991
Thyroid Blood *VTBc* (%VT)	9.1	20.3	Malendowicz and Bednarek, 1986; Conde et al., 1991
Plasma *VPlasc* (%BW)	4.7	4.7	Brown et al., 1997; Altman and Dittmer, 1971
Red Blood Cells *VRBCc* (%BW)	2.74	2.74	Brown et al., 1997; Altman and Dittmer, 1971
Placenta *VPl ac* (%BW)	0.0 - 2.57	—	O'Flaherty et al., 1992
Mammary Tissue *VMc* (%BW)	1.0 - 5.5	—	Knight et al., 1984; O'Flaherty et al., 1992
Blood Flows (%QC)			
Cardiac Output *QCc* (L/hr-kg)	14	14.0	Buelke-Sam, 1982a & b; O'Flaherty et al., 1992
Slowly Perfused *QSc* (%QC)	24.0	24.0	Brown et al., 1997
Richly Perfused *QRc* (%QC)	76.0	76.0	Brown et al., 1997
Fat *QFc* (%QC)	7 - 8.1	0.0	Brown et al., 1997
Kidney *QKc* (%QC)	14.0	14.0	Brown et al., 1997
Liver *QLc* (%QC)	18.0	18.0	Brown et al., 1997
GI *QGc* (%QC)	13.60	13.60	Brown et al., 1997
Thyroid *QTc* (%QC)	1.6	1.6	Brown et al., 1997
Mammary *QMc* (%QC)	0.2 - 1.2	—	Hanwell and Linzell, 1973
Placenta *QPlc* (%QC)	0.0 - 12.3	—	O'Flaherty et al., 1992

TABLE 6-4. PERCHLORATE-SPECIFIC PARAMETERS FOR THE PREGNANT RAT AND FETUS PBPK MODEL (Clewell, 2001a)[a]

Pregnancy Parameters	Perchlorate Values		
Partition Coefficients (unitless)	**Dam**	**Fetus**	**Source**
Slowly Perfused/Plasma PS_	0.31	0.31	Yu et al., 2000
Rapidly Perfused/Plasma PR_	0.56	0.56	Yu et al., 2000
Fat/Plasma PF_	0.05	—	Pena et al., 1976
Kidney/Plasma PK_	0.99	0.99	Yu et al., 2000
Liver/Plasma PL_	0.56	0.56	Yu et al., 2000
Gastric Tissue/Gastric Blood PG_	0.50	1.80	Yu et al., 2000
GI Contents/GI Tissue PGJ_	1.30	2.30	Yu, 2000
Skin Tissue/Skin Blood PSk_	1.15	1.15	Yu, 2000
Thyroid Tissue/Thyroid Blood PT_	0.13 / 2.25	0.13 / 2.25	Chow and Woodbury, 1970[b]
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	Chow and Woodbury, 1970
Red Blood Cells/Plasma	0.73	0.73	Yu et al., 2000
Placenta/ Plasma PPL_	0.56	—	Assume same as richly perfused
Mammary/Plasma PMam_p	0.66	—	Anbar et al., 1959
Max Capacity, Vmaxc (ng/hr-kg)			
Thyroid Follicle Vmaxc_T	1.80E+03	1.80E+03	Fitted[c]
Thyroid Colloid Vmaxc_DT	1.00E+04	1.00E+04	Fitted[c]
Skin Vmaxc_S	6.00E+05	4.00E+05	Fitted
Gut Vmaxc_G	8.00E+05	8.00E+05	Fitted
Mammary Gland Vmaxc_M	3.90E+04	---	Molar equivalent to Vmaxc_M*i*
Plasma Binding Vmaxc_Bp	5.00E+03	1.50E+03	Fitted
Affinity Constants, Km (mg/L)			
Thyroid Follicle Km_T	1.00E+05	1.00E+05	Wolff, 1998
Thyroid Colloid Km_DT	1.00E+08	1.00E+08	Golstein et al., 1992; Wolff, 1998
Skin Km_S	1.00E+05	1.00E+05	Wolff, 1998
Gut Km_G	1.00E+05	1.00E+05	Wolff, 1998
Mammary Gland	1.00E+5	—	Wolff, 1998
Plasma Binding Km_Bp	1.00E+05	1.00E+05	Fitted
Permeability Area Cross Products, (L/hr-kg)			
GI Blood to GI Tissue PAGc_	1.00	1.00	Fitted
GI Tissue to GI Contents PAGJc_	1.00	1.00	Fitted
Thyroid Blood to Thyroid Tissue PATc_	4.0E-5 / 6.0E-4	4.0E-5 / 6.0E-4	Fitted[b]
Thyroid Tissue to Thyroid Lumen PADTc_	0.01	0.01	Fitted
Skin Blood to Skin Tissue PASkc_	1.00	1.00	Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted
Clearance Values, (L/hr-kg)			
Urinary Excretion CLUc_	0.07	—	Yu et al., 2000
Transfer from Placenta to Fetus Cltrans1c_	0.10	0.10	Yu, 2000
Transfer from Fetus to Placenta Cltrans2c_	0.19	0.19	Yu, 2000
Dissociation from Plasma Binding Sites Clunbc_p	0.034	0.010	Yu, 2000

[a]All parameters listed are notated in the model by either an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR_*i*, PR_*p*, Vmaxc_T*i*, etc.)
[b]Parameters with two values indicate acute and drinking water parameters, respectively.
[c]Fetus was given maternal values for Vmax (scaled by fetal body weight) in the absence of data.

TABLE 6-5. IODIDE-SPECIFIC PARAMETERS FOR THE PREGNANT RAT AND FETUS PBPK MODEL (Clewell, 2001a)[a]

Pregnancy Parameters	Iodide Values		
Partition Coefficients (unitless)	**Dam**	**Fetus**	**Iodide Source**
Slowly Perfused/Plasma PS_	0.21	0.21	Halmi et al., 1956
Rapidly Perfused/Plasma PR_	0.40	0.40	Halmi et al., 1956
Fat/Plasma PF_	0.05	—	Pena et al., 1976
Kidney/Plasma PK_	1.09	1.09	Yu et al., 2000
Liver/Plasma PL_	0.44	0.44	Yu et al., 2000
GI Tissue/GI Blood PG_	1.0	1.0	Yu, 2000
GI Contents/GI Tissue PGJ_	2.0	2.0	Yu, 2000
Skin Tissue/Skin Blood PSk_	0.70	0.70	Perlman et al., 1941
Thyroid Tissue/Thyroid Blood PT_	0.15	0.15	Chow and Woodbury, 1970
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	Chow and Woodbury, 1970
Red Blood Cells/Plasma	1.00	1.00	Yu et al., 2000
Placenta/Plasma PPL_	0.99	—	Unpublished GD20 data
Mammary/Plasma PMam_p	0.66	—	Anbar et al., 1959 (for ClO_4^-)
Max Capacity, Vmaxc (ng/hr-kg)			
Thyroid Follicle Vmaxc_T	4.00E+04	0.0 – 7.5E+04	Fitted
Thyroid Colloid Vmaxc_DT	6.00E+07	6.00E+07	Fitted
Skin Vmaxc_S	6.00E+04	3.00E+05	Fitted
Gut Vmaxc_G	1.00E+06	2.00E+05	Fitted
Mammary Gland Vmaxc_M	5.00E+04	—	Fitted
Affinity Constants, Km (mg/L)			
Thyroid Follicle Km_T	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Thyroid Colloid Km_DT	1.00E+09	1.00E+09	Golstein et al., 1992
Skin Km_S	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Gut Km_G	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Mammary Gland Km_M	4.00E+06	—	Gluzman and Niepomniszcze, 1983
Permeability Area Cross Products, (L/hr-kg)			
GI Blood to GI Tissue PAGc_	0.80	0.10	Fitted
GI Tissue to GI Contents PAGJc_	0.60	0.30	Fitted
Thyroid Blood to Thyroid Tissue PATc_	1.000E-04	1.000E-04	Fitted
Thyroid Tissue to Thyroid Lumen PADTc_	1.00E-04	1.00E -04	Fitted
Skin Blood to Skin Tissue PASkc_	0.10	0.02	Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted
Clearance Values, (L/hr-kg)			
Urinary excretion CLUc_	0.03	—	Fitted
Transfer from Placenta to Fetus Cltrans1c_	0.06	0.06	Unpublished GD 20 Iodide iv Data
Transfer from Fetus to Placenta Cltrans2c_	0.12	0.12	Unpublished GD 20 Iodide iv Data

[a]All parameters listed are notated in the model by either an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR_*i*, PR_*p*, Vmaxc_T*i*, etc.)

and blood, so that Clewell (2001a) described the two-subpartment mammary gland with both diffusion of iodide and active uptake by the NIS.

Although it has been suggested that the placenta may contain the capability for active uptake in the rat, AFRL/HEST data did not indicate placenta:plasma levels greater than one for perchlorate or iodide (Yu, 2000), and unpublished iodide time course data indicate that the behavior of iodide in the placenta closely mirrors that of the plasma (Clewell, 2001a). Thus, the placenta was simulated with a single, flow-limited compartment.

Partitioning into the mammary, placenta, and other diffusion-limited compartments was based on effective partitioning. This effective partitioning is probably very similar to that in the thyroid where an electrochemical gradient is responsible for allowing the ClO_4^- anion to move between the serum and the tissue (Chow and Woodbury, 1970). Urinary clearance and placental-fetal transfer of the anions were represented by first order clearance rates.

The structure of the fetal perchlorate model is similar to that of the pregnant rat, with the exception of the mammary gland and placenta compartments. In order to simplify the model, all of the fetuses from a single litter were combined in the structure of the model, essentially viewing the individual fetuses as one entity, or one large fetus. The dose to the fetus is based on the transfer of perchlorate from the maternal placenta to the serum of the fetus, rather than through direct exposure to the drinking water. Though a kidney is included in the fetal model, urinary excretion is not used to identify the loss of perchlorate for the fetus. Since the ability to produce urine is not well developed until after parturition, the loss from the fetus is described as first order clearance from the fetal arterial blood to the placenta (Clewell, 2001a).

The pregnancy model attempts to describe perchlorate distribution in a highly dynamic system. In addition to total body weight changes in the dam and fetus, maternal mammary tissue and blood flow, cardiac output, fractional body fat, placenta and fetus body weight, and fractional body fat are also changing with respect to time. Growth equations, based on O'Flaherty et al. (1992) were used to account for these changes (Clewell, 2001a). All tissue volume and blood flow values were adjusted with respect to the changing parameters.

6.3.1 Data and Methods

This section summarizes the data that Clewell (2001a) used for development and validation of the pregnant and fetal rat model structures. Details on experimental methods, including:

protocol design, exposure regimen, chemical source and purity, animals (housing, feeding, surgical procedures, etc.), and the analytical methods can be found in the consultative letter and associated reports from AFRL/HEST or cited papers therein.

6.3.1.1 AFRL/HEST Experiments in Laboratory Rats

These studies are described in the consultative letters and reports of Clewell (2001a), Yu (2000, 2001, 2002) and Yu et al. (2000).

6.3.1.1.1 Drinking Water Study

Perchlorate drinking water experiments used in model development were performed at AFRL/HEST and described in detail in the report Yu (2000). Pregnant dams of the Sprague-Dawley strain were exposed to drinking water treated with perchlorate from gestational day (GD) 2 through 20, at perchlorate doses of 0.0, 0.01, 0.1, 1.0 and 10.0 mg/kg-day. GD0 was determined by the presence of a vaginal plug. Both dams and fetuses were sacrificed on GD20 and maternal and fetal serum analyzed for free and total thyroxine (fT4 and tT4), triiodothyronine (T3), and TSH. Maternal serum, thyroid, skin, GI contents, placenta, and amniotic fluid were analyzed for perchlorate at all of the above doses. Fetal serum, skin and GI tract were also analyzed for perchlorate at all of the doses. Two hours before sacrifice, the dams were given iv doses of 33 mg/kg radiolabeled iodide ($^{125}I^-$) with carrier. Tissue concentrations of iodide were measured in order to determine the inhibition in the various tissues after long-term exposure to perchlorate.

6.3.1.1.2 Preliminary Iodide Kinetics Study

A preliminary study of radiolabeled ($^{125}I^-$) kinetics was performed by AFRL/HEST in which timed-pregnant dams of the Sprague-Dawley strain were exposed via tail-vein injection to a tracer dose (average dose = 2.19 ng/kg body weight) of the radiolabeled anion on GD20. Dams (n=6) were sacrificed at 0.5, 2, 4, and 8 hours post-dosing. Maternal serum, thyroids, skin, GI contents, placenta and mammary gland tissue, as well as fetal serum, skin, and GI tract were collected and analyzed for iodide content at each time point. Serum was pooled for all fetuses within a litter, due to limited sample volume. Fetal skin and GI tract were analyzed individually.

6.3.1.1.3 Iodide Inhibition Kinetics Study

A more in-depth study was performed by AFRL/HEST, in which Sprague-Dawley timed-pregnant dams were given 1.0 mg/kg body weight perchlorate via tail-vein injection on GD20; control rats were given saline. The perchlorate or saline dose was followed two hours post dosing with a tail-vein injection of carrier free $^{125}I^-$ at an average dose of 1.87 ng/kg BW. Dams (n=6) were sacrificed after 0.5, 1, 2, 4, 8, 12, and 24 hours. Maternal serum, thyroids, skin, GI contents, placenta, mammary gland tissue, and fetal serum, skin, and GI tract were collected and analyzed for iodide content at each time point. Serum was again pooled for all fetuses within a litter. Fetal skin and GI tract were analyzed individually. At this time, only the maternal serum, maternal thyroids and fetal serum from this study were available for use with the model. Clewell (2001a) states that further validation of the model structure will be performed at a later time with the remaining data, but no further work has been provided to the EPA. Additional data were provided by Yu (2002).

6.3.1.2 Data Published in the Literature

Data available in the literature used in a validation exercise of the model are described briefly in this section.

6.3.1.2.1 Versloot et al., 1997

Versloot and coauthors measured $^{125}I^-$ as percent of dose in maternal and fetal thyroid, mammary gland, placenta, and fetal carcass without the thyroid. Pregnant Wistar rats (body weight [BW] = 300 ± 5 g) were given an injection of 10 μCi carrier free $^{125}I^-$ into the right vena jugularis on GD19. Measurements of the maternal thyroid were taken at 4 and 24 hours post dosing. Mammary gland, placenta, fetal thyroid, and fetal carcass minus the thyroid were taken only 24 hours post dosing.

6.3.1.2.2 Sztanyik and Turai 1988

Sztanyik and Turai measured the uptake of iodide into the placenta and fetal whole body 24 hours post dosing. Five groups of CFY albino rats (BW = 200 to 250 g) were dosed ip with 370 kBq (0.081 ng) carrier free radiolabeled iodide ($^{131}I^-$) on GDs 17, 18, 19, 20, and 22. Although this is a different strain of rat, the GD20 fetal weights (average BW = 4.088 g) compare

favorably with those seen on GD20 in the Sprague-Dawley fetus. As a result, Clewell (2001a) used the GD20 time point as a means of validating GD20 parameters for iodide across different data sets and doses. Placental and whole body fetal $^{131}I^-$ were measured in a well-type scintillation detector.

6.3.1.2.3 Feldman et al., 1961

Feldman and coauthors measured the uptake of iodide into the fetal thyroid and rest of body carcass on GDs 16, 17, 18, and 19 in pregnant female Holtzman rats. A single subcutaneous injection was given to the dam, containing 50 μCi of $^{131}I^-$ on each of the days mentioned above. Fetal thyroid and carcasses were measured at 24 hours post dosing.

6.3.2 Pregnant Rat and Fetus Model Development

This section summarizes only the key features that were different than the adult male rat model previously described in Section 6.2.

6.3.2.1 Physiological Parameters and Tissue Partition Coefficients

Maternal parameters were scaled allometrically based on body weight as previously described for the male rat. Fetal values were scaled in the same manner as the maternal parameters. However, since the model actually represents several fetuses, it was necessary to first scale the values for the individual fetus and then adjust for the total number of fetuses in the litter (Clewell, 2001a).

Clewell (2001a) based the physiological description of the maternal and fetal rat during gestation on O'Flaherty et al. (1992). However, growth descriptions, body weights, and organ descriptions were optimized for use within this particular model structure. The model is able to account for differences in gestation time, pup birth weight, and litter size between experiments and strains of rats. Growth equations and parameters that change over time were described with mathematical descriptions of available literature and experimental data. Details and equations are provided in the consultative letter (Clewell, 2001a).

6.3.2.1.1 Maternal Tissues

The body weight of the dam is known to change significantly throughout the relatively short gestation time in the rat (21 days). However, the traditional approach utilizing allometric scaling to describe tissue growth in relation to the change in body weight is not a sufficient description for the changes taking place during pregnancy. As opposed to the typical growth scenario, organs and tissues cannot be assumed to increase at the same rate in this dynamic system (Clewell, 2001a). The placenta, fetal volume, and mammary tissue grow at an accelerated rate in comparison to the other organs. These require additional descriptions for their growth beyond the previously described allometric scaling by body weight.

Since the growth of the other tissues is negligible in comparison to the change in the placenta, mammary gland, fat and fetal volume, Clewell (2001a) described the total change in the maternal body weight as simply the change in these four volumes added to the initial (pre-pregnancy) body weight (BW_{init}). All other maternal organs were assumed to remain constant and were scaled allometrically relative to the initial body weight (see Table 6-5).

Mammary tissue growth during gestation was described by Knight and Peaker (1982). Based on this work, Clewell (2001a) described mammary tissue growth as a linear process during which the mammary gland reaches a maximum volume for gestation on GD21 of 4.6% of the maternal body weight.

Clewell (2001a) also described the growth of maternal fat as a linear process throughout gestation based on the work of Naismith et al. (1982). Naismith reported a 40% increase in body fat throughout gestation. Thus, in the model a linear equation was employed to describe a 40% increase in body fat during the length of gestation with an initial (non-pregnant) value of 7.0% body weight for Sprague-Dawley rats (Brown et al., 1997).

Placental volume was described in the model as a sum of three stages of growth, based on the data of Buelke-Sam et al. (1982a), Sikov and Thomas (1970), and the mathematical description of data provided in O'Flaherty et al. (1992). The placenta volume is negligible during gestational days 1 through 5. Individual yolk sac placenta enter a stage of rapid growth between days 6 and 10 of gestation, and was described by an equation that accounted for yolk sac placenta, the total volume of the placenta during this time period, and the number of fetuses present. Placental growth during gestational days 6 through 10 is defined solely by this equation. Total placenta volume changes during gestational days 10 through 21 (parturition) were defined

by two separate processes: the exponential decline in yolk sac volume and the increase in chorioallantoic placenta (Clewell, 2001a).

O'Flaherty et al. (1992) also described the growth of the uterus and liver during gestation. However, Clewell (2001a) did not include a specific description of growth in these organs because the liver is not believed to have a major role in perchlorate kinetics. Further, because the iodide model does not describe deiodination, the description of liver growth was deemed unnecessary. The use of a uterine compartment was also not included in the Clewell (2001a) model due to the lack of available perchlorate and iodide data. The uterus was considered to be part of the lumped richly perfused tissue. EPA agrees that adding a description of liver growth would only bring additional complexity to the model structure without providing a real benefit to the description of perchlorate and total iodide kinetics and that the uterine compartment would be purely hypothetical and could not be validated without pertinent data.

6.3.2.1.2 Maternal Blood Flow

Clewell (2001a) described temporal changes in maternal cardiac output during gestation as the sum of the initial cardiac output, given in Brown et al. (1997) for a non-pregnant rat, and the change in blood flow to the placenta, mammary, and fat tissues. The approach of O'Flaherty et al. (1992) to changing blood flows was utilized in placental, mammary, and fat blood flows. The fraction of cardiac output to the mammary gland and fat tissues are described as proportional to the change in volume of the tissue. The change in blood flow to the yolk sac placenta is approximately proportional to the change in volume of the yolk sac. However, the blood flow to the chorioallantoic placenta increases at a faster rate than the change in volume, so three different equations were used to describe the blood flow for each different stage of placental growth (GD1 to GD6, GD7 to GD10, GD11 to GD12, and GD13 to GD21).

6.3.2.1.3 Fetal Tissues

A three stage description of fetal growth was also described in O'Flaherty et al. (1992) in order to mathematically reproduce data obtained from Beaton et al. (1954), Sikov and Thomas (1970), Goedbloed (1972), and Buelke-Sam et al. (1982a). Because data are not available for fetal volume between gestational days 1 through 11, an exponential growth curve was used as a reasonable approximation of fetal growth and was fit to the first available data for fetal volume

(Clewell, 2001a). The second stage of growth describes a slower increase in fetal volume, beginning on GD11, based on the same data. Clewell (2001a) described the third stage of fetal growth as a linear increase between days 18 and the day of parturition. The equation is dependent on the weight of the pup at the time of birth so that the model can account for the differences in birth weight encountered when simulating different data sets.

Individual fetal organ weights were assumed to increase linearly with respect to change in fetal body weight and were therefore scaled allometrically to account for changes in tissue volumes. Values for tissue volumes were taken from the literature and from experimental data for the fetus when available. However, most volumes were taken from adult rat data and scaled allometrically for the fetus due to the lack of tissue data in fetuses.

Florsheim et al. (1966) measured thyroid and body weight of the rat fetus and pup from GD18 through PND22 and reported a linear relationship between the thyroid weight and body weight throughout the time period. The value given for the thyroid of the fetus in %fetal body weight for GD19 was used in the Clewell (2001a) model. On the other hand, the physiology of the developing thyroid was found by Conde et al. (1991) to change significantly between birth and PND120. Conde reported a decrease in follicle volume from 61.4% to 37.2% of the total volume of the thyroid from birth to 120 days. An increase in colloid volume from 18.3% of the total thyroid volume at birth to 32.5% at 120 days was also reported. In the absence of histometric data in the fetal thyroid, the follicle, colloid, and stroma volumes for the fetus were described using the thyroid fractions measured at birth. The value for thyroid stroma was calculated within the model by subtracting the colloid and follicle volumes from the total thyroid volume.

The fetal body fat content was assumed to be zero in the Clewell (2001a) model. This assumption is reasonable in light of the data given in Naismith et al. (1982). Naismith et al. (1982) measured values for the body fat of PND2 and 16 rat pups, corresponding to 0.16% and 3.7% of the body weight. Given that body fat quickly increases in the neonatal period, it is not unreasonable to assume that body fat in the fetus is negligible. The volume is certainly not large enough to interfere with iodide or perchlorate kinetics. All other parameters were scaled allometrically by fetal weight from the adult male rat. The male rat physiological parameters were used rather than female parameters for several reasons. First, the male rat pups have been shown to be more sensitive to perturbation of hormone homeostasis by perchlorate, and therefore

are considered the sensitive endpoint (Yu, 2000). Additionally, Clewell (2001a) asserts that sufficient evidence was not found to indicate that physiological parameters between male and female rats were present in the fetus.

6.3.2.1.4 Fetal Blood Flow

Fetal blood flow was assumed to operate independently from the mother. The transfer of the chemical was accomplished via diffusion between the placenta and fetal blood. Therefore, the fetal cardiac output and blood flow to organs (as % cardiac output) were scaled allometrically from the male rat values relative to the fetal volume.

6.2.2.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities, and clearance rates (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the pregnant and fetal rat model were derived. The values can be found in Tables 6-4 and 6-5; details on the derivation can be found in Clewell (2001a).

6.3.2.2.1 Affinity Constants and Maximum Velocities for Active Uptake Processes

These were developed as described previously for the adult male rat model (Merrill, 2001c) in Section 6.2. The chemical specific parameters were kept the same in male, female, neonatal and fetal rats, and humans whenever possible. However, it was necessary to change a few of the parameters, including the maximum velocities (Vmaxc's) in the Clewell (2001a) model for pregnant rat and fetus. The Km values were similar between tissues and between female and male rat and human models. However, the maximum velocity or capacity differs between tissues (Wolff and Maurey, 1961). Since Vmaxc values for perchlorate were not given in literature, the values were estimated with the model. In order to determine Vmaxc using the model, the simulation for the tissue of interest was compared to various data sets with several different perchlorate dose levels. The value for Vmaxc within a given compartment was then determined by the best fit of the simulation to the data.

6.3.2.2.2 Effective Partitioning Permeability Area Cross Products and Clearance Values

These were developed as described previously for the adult male rat model (Merrill, 2001c) in Section 6.2. The value of 0.05 was used to represent the partitioning of perchlorate into the fat for the pregnant rat and fetus (Clewell, 2001a). This value was based on the data of Pena et al. (1976) who measured tissue:blood ratios in the laying hen after intra-muscular dosing with either a single injection of 10 μCi or 3 sequential doses of 10 μCi radiolabeled perchlorate. Although the hen is a very different species, several other tissues were reported to have values comparable to those found by Yu (2000) and Yu et al. (2000) in the male and female rat (0.3 vs. 0.31 in muscle, 0.1 vs. 0.1 in brain, 0.8 vs. 0.99 in the kidney). Clewell (2001a) noted that the use of this value is supported by the fact that the polarity of the perchlorate anion would severely limit the movement of perchlorate into fatty lipophilic tissue. Anbar et al. (1959) measured the mammary gland:blood ratios in the rat four hours after ip injection of radiolabeled perchlorate (100 mg $KClO_4$), and they reported an effective partition of 0.66 for the rat mammary gland. This value is in general agreement with that chosen by Clewell (2001a).

Maternal and fetal skin were described using the value Perlman et al. (1941) determined after a sc tracer dose of iodide for the partition coefficient in this compartment. Iodide partition coefficients were calculated from the tissue:blood ratios measured during the clearance phase of iodide data in the tissue of interest. The preliminary iodide kinetics study described in the supporting experiments was utilized for the determination of the placenta partition coefficients. For example, values for the GI tract and its contents were determined from the clearance portion of the iodide kinetic study in the adult male rat (Yu et al., 2000).

For all tissues in which a clearance was described (urinary clearance, transfer between placenta and fetal serum, and dissociation of perchlorate from the binding sites), a clearance value was determined. Since perchlorate is quickly excreted in urine and binding has little effect on serum levels at high doses, the simulation for the 10 mg/kg-day dose group was primarily dependent on the urinary clearance value (ClUc_*p*). The urinary clearance value for perchlorate was therefore based on the fit of the model to the serum data at the high dose. Iodide is incorporated into many of the constituents in plasma. However, it is not bound to the plasma proteins (i.e., albumin) in the same manner as perchlorate. Additionally, the iodide model is currently simplified to account for the distribution of total iodine. Therefore, the urinary clearance value (ClUc_*i*) was determined primarily by fitting the model simulation to the iodide

serum data, as blood levels were more dependent on excretion than on the amount of iodide in other tissues. The clearance of both iodide and perchlorate between the fetal serum and maternal placenta were based on the fit of the model simulation to the fetal and maternal blood levels and to the placenta concentration.

6.3.2.3 Pregnant Rat and Fetus Model Parameterization and Validation

This section summarizes how Clewell (2001a) used the various data sets to parameterize the model and the validation exercises performed.

6.3.2.3.1 Perchlorate Model Parameterization

Clewell (2001a) performed model parameterization for perchlorate using the data obtained from the AFRL/HEST drinking water studies on GD20. Optimized kinetic parameters (Vmax and permeability area values) were determined by fitting the model simulation to the experimental data. As for the adult male rat and human, it was necessary to account for the serum binding of perchlorate in order to adequately describe the blood perchlorate concentrations at the lower doses (0.01 and 0.1 mg/kg-day). Figure 6-28 illustrates the importance of binding in the model simulations of both maternal (A) and fetal (B) serum at 0.01 (left) versus the 10.0 (right) mg/kg-day dose. Binding does not have a noticeable effect on the plasma concentrations in the highest dose. However, as the perchlorate dose decreases, the effect of binding is more pronounced. Therefore, at lower levels, a larger percent of the injected dose will be bound. As the amount consumed is increased, the binding process is saturated and eventually the amount of perchlorate that is bound is negligible in contrast to the large amount of free perchlorate in the plasma. This is to be expected because the number of binding sites is limited.

Figure 6-29 shows the fit of the model to the maternal serum (left) and thyroid (right) perchlorate concentration (mg/L) in the dam on GD20. Since saturation of the symporter occurs between the 1.0 and 10.0 mg/kg-day dose groups, the influence of Vmaxc in the tissues was primarily in the 0.01 to 1.0 mg/kg-day doses. Thus, the fit of the model simulation to the data in the lower three doses was used to determine the values for Vmaxc in the tissues. On the other hand, the Vmaxc did not have a significant effect on the highest dose. The model fits to the 10 mg/kg-day dose group were primarily affected by the partition coefficients and permeability area values. Clewell (2001a) obtained the permeability area values in the tissues by fitting the

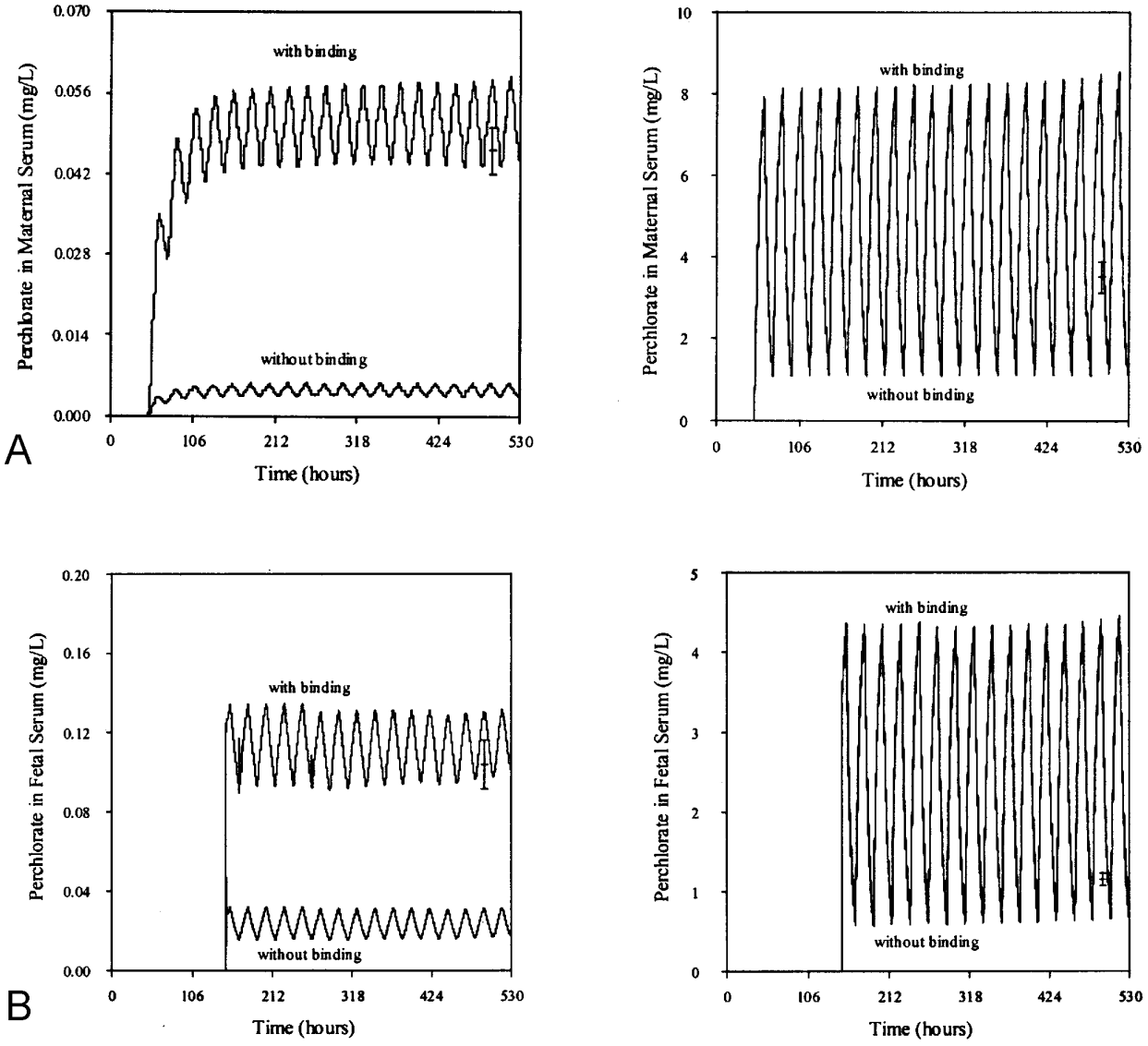

Figure 6-28. Simulations illustrating the necessity of including plasma binding in the pregnant dam and fetal rat PBPK model structure (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) are shown with and without plasma binding for maternal (A) and fetal (B) serum concentrations (mg/L) at two different doses, 0.01 mg/kg-day (left) and 10.0 mg/kg-day (right).

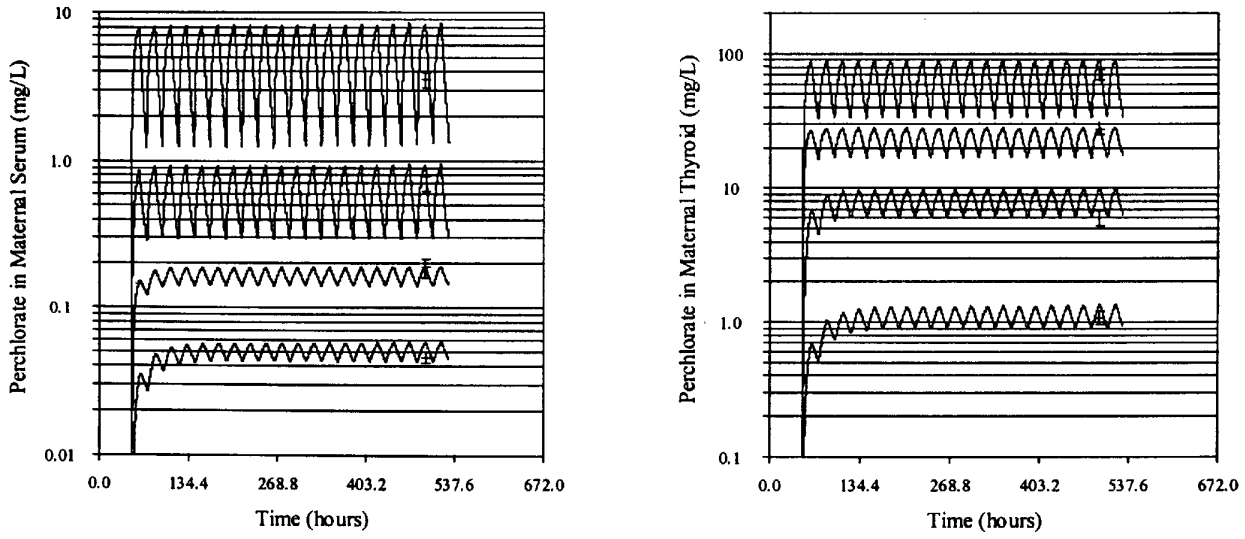

Figure 6-29. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in maternal serum (left) and thyroid (right) on GD20 (Clewell, 2001a). Pregnant rats were dosed in drinking water with 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate. Data of Yu (2000).

highest dose to the 10 mg/kg-day data in the tissues. Maternal placenta, mammary gland, and GI tract concentrations were available at the 10 mg/kg dose only. These tissues were used to verify the applicability of the assigned partition coefficients to the model. Since mammary glands were not available for the 0.01 through 1.0 mg/kg-day dose groups, it was not possible to fit the Vmaxc value to data at which the symporter has a significant effect. Therefore, the Vmaxc in the mammary gland was assigned the molar equivalent of the iodide Vmaxc. This is probably a reasonable value in the non-lactating gland. Clewell (2001a) provides additional figures that demonstrate the fit of the model to the GI tract, mammary glands, and placenta in the pregnant dam.

Fewer data were available for perchlorate distribution in the fetus than in the dam due to the experimental difficulty involved in sampling the small fetal tissues. Figure 6-30 depicts the model simulation of the fetal serum concentration (mg/L) compared to the data obtained in the drinking water study. Fetal serum and skin were pooled by litter. Fits to additional compartments are provided in Clewell (2001a).

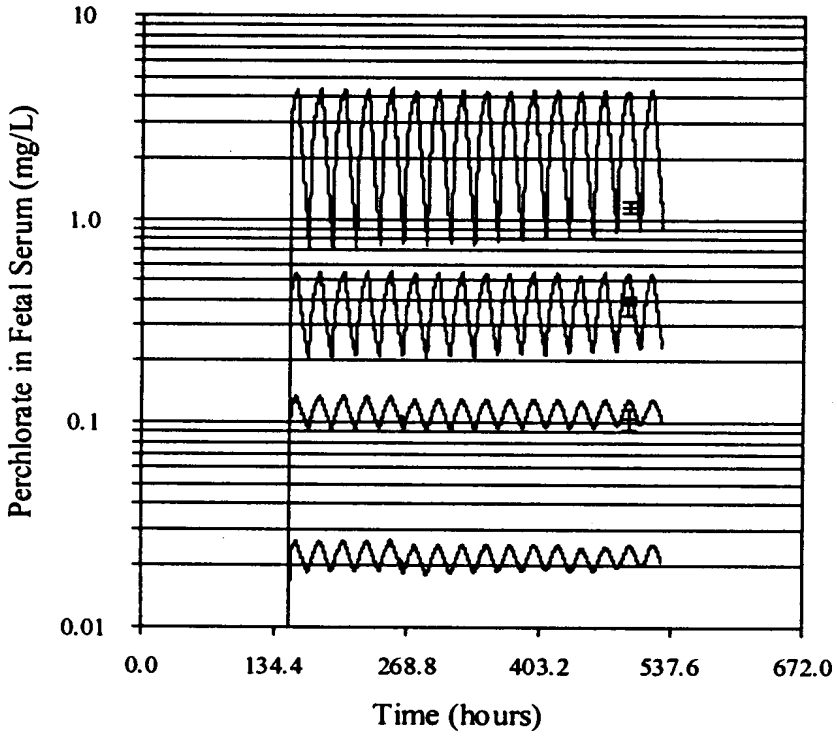

Figure 6-30. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in pooled fetal serum on GD20 (Clewell, 2001a). Pregnant rats were dosed in drinking water with 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate. Data of Yu (2000).

6.3.2.3.2 Iodide Model Parameterization

Development of the iodide model was performed by fitting the model to the kinetic data in the tissues of the dam and fetus from the preliminary iodide study. Only the values for Vmaxc and permeability area needed to be fit with the model. The clearance value for urinary excretion was determined by fitting the maternal serum prediction to the above data while keeping good fits in the other tissues, such as the maternal skin and gut and the fetal skin. Permeability area values were adjusted to describe the behavior of the iodide data, where varying the permeability area values toward 1.0 L/hr-kg generally increased the rate at which uptake and clearance in a particular tissue occurred; and decreasing permeability area slowed the uptake and clearance. Figure 6-31 shows the model simulation of the iv injection of 2.19 ng/kg $^{125}I^-$ on GD20 versus the experimental data for the maternal iodide concentrations in serum (top left), thyroid (top right), mammary gland (bottom left) and placenta (bottom right). The data are described well by the

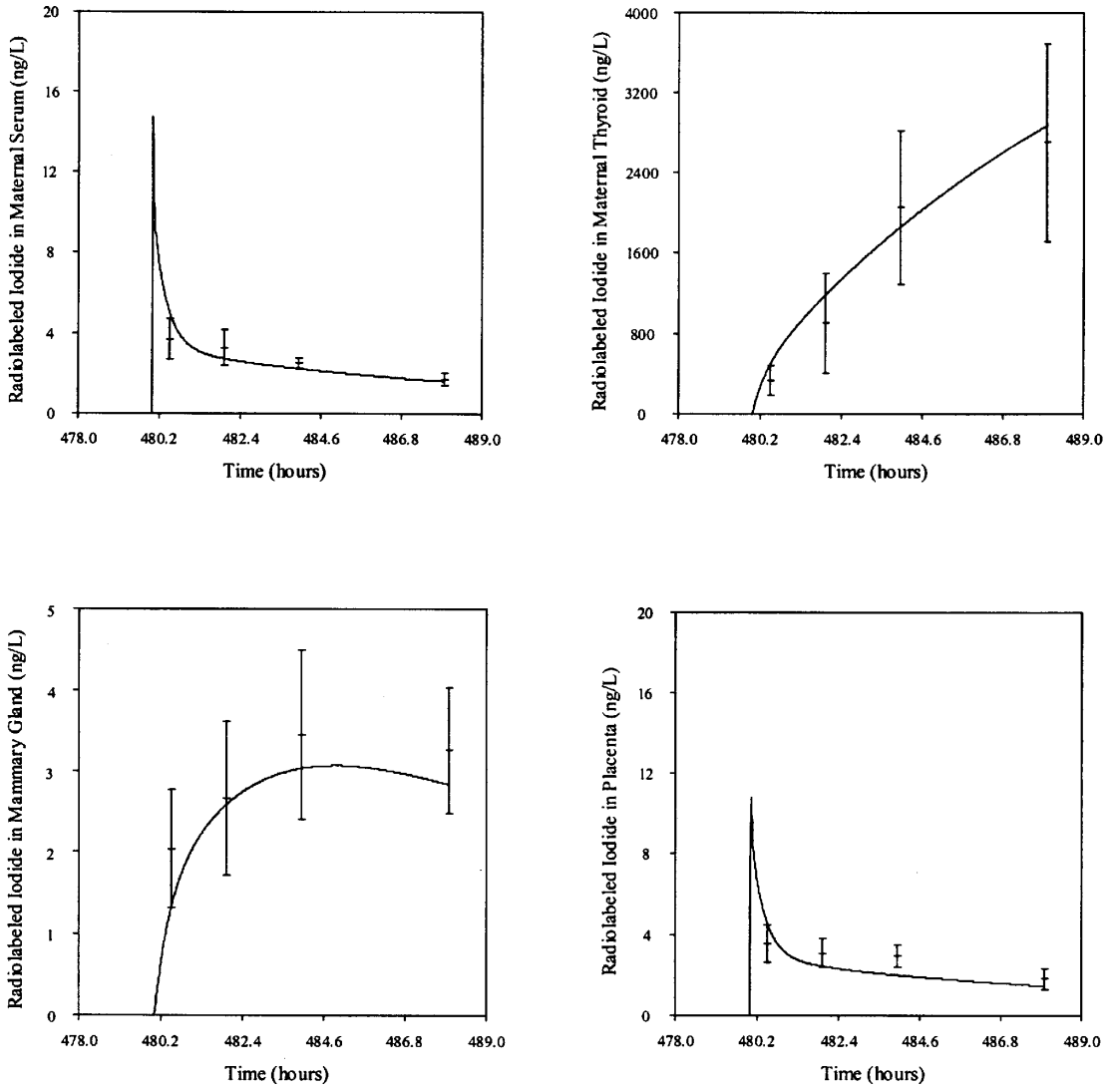

Figure 6-31. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in maternal serum (top left), thyroid (top right), mammary gland (bottom left), and placenta (bottom right) on GD20 (Clewell, 2001a) Pregnant rats were dosed by iv injection with 2.19 ng/kg $^{125}I^-$ on GD20. Data of Yu (2002).

model. The behavior of these mammary tissue data indicate that iodide is maintained in the mammary gland well into the clearance phase of the serum. In order to simulate this behavior, it was necessary to use a low permeability area value (0.02 L/hr-kg) in the mammary gland

(Clewell, 2001a). The mammary:plasma ratios of greater than one were fit with the Vmaxc for mammary NIS.

Clearance values for the transfer of iodide between the placenta and fetal blood were determined by optimizing the fit of the fetal serum to the data points while maintaining the fit of the simulations of the maternal blood and fetal tissue data. Figure 6-32 shows the model simulation versus the fetal data in the preliminary iodide time course study for radiolabeled iodide in fetal serum (ng/L). Clewell (2001a) shows additional simulations for fetal skin and fetal GI tract.

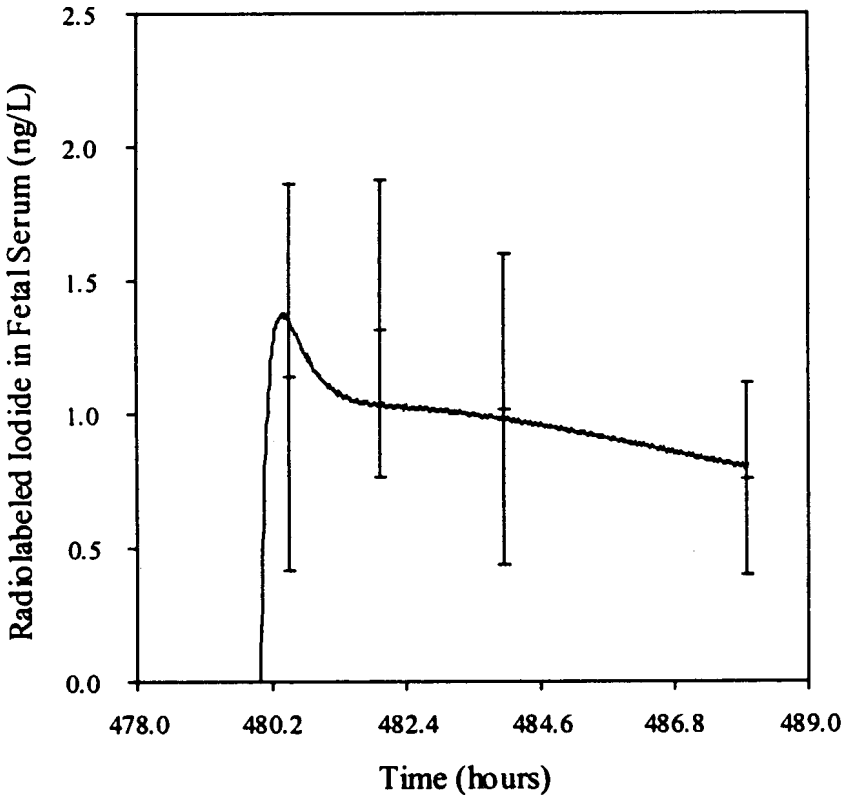

Figure 6-32. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in fetal serum on GD20 after an iv injection to the dam with 2.19 ng/kg $^{125}I^-$ (Clewell, 2001a). Data of Yu (2002).

The data of Feldman et al. (1961) were used by Clewell (2001a) to determine the values for maximum velocity of iodide uptake in the fetal thyroid. An exponential function was fit to the experimental values and time points where time was gestation in hours. This equation was then used in the model to account for the increasing ability of the fetal thyroid to incorporate iodide. Iodide levels were negligible on GD16 but increased dramatically from GD17 to GD19 (see Clewell, 2001a; Figure 25).

6.3.3 Model Validation

The Clewell (2001a) model predictions for the inhibition of iodide uptake into the thyroid and the resulting effect on the maternal and fetal serum was validated against the data collected by AFRL/HEST during the inhibition study on GD20. The kinetic parameters derived from the perchlorate drinking water and preliminary iodide data sets were used in the model. Because the inhibition study was performed with an acute perchlorate dose, it was necessary to make some slight changes in the parameters describing thyroid perchlorate kinetics. The long-term exposure to perchlorate in the drinking water studies (18 days) that were used to determine the perchlorate parameters is sufficient to induce up-regulation in the thyroid (Yu, 2000). Thus, it was determined that the thyroid parameters in the dam at this point would be different from those seen in an acute situation. The only parameters altered in order to model the acute perchlorate were the partition coefficient (from 2.25 to 0.13) and permeability area value (from 6.0E-4 to 4.0E-5) into the thyroid at the basolateral membrane (thyroid follicle). The value for the partitioning into the follicle in a naive thyroid was calculated as described previously from Chow and Woodbury (1970). The permeability area value in the naive thyroid follicle was determined with the lactation model, which is described in another consultative letter describing model development for the lactating rat (Clewell, 2001b).

The model simulation was fit to the available kinetic data in the thyroid while keeping all other thyroid parameters identical to those in the pregnancy model. Figure 6-33 illustrates the model prediction of thyroidal iodide uptake with and without perchlorate inhibition, utilizing these pre-set parameters. The model prediction of inhibition in the thyroid gland at 0.5, 1., 2, 4, 8, 12, and 24 hours after dosing with iodine shows an excellent fit to the data. The use of parameters derived from the drinking water perchlorate data for acute iodide uptake kinetics is well supported by the inhibition of iodide because inhibition is highly dependent on the

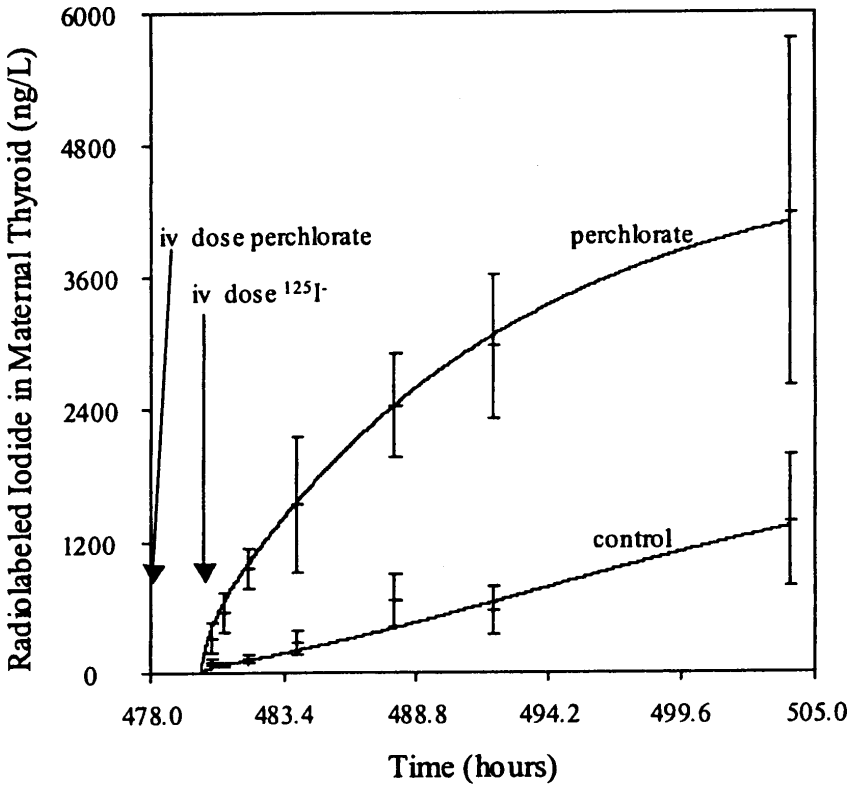

Figure 6-33. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in maternal thyroid with and without 1.0 mg/kg perchlorate administered by iv injection to the dam 2 hours prior to an iv injection with 1.87 ng/kg $^{125}I^-$ (Clewell, 2001a). The top simulation represents the control thyroid and the lower indicates the inhibited thyroid. Data of Yu (2000, 2002).

perchlorate concentration in the thyroid and the perchlorate affinity constants in the apical and basolateral membranes of the thyroid. Figure 6-34 illustrates the effect of perchlorate thyroid inhibition on the maternal (top) and fetal (bottom) blood iodide levels. Significant differences were found in the maternal serum iodide concentrations collected at the 1, 4, and 24 hour time points. Fetal serum, however, did not show any significant differences in the total serum iodide between the control and inhibited groups. Additional statistical analysis of these data are provided as Attachment #2 in Clewell (2001a).

Clewell (2001a) performed a model simulation of data presented by Versloot et al. (1997) in order to test the ability of the model to predict diverse data sets collected under different

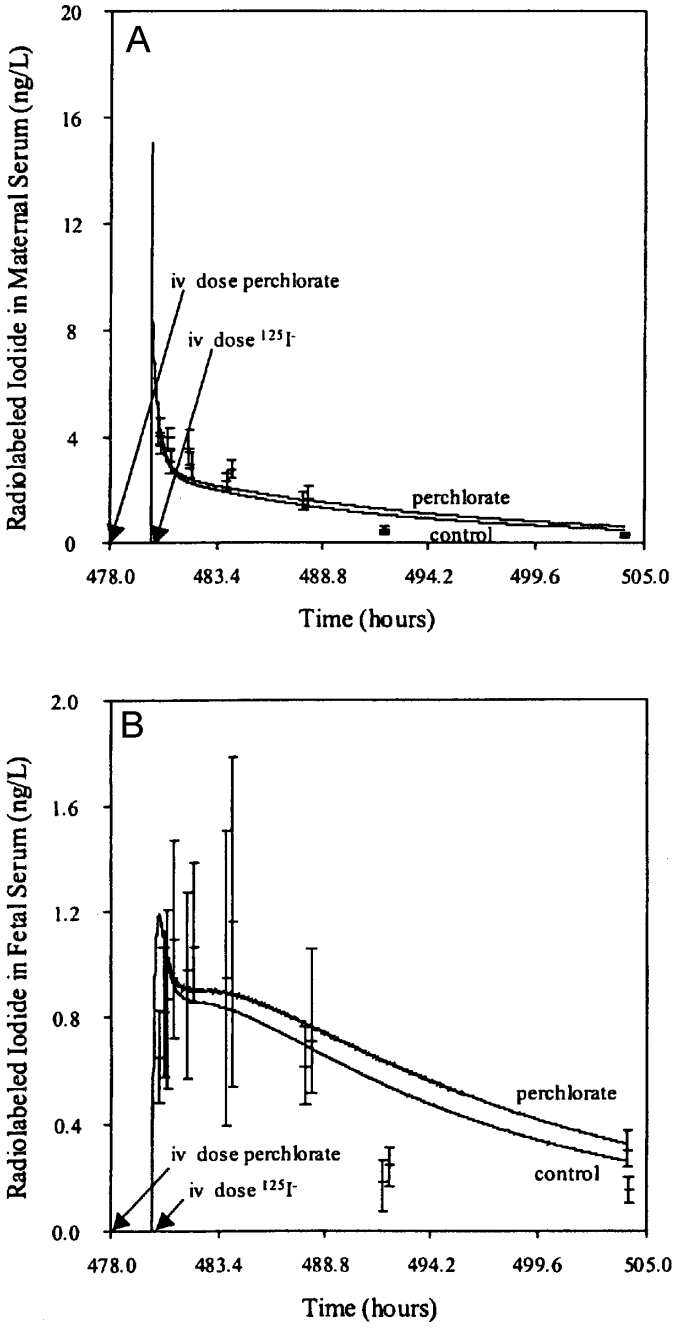

Figure 6-34. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in maternal (A) and fetal (B) serum with and without a 1.0 mg/kg perchlorate dose administered by iv injection to the dam 2 hours prior to an iv injection with 1.87 ng/kg $^{125}I^-$ (Clewell, 2001a). The top simulations in each represents the serum during thyroid inhibition and the lower represents the control serum. Data of Yu (2000, 2002).

conditions. This data set provided an additional time point for the iodide model validation (GD19). Dams were exposed by iv injection to 10 μCi (1.74 ng/kg) carrier-free radiolabeled iodide ($^{125}I^-$) on GD19. Figure 6-35 shows the model predictions versus data (mean ± SD) for the amount (ng) of iodide taken up in maternal thyroid (A), mammary gland (B), and placenta (C), or fetal thyroid (D). The model is able to accurately describe these tissues of interest and fits other compartments (data shown in Clewell, 2001a) within a two-fold factor without changing any parameters. This illustrates its predictive power and usefulness to the extrapolations required.

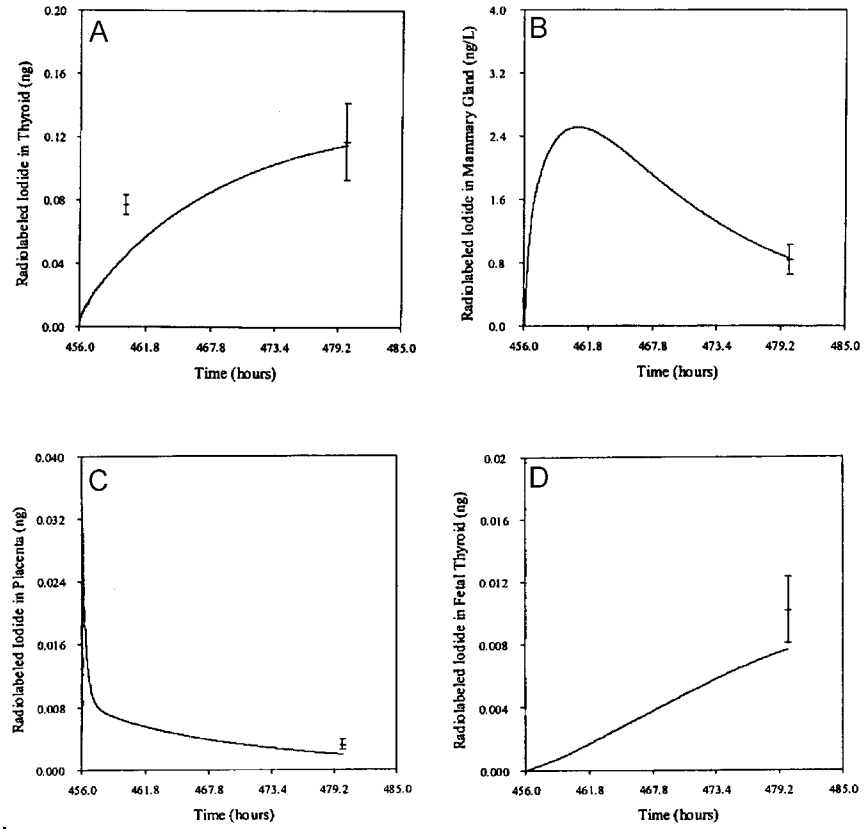

Figure 6-35. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of total $^{125}I^-$ radiolabeled iodide in the maternal thyroid (A), mammary gland (B), placenta (C), or fetal thyroid (D) at 24 hours afer exposure to the dam by iv injection of 10 μCi (1.74 ng/kg carrier-free) $^{125}I^-$ in GD19 dams. Data of Versloot et al. (1997).

Model predictions were also shown to be in good agreement with another unrelated data set, that of Sztanyik and Turai (1988), who measured carrier-free radiolabeled iodide ($^{131}I^-$) in GD20 dams and in the total (whole body) fetuses afer an iv injection (Clewell, 2001a). This validation illustrated adequate model fit to another time point and radioactive species of iodide. The model was additionally validated against AFRL/HEST data for dams and fetuses after administration of radiolabeled iodide ($^{125}I^-$) with carrier at doses four orders of magnitude greater than that used to parameterize the model (33000 ng/kg versus 2.19 ng/kg). These validation simulations are shown in Clewell (2001a).

As a final validation exercise, the model was used to predict radiolabeled iodide uptake inhibition after perchlorate exposures in drinking water for 18 days at 0.0, 0.01, 1.0, and 10 mg/kg-day (Yu, 2000). It was apparent that even at the lowest dose, the hormonal system had experienced a perturbation and was attempting to compensate for the interruption caused by the perchlorate exposure (Clewell, 2001a). Maternal T4 decreased in a dose-dependent manner, while TSH increased. The maternal total T4 and TSH changes were statistically significant at all doses. Free T4 was significantly increased at the 0.1, 1.0, and 1.0 mg/kg-day doses and total T3 was significantly decreased at the 1.0 and 10.0 mg/kg-day doses. The fetus appeared to follow the same trends as those seen in the dam. However, only the 1.0 and 10.0 mg/kg-day dose groups show significant decreases in total T4 and the 0.01, 1.0, and 10.0 mg/kg-day doses resulted in significantly increases in fetal free T4 and TSH. No significant decrease was seen in fetal T3. The statistical analysis of the hormone data is provided as Attachment #3 in Clewell (2001a).

From the perspective of iodide kinetics, these hormone changes are important indicators of thyroid up-regulation. When TSH is increased, the thyroid is stimulated to increase iodide uptake. It is evident, then, that after exposure to perchlorate in drinking water for 18 days, the thyroid of the pregnant dam has experienced both inhibition and up-regulation and has successfully compensated for the competition of perchlorate for binding sites of NIS. Therefore, it is not surprising that no inhibition was reported on GD20. It is not that the inhibition is not taking place, but rather that the system has compensated for the effect.

None of the models is currently equipped with the capability to account for up-regulation of the thyroid. Therefore, when a simulation of the inhibition is performed with the model, the concentration of iodide is under-predicted in a perchlorate-dose dependent manner (Clewell,

2001a). Figure 6-36 shows the model prediction of iodide in the thyroid of the dam at drinking water doses of 0.0, 0.1, 1.0, and 10.0 mg/kg-day. The Vmaxc for iodide was decreased to 2.5×10^4 to fit the mean from the control data with the control simulation in order to make the comparison of the inhibition data and simulations clearer. All experimental data were actually taken two hours post dosing. However, the data points were separated slightly by time on the plot in order to make them more visible. The prediction of thyroid perchlorate levels from this same study can be seen in Figure 6-29 (right).

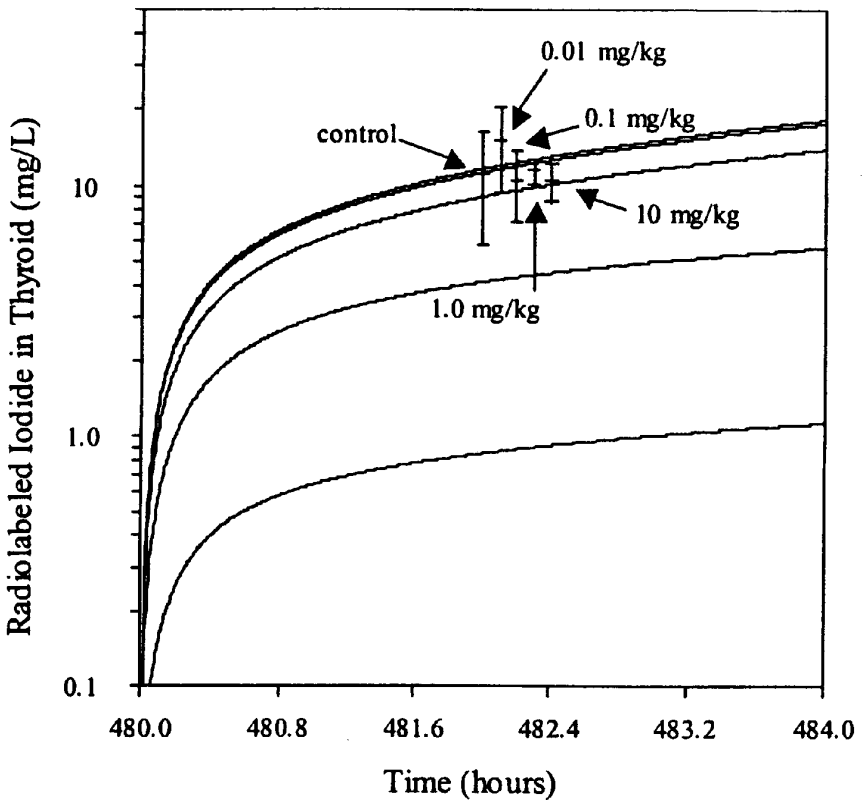

Figure 6-36. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of radiolabeled iodide in the maternal thyroid of the dam at doses of perchlorate in drinking water at 0.0, 0.01, 1.0, and 10.0 mg/kg-day for 18 days. Data of Yu (2000).

6.3.4 Summary

The proposed model for the pregnant rat and fetus developed by Clewell (2001a) appears to adequately describe perchlorate and iodide distribution in a highly dynamic, changing system, by accounting for growth with age-specific functions. The model predicts the transfer of perchlorate to the fetus and is also able to describe the uptake into fetal tissues of interest, such as the serum and thyroid. Fetal and dam tissues were predicted well by fitting data that spans three orders of magnitude (i.e., 0.01 to 10.0 mg/kg-day).

In addition to the requisite compartments for pregnancy (mammary gland, placenta, fetus), some differences exist that affect the kinetics of both perchlorate and iodide. The thyroidal maximum capacities are lower in the pregnant dam than in the male rat. Model parameterization in the male rat indicated the need for Vmax values for uptake into the follicle of the thyroid of 2.2×10^3 L/hr-kgr for perchlorate and 5.5×10^4 L/hr-kg for iodide, while the gestation model required values of 1.8×10^3 L/hr-kg and 4.0×10^4 L/hr-kg for the same parameters. This difference is supported in the literature. Versloot et al. (1997) suggest that the pregnant rat may have a lowered reserve of iodide in the thyroid toward the end of pregnancy, causing increased activity in the thyroid. The increased response of the pregnant rat was also seen in the studies performed by Yu (2000) and Yu et al. (2000) that reported a greater than average inhibition in the thyroid of the pregnant dam than in the male rat at the same perchlorate dose (78% vs. 70% over 8 hours). The skin of the pregnant dam also required a smaller value for Vmaxc than the male rat. This is supported by the work of Brown-Grant and Petes (1959), which reported higher levels of iodide in the skin male rats than in female rats. Skin, therefore, appears to be a more important iodide reserve in the male rat than the female. It is reassuring that the model is able to account for the majority of differences in the uptake, distribution, and excretion between the male and pregnant female by incorporating known differences in physiology.

Clewell (2001a) notes that at this time the amount of data concerning perchlorate kinetics in the pregnant rat is very limited. Although perchlorate has been used extensively in literature to study the thyroidal uptake of iodide, it has not been commonly used in rat gestation studies. As such, the perchlorate model was limited to utilizing the drinking water studies for parameterization. However, acute kinetic data were available for perchlorate in the lactating dam and were utilized in the development of the rat lactation model (Clewell, 2001b; see Section 6.4). This system is similar to that of the pregnant dam. Consequently, it was possible to simulate the

perchlorate kinetics of the dam with the same general model structure, changing only the physiological parameters. Therefore, it seemed reasonable to use the acute perchlorate parameters from the lactation model. The use of the described parameters for acute perchlorate kinetics is also supported by the ability of the model to predict inhibition in the pregnant dam. Clewell (2001a) discusses that acute perchlorate kinetic data to further verify the model are currently being analyzed by AFRL/HEST, and these were provided to the EPA too late for evaluation (Yu, 2002). In these studies, tissues were collected from pregnant dams and fetuses at various time points after iv injections of perchlorate. The use of these data in the modeling effort may be described in draft manuscripts provided to the external peer review.

The kinetic behavior of iodide was also accurately simulated with a range of doses that spans nearly five orders of magnitude (0.36 to 33,000 ng/kg). The active sequestration of iodide in maternal and fetal tissues and the transfer of iodide between mother and fetus was described kinetically with the model, and data have been simulated at a variety of doses and at various time points up to 24 hours post exposure. The fact that the model was able to simulate data from other laboratories under a variety of different conditions attests to the validity of the model structure and its applicability to other studies. The ability of the model to predict iodide was indicative of the usefulness of the model for predictive purposes. It was possible to predict inhibition out to 24 hours while simulating the serum and thyroid perchlorate and iodide levels with satisfactory accuracy. This provides support for the chosen model structure, as well as validation for the physiological and chemical descriptions used.

Clewell (2001a) notes that the inability of the model to respond to this auto-regulation presents a considerable need for further model development since drinking water scenarios would allow time for the hypothalamic-pituitary-feedback system to upregulate. Given that the temporal windows of developmental susceptibility are not well established across species, this issue may have to wait for further fundamental neurodevelopmental research.

The EPA has also become aware of a recent human biokinetic model for iodine and radionuclides at various ages (fetus, children, mothers) that may provide some additional information with which to validate the iodide kinetic components of the proposed models from AFRL/HEST scientists (International Commission on Radiological Protection, 2001, 1989).

6.4 LACTATING AND NEONATAL RAT MODEL STRUCTURE

This section describes the model developed by AFRL/HEST in response to concerns about interspecies extrapolation of effects observed in laboratory rats immediately after parturition up to about PND22 (Clewell, 2001b) and updates the preliminary structure provided to EPA (Clewell, 2000). The model predicts the distribution of perchlorate within the lactating dam and neonatal rat during these first few weeks of life, and also predicts the short-term effect of acute perchlorate exposure on iodide kinetics, including iodide uptake in the maternal thyroid.

Concern regarding the kinetics of perchlorate in lactating dams and neonates was motivated by the knowledge that the mammary gland is another tissue with active transport via the NIS, as described in Section 6.3. Perchlorate can thus competitively inhibit the uptake of iodide into the mammary gland in a manner reminiscent of the thyroid, and reduce the amount of available iodide to the infant. Studies utilizing radiolabeled iodide in lactating rats have shown perchlorate to be an effective inhibitor of iodide secretion of into breast milk (Potter et al., 1959, Brown-Grant, 1961). The fact that perchlorate not only inhibits the uptake of iodide, but is also taken up itself into the mammary tissue by way of the NIS, results in an additional risk to the neonate. The perchlorate is then concentrated in the milk and transferred to the litter through suckling.

Although early papers suggest that perchlorate is not transferred in milk (Zeghal et al., 1992), newer technology with better analytical sensitivity has detected perchlorate in the milk of rats dosed with as little as 0.01 mg/kg-day perchlorate in drinking water at the AFRL/HEST. The perchlorate levels in 5- and 10-day old neonate serum are comparable to those of the mother (Yu et al., 2000), indicating that the pups are in fact exposed to significant levels of perchlorate through the maternal milk. This information highlighted the need for more information regarding the effect of perchlorate exposure on the neonate.

The model structure is shown in Figure 6-37. Table 6-6 provides the physiological parameters used in the lactating and neonatal rat PBPK models. Table 6-7 provides the perchlorate-specific parameters, and Table 6-8 provides the iodide-specific parameters for each.

The model structure was developed to be consistent with the previously discussed structures for the adult male rat, pregnant rat, and fetus. In fact, an important linking to the pregnancy model was required. Since the experimental data used to develop the lactation model were taken from drinking water studies in which the dosing began on GD2, it was necessary to include initial perchlorate concentrations in the tissues at the time of birth (0 hours). In order to

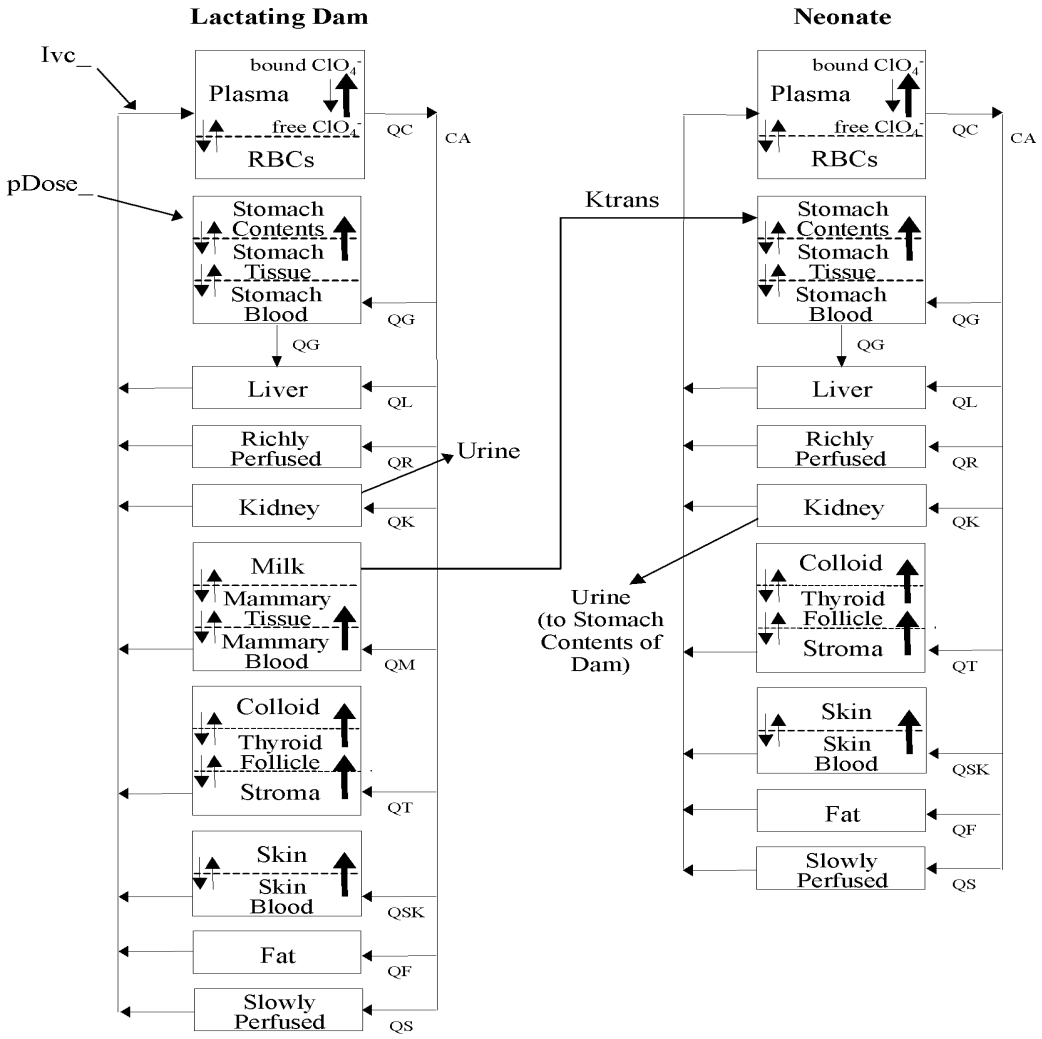

Figure 6-37. Schematic for the lactating dam and neonatal rat PBPK model of perchlorate and iodide distribution (Clewell, 2001b). Boxes represent specific compartments in the model structure. The thyroid consists of the stroma, the follicle, and the colloid, and the stomach consists of the capillary bed, stomach wall, and contents. The skin contains two subcompartments representing the capillary bed and skin tissue. Bold arrows indicate active uptake at NIS sites in the thyroid, skin, mammary gland and GI tract. Plasma binding was also described with Michaelis-Menten terms for the association of perchlorate anion to binding sites with first-order clearance rates for dissociation. Sequestration of the perchlorate (ClO_4^-) and iodide (I^-) anions into milk was also described with Michaelis-Menten kinetics. Permeability area cross products and partition coefficients were used to describe the first-order movement of the perchlorate (ClO_4^-) anion into deeper subcompartments which results from the inherent electrochemical gradient within the tissues. Urinary clearance and transfer of the anions through suckling were represented by first order clearance rates.

TABLE 6-6. PHYSIOLOGICAL PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)

Physiological Parameters	Lactation		
Tissue Volumes[a]	**Dam**	**Neonate**	**Source**
Body Weight *BW* (kg)	0.277 - 0.310	0.0075 - 0.1985	Yu, 2000
Slowly Perfused *VSc* (%BW)	37.07-40.42	53.92-49.31	Brown et al., 1997
Richly Perfused *VRc* (%BW)	5.35	5.36	Brown et al., 1997
Fat *VFc* (%BW)	12.45 - 6.9	0.0 - 4.61	Naismith et al., 1982
Kidney *VKc* (%BW)	1.7	1.7	Brown et al., 1997
Liver *VLc* (%BW)	3.4	3.4	Brown et al., 1997
Stomach Tissue *VGc* (%BW)	0.54	0.54	male rat ClO_4^- kinetics
Gastric Juice *VGJc* (%BW)	1.68	1.68	Yu, 2000
Stomach Blood *VGBc* (%VG)	2.9	2.9	Altman & Dittmer, 1971
Skin Tissue *VSkc* (%BW)	19.0	19.0	Brown et al., 1997
Skin Blood *VSkBc* (%VSkc)	2.0	2.0	Brown et al., 1997
Thyroid Total *VTtotc* (%BW)	0.0105	0.0125	Malendowicz & Bednarek, 1986; Florsheim et al., 1966
Thyroid Follicle *VTc* (%Vttot)	45.89	37.2	Malendowicz & Bednarek, 1986; Conde et al.,1991
Thyroid Colloid *VDTc* (%VTtot)	45	13.8	Malendowicz & Bednarek, 1986; Conde et al.,1991
Thyroid Blood *VTBc* (%VTtot)	9.1	49.0	Malendowicz & Bednarek, 1986; Conde et al.,1991
Plasma *VPlasc* (%BW)	4.7	4.7	Brown et al., 1997, Altman & Dittmer, 1971
Red Blood Cells *VRBCc* (%BW)	2.74	2.74	Brown et al., 1997, Altman & Dittmer, 1971
Mammary Tissue *VMc* (%BW)	4.4 - 6.6	—	Knight et al., 1984
Mammary Blood *VMBc* (%VM)	18.1	—	Assume same % as Thyroid Blood
Milk *VMk* (L)	0.002	—	Fisher et al., 1990
Blood Flows			
Cardiac Output *QCc* (L/hr-kg)	14.0 - 21.0	14.0	Hanwell & Linzell, 1973; Brown et al., 1997
Slowly Perfused *QSc* (%QC)	7.9-1.9	16.9	Brown et al., 1997
Richly Perfused *QRc* (%QC)	40.8	40.8	Brown et al., 1997
Fat *QFc* (%QC)	7.0	7.0	Brown et al., 1997
Kidney *QKc* (%QC)	14.0	14.0	Brown et al., 1997
Liver *QLc* (%QC)	18.0	18.0	Brown et al., 1997
GI *QGc* (%QC)	1.61	1.61	Brown et al., 1997
Skin QSkc (%QC)	0.058	0.058	Brown et al., 1997
Thyroid *QTc* (%QC)	1.6	1.6	Brown et al., 1997
Mammary *QMc* (%QC)	9.0 - 15.0	—	Hanwell & Linzell, 1973

[a]For calculation of volumes from body weight, a density of 1.0 g/mL was assumed.

TABLE 6-7. PERCHLORATE-SPECIFIC PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)[a]

Perchlorate Parameters	Lactation Values		
Partition Coefficients (unitless)	**Dam**	**Neonate**	**Source**
Slowly Perfused/Plasma PS_	0.31	0.31	Yu et al., 2000
Rapidly Perfused/Plasma PR_	0.56	0.56	Yu et al., 2000
Fat/ Plasma PF_	0.05	0.05	Pena et al., 1976
Kidney/ Plasma PK_	0.99	0.99	Yu et al., 2000
Liver/Plasma PL_	0.56	0.56	Yu et al., 2000
Gastric Tissue/Gastric Blood PG_	1.80	3.21	Yu, 2000; Yu et al., 2000
Gastric Juice/Gastric Tissue PGJ_	2.30	5.64	Yu, 2000; Yu et al., 2000
Skin Tissue/Skin Blood PSk_	1.15	1.15	Yu et al., 2000
Thyroid Tissue/Thyroid Blood PT_	0.13/2.0	0.13/2.0	Chow and Woodbury, 1970; Yu, 2000[b]
Thyroid Lumen/Thyroid Tissue PDT_	7.0	7.0	Chow and Woodbury, 1970; Yu, 2000
Red Blood Cells/Plasma PRBC_	0.73	0.73	Yu et al., 2000
Mammary Tissue/Mammary Blood PM_	0.66	—	Anbar et al., 1959
Milk/Mammary Tissue PMk_	2.39	—	Yu, 2000
Max Capacity, Vmaxc (ng/hr-kg BW)			
Thyroid Follicle Vmaxc_T	1.50E+03	1.50E+03	Fitted[c]
Thyroid Colloid Vmaxc_DT	1.00E+04	1.00E+04	Fitted[c]
Skin Vmaxc_S	8.00E+05	8.00E+05	Fitted
Gut Vmaxc_G	1.00E+06	1.00+06	Fitted
Mammary Tissue Vmaxc_M	2.0E+5/2.0E+4	—	Fitted[b,c]
Milk Vmaxc_Mk	2.00E+04	—	Fitted
Plasma Binding Vmaxc_B	9.00E+03	1.00E+03	Fitted
Affinity Constants, Km (ng/L)			
Thyroid Follicle Km_T	1.00E+05	1.00E+05	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Thyroid Colloid Km_DT	1.0E+09	1.0E+09	Golstein et al., 1992; Wolff, 1998
Skin Km_S	1.00E+05	1.00E+05	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Gut Km_G	1.00E+05	1.00E+05	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Mammary Km_M	1.0E+05	—	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Milk Km_Mk	1.00E+06	—	Fitted
Plasma Binding Km_B	1.00E+04	1.00E+04	Fitted

TABLE 6-7 (cont'd). PERCHLORATE-SPECIFIC PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)[a]

Perchlorate Parameters	Lactation Values		Source
	Dam	Neonate	
Permeability Area Cross Products, (L/hr-kg)			
Gastric Blood to Tissue PAGc_	1.00	1.00	Fitted
Gastric Tissue to Juice PAGJc_	1.00	1.00	Fitted
Thyroid Blood to Tissue PATc_	4.0E-05/6.0E-04	4.0E-05/6.0E-05	Fitted[b,c]
Thyroid Tissue to Colloid PADTc_	0.01	0.01	Fitted
Skin Blood to Tissue PASkc_	0.50	1.00	Fitted
Mammary Blood to Tissue PAMc_	0.01	—	Fitted
Mammary Tissue to Milk PAMkc_	0.001/1.0		Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted
Clearance Values, (L/hr-kg)			
Urinary excretion CLUc_	0.07	0.005	Fitted
Dissociation from Binding Sites Clunbc_	0.034	0.034	Fitted
Transfer from Milk to Pup Ktransc	6.4E-04/1.04E-03	6.4E-04/1.04E-03	Sampson & Jansen, 1984

[a]All parameters listed are notated in the model either by an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR_*i*, PR_*p*, Vmaxc_T*i*, Vmaxc_T*p*, etc.).
[b]Neonate was given maternal values for Vmax (scaled by body weight) in the absence of data.
[c]Parameters with two values indicate acute and drinking water parameters, respectively.

obtain these initial values for tissue loading at birth, the pregnancy model had to include all of the compartments contained in the lactation model (Clewell, 2001a). The pregnancy model was then allowed to run until the day of birth (GD22), and the average tissue concentrations of perchlorate or iodide for the final day of gestation were used as the starting values for the respective tissues in the lactation model (Clewell, 2001b).

As discussed, the mammary tissue has been shown to concentrate both perchlorate and iodide during lactation via the NIS symporter. Additionally, hormones produced during lactation such as prolactin which stimulates milk production, have been shown to regulate the mammary NIS. Suckling of the neonatal rats has also been shown to stimulate mammary NIS activity (Tazebay et al., 2000). An additional symporter has been identified in the experiments of Shennan (2001). *In vitro* studies of iodide transport into the mammary gland and the resulting efflux of sulfate from the cells in the absence of sodium cation ($Na^{+)}$, indicates that another form of transport exists for iodide in the mammary gland in addition to the NIS. Shennan suggests

TABLE 6-8. IODIDE-SPECIFIC PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)[a]

Iodide Parameters	Lactation Values		
Partition Coefficients (unitless)	**Dam**	**Neonate**	**Source**
Slowly Perfused/Plasma PS_	0.21	0.21	Halmi et al., 1956
Rapidly Perfused/Plasma PR_	0.40	0.40	Halmi et al., 1956
Fat/Plasma PF_	0.05	0.05	Pena et al., 1976
Kidney/Plasma PK_	1.09	1.09	Perlman et al., 1941
Liver/Plasma PL_	0.44	0.44	Perlman et al., 1941
Gastric Tissue/Gastric Blood PG_	1.00	1.00	Unpublished Lactation Inhibition Study
Gastric Juice/Gastric Tissue PGJ_	1.00	3.50	Unpublished Lactation Inhibition Study
Skin Tissue/Skin Blood PSk_	0.70	0.70	Perlman et al., 1941
Thyroid Tissue/Thyroid Blood PT_	0.15	0.15	Chow and Woodbury, 1970
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	Chow and Woodbury, 1970
Red Blood Cells/Plasma	1.00	1.00	Rall et al., 1950
Mammary Tissue/Mammary Blood PM_	0.66	—	Anbar et al., 1959
Milk/Mammary Tissue PMk_	4.00	—	Yu, 2000
Max Capacity, Vmaxc (ng/hr-kg BW)			
Thyroid Follicle Vmaxc_T	4.00E+04	4.00E+04	Fitted[b]
Thyroid Colloid Vmaxc_DT	6.00E+07	6.00E+07	Fitted[b]
Skin Vmaxc_S	6.00E+04	2.50E+05	Fitted
Gut Vmaxc_G	1.00E+06	2.00E+05	Fitted
Mammary Tissue Vmaxc_M	8.00E+05	—	Fitted
Milk Vmaxc_Mk	5.00E+06	—	Fitted
Affinity Constants, Km (ng/L)			
Thyroid Follicle Km_T	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Thyroid Colloid Km_DT	1.00E+09	1.00E+09	Golstein et al., 1992
Skin Km_S	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Gut Km_G	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Mammary Km_M	4.00E+06	—	Gluzman and Niepomniszcze, 1983
Milk Km_Mk	1.00E+06	—	Fitted
Permeability Area Cross Products, (L/hr-kg)			
Gastric Blood to Gastric Tissue PAGc_	0.80	0.05	Fitted
Gastric Tissue to Gastric Juice PAGJc_	0.60	0.06	Fitted
Thyroid Blood to Thyroid Tissue PATc_	1.00E-04	1.00E-04	Fitted[b]
Thyroid Tissue to Thyroid Colloid PADTc_	1.00E-04	1.00E-04	Fitted[b]
Skin Blood to Skin Tissue PASkc_	0.50	0.02	Fitted
Mammary Blood to Tissue PAMc_	0.02	—	Fitted
Mammary Tissue to Milk PAMkc_	1.00	—	Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted
Clearance Values, (L/hr-kg)			
Urinary excretion CLUc_	0.03	0.02	Fitted
Transfer from Milk to Pup Ktransc	6.4E-04 - 1.04E-03		Sampson & Jansen, 1984

[a]All parameters listed are notated in the model either by an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR_*i*, PR_*p*, Vmaxc_T*i*, Vmaxc_T*p*, etc.).
[b]Neonate was given maternal values for Vmax (scaled by body weight) in the absence of data.

that this anion transport mechanism is able to transfer perchlorate and iodide into the secretory cells against a concentration gradient. Since the secretory cells are responsible for secreting their contents into the milk, the anion transport mechanism was included in the milk compartment of the Clewell (2001b) model.

The structure of the Clewell (2001b) neonatal model is similar to that of the pregnant and fetal rat model, with the exception of the mammary gland compartment as will be described in 6.4.2.1.1. In order to simplify the model, all neonates from a single litter were combined in the structure of the model, essentially viewing the entire litter as one entity, or one large neonate. The dose to the neonate is based on the transfer of perchlorate from the maternal milk to the GI contents of the neonate rather than through direct exposure to the drinking water. The 60% of urinary excretion of the neonate is then entered back into the GI contents of the dam in order to account for maternal ingestion of the pup's urine during cleaning, based on the work of Samuel and Caputa (1965).

The same challenge posed by the pregnancy model (i.e., to describe perchlorate and iodide distribution in a highly dynamic system) was the objective of the lactating and neonatal rat model (Clewell, 2001b). In addition to total body weight changes in the dam and neonate, maternal mammary tissue and blood flow, cardiac output, fractional body fat and neonatal body weight, and fractional body fat change with respect to time. All tissue volume and blood flow values were adjusted with respect to the changing parameters.

Clewell (2001b) assumed the neonate to be nursing at a constant rate, 24 hours a day. This assumption is based on the fact that young nursing rats are unable to go for long periods of time without suckling. The loss through suckling was then described with a first order clearance rate from the mother's milk to the gastric juice of the neonate, based on the experiments of Sampson and Jansen (1984). The milk production rate was assumed to be equal to the amount of milk ingested by the litter.

6.4.1 Data and Methods

This section summarizes the data that Clewell (2001b) used for development and validation of the lactating and neonatal rat model structures. Details on experimental methods, including: protocol design, exposure regimen, chemical source and purity, animals (housing, feeding,

surgical procedures, etc.), and the analytical methods can be found in the consultative letter and associated reports from AFRL/HEST or papers cited therein.

6.4.1.1 AFRL/HEST Experiments in Laboratory Rats

These studies are described in the consultative letter and reports of Clewell (2001b), Yu (2000, 2002), Yu et al. (2000), and Mahle (2000; 2001).

6.4.1.1.1 Drinking Water Study

Perchlorate drinking water experiments used in development of the Clewell (2001b) model included this study in which pregnant Sprague-Dawley dams were exposed to drinking water treated with perchlorate from GD 2 through PND5 or PND10 at perchlorate doses of 0.0, 0.01, 0.1, 1.0, and 10.0 mg/kg-day. GD0 was determined by the presence of a vaginal plug. Litters were standardized to eight pups (four male and four female, when possible) on PND2. Dams and their litters were euthanized on either PND5 or PND10; maternal and neonatal serum was analyzed for fT4, tT4, T3, and TSH. Maternal serum, thyroid, skin, and gastric contents were analyzed for perchlorate at all doses. Neonatal serum, skin, and GI contents were also analyzed for perchlorate at all doses. Milk was analyzed only on PND10 at all doses. Perchlorate analysis was performed only on maternal gastric tract, mammary tissue, and neonatal gastric tract samples from the PND5 study at the 10.0 mg/kg-day dose. Two hours before euthanization, the dams were given iv doses of 33 mg/kg radiolabeled iodide ($^{125}I^-$) with carrier. Tissue concentrations of iodide were measured in order to determine the inhibition in the various tissues after long-term exposure to perchlorate. This study is described in detail in the consultative letter (Yu, 2000).

6.4.1.1.2 Cross-fostering Study

The cross-fostering study involved four groups of rats with varied experimental conditions: true control, control, exposed, and true exposed. True control rats were never dosed with perchlorate. Neonates remained with the dam after birth. In the control group, dams were never exposed to perchlorate in drinking water. However, at the time of birth, the neonates were replaced with pups (less than 24 hours old) that had been exposed to perchlorate throughout gestation (1.0 mg/kg-day to mother through drinking water). In the exposed group, the dams were dosed with 1.0 mg/kg-day perchlorate in drinking water from GD2 to PND10. At the time

of birth, the neonates were replaced with pups (less than 24 hours old) that had never been exposed to perchlorate. The true exposed dams were dosed with 1.0 mg/kg-day perchlorate from GD2 to PND10. Neonates remained with their mother after birth. All dams and pups were euthanized on PND10. The skin, GI contents, and serum from the neonates and dam were analyzed for perchlorate. Results indicated that both true control and control (exposed neonates with control dams) showed no perchlorate present on PND10. True exposed and exposed (exposed dams with control litters) showed comparable perchlorate levels on PND10. This study is described in detail in the consultative letters (Mahle, 2000; 2001).

6.4.1.1.3 Perchlorate Kinetics Study

In order to evaluate the acute kinetics of perchlorate in the lactating dam and neonate, AFRL/HEST performed a study of the kinetic behavior of perchlorate after the administration of an acute dose. PND10 Sprague-Dawley dams were given 0.1 mg/kg perchlorate by tail-vein injection. The dams were left with their neonates until the time of euthanization at 0.5, 1, 2, 4, 8, or 12 hours post-dosing. Maternal serum, thyroid, stomach contents, skin, and mammary gland were collected and analyzed for perchlorate content at all time points. Neonate serum, stomach contents, and skin were also collected for perchlorate analysis at all time points. Fat, liver, kidney and bladder tissues were also collected from the dam at the eight hour time point. Perchlorate analysis was performed on the serum of the dam and neonates and the maternal thyroid, mammary gland, GI contents, and skin.

6.4.1.1.4 Iodide Inhibition Kinetics Study

A study of iodide time course and inhibition kinetics was performed by AFRL/HEST in which Sprague-Dawley timed-pregnant dams were given 1.0 mg/kg body weight perchlorate via tail-vein injection on PND10. The perchlorate dose was followed at two hours post-dosing with a tail-vein injection of carrier free radiolabeled iodide ($^{125}I^-$) at an average dose of 2.10 ng/kg. Dams (n=6) were euthanized after 0.5, 1, 2, 4, 8, and 24 hours. Maternal and neonatal serum, skin, GI contents and tract, as well as the maternal thyroid and mammary gland tissue, were collected and analyzed for total iodide content at each time point. Neonatal serum was pooled by sex in each litter. Neonatal skin and GI contents and tract were analyzed individually.

6.4.1.2 Data Published in the Literature

Data available in the literature and used in development and validation of the model are described briefly in this section.

6.4.1.2.1 Sztanyik and Turai, 1988

Five groups of CFY albino rats (BW = 200 to 250 g) were dosed ip with either 370 kBq (0.081 ng) or 740 kBq (1.61 ng) carrier-free radiolabeled iodide ($^{131}I^-$) on PND1 (after 24 hours). Sztanyik and Turai measured the total iodide burden of each litter at 29 hours and on PNDs 2, 5, 7, 9, and 14. Since the litters were not standardized, the number of pups in each litter varied.

6.4.1.2.2 Potter et al., 1959

Four dams of the Long-Evans strain (PND 17-18) were dosed ip with 500 μCi of carrier-free radiolabeled iodide ($^{131}I^-$). Iodide uptake was measured in the milk and plasma of the dam 3, 6, and 24 hours postdosing and in the maternal thyroids 24 hours postdosing.

6.4.2 Lactating and Neonatal Rat Model Development

This section summarizes only the key features that were different than the preceding model structures described in Sections 6.2 and 6.3.

6.4.2.1 Physiological Parameters and Partition Coefficients

Maternal parameters were scaled allometrically based on body weight as previously described for the male rat. Neonatal values were scaled in the same manner as the maternal parameters. However, since the model actually represents several neonates, it was necessary to scale the values for the individual pup first, then to adjust for the total number of pups in the litter as was done in an analogous fashion as for the fetuses in the pregnant rat model (Clewell, 2001a,b).

6.4.2.1.1 Maternal Tissues

During lactation, the mammary gland grows in response to the increased need for milk production by the growing neonates. Knight et al. (1984) measured the mammary gland on several days during lactation. They found the mammary tissue to be 4.4, 5.6, 6.3, and 6.6% of

the maternal body weight on days 2, 7, 14, and 21, respectively. The residual milk was assumed to be 0.002 L based on the model of Fisher et al. (1990). Naismith et al. (1982) examined the change in body fat content of the lactating rat. They reported values for the volume of maternal body fat of 15.2 and 6.9% of the body weight on PND 2 and 16, respectively. The body fat composition of the dam on PND1 was calculated to be 12.4% from the PBPK model for perchlorate and iodide kinetics in the pregnant rat model described in Section 6.3 (Clewell, 2001a).

In order to describe the changes in the physiology of the lactating rat, it was not sufficient to simply scale some of the parameters allometrically. As opposed to the typical growth scenario, some of the tissues in the lactating rat cannot be assumed to increase at the same rate in this dynamic system. Rather, a few tissues, such as the mammary gland and fat, are changing at an accelerated rate in comparison to the other organs. These parameters required additional descriptions for their growth beyond the previously described allometric scaling by body weight. Clewell (2001b) based the approach to modeling these changing parameters on the work of Fisher et al. (1990) with trichloroethylene.

Additionally, the thyroid of the female rat was found by investigators to be significantly larger than that of the male rat (Malendowicz and Bednarek, 1986). Clewell (2001b) assigned values to these parameters based on these data and relevant to the gender and condition (i.e., lactation) of the animal. A value of 1.05% of the maternal body weight was used for the thyroid in the lactation model. The volume fractions of the colloid, follicle, and stroma were given values of 45, 46, and 9% of the thyroid volume. These are significantly different from the values given for the male rat. The volume of the colloid in particular is much greater in the female than the male rat (46 vs. 24% of the thyroid volume). Parameters that were not available specifically for the female were described by adjusting the values for the male rat by body weight.

In the PND10 drinking water study performed by AFRL/HEST (see Attachment #2; Clewell, 2001b), the body weight of the dam showed an average increase of 12% between PND1 and PND10, but did not show a significant difference in weight between dose groups. As a result, Clewell (2001b) calculated the average body weight of the dams for all dose groups for each day of the study and then programmed this changing body weight into the model as a table function.

6.4.2.1.2 Neonatal Tissues

As for the lactating rats, Clewell (2001b) programmed the overall average body weights of the neonates measured on PNDs 3, 5, 7, 9 and 10 into the model is a table function, in order to estimate growth. Naismith et al. (1982) reported the body fat in the pup at PND2 and PND16 to be 0.167 and 3.65% of the neonatal body weight. The amount of body fat in a 41-day old rat was given in Brown et al. (1997) as 4.61% of the body weight.

The volume of the thyroid was studied by Florsheim et al. (1966). The volume of the thyroid was found to increase in a fairly linear relationship with body weight between PND1 and PND22. These investigators reported thyroid volumes of 0.0125, 0.0146, 0.0120, 0.0137, 0.0130, 0.0130, and 0.0131% body weight for neonates on PND1 through 5, 7, and 11. These values were used in a table function in the model to describe the growth of the neonatal thyroid (Clewell, 2001b). The histometry of the thyroid in the neonate was examined by Conde et al. (1991). The authors found a significant difference between the volume fractions of the colloid, follicle and stroma in the neonatal rat versus those in the adult. The reported values of 18.3, 61.4, and 20.3% thyroid volume were used to describe the colloid, follicle, and stroma fractions in the neonatal rat (Clewell, 2001b).

The suckling rate of the neonatal rat has been examined in more than one literature study and has been shown to change over time in response to the growth of the neonatal rats. As the pups grow, they require larger amounts of milk. Sampson and Jansen (1984) measured the amount of milk suckled in rats by removing neonates from the dams for two hours and then allowing the pups to suckle for two hours. This process was repeated throughout the day on several days of lactation. By assuming that the weight gained by the neonates during the suckling period was due to the milk intake and the weight lost while separated from the dam was through excretion, Sampson and Jansen were able to develop an equation that describes the suckling rate of the neonatal rat. Since this equation is dependent on the body weight and growth rate of the neonates, it is able to account for the change over time and the difference between strains and studies. The equation was used in the Clewell (2001b) model which assumed the milk yield of the dam was equal to the suckling rate of the neonate.

6.4.2.1.3 Blood Flows

All maternal and neonatal blood flows that were not directly affected by the changes induced by lactation were scaled by weight from the adult male rat parameters. For those blood flow parameters that change in response to lactation, some additional description was required (Clewell, 2001b). Cardiac output has been shown to increase during lactation (Hanwell and Linzell, 1973). The values given by Hanwell and Linzell (1973) of 14.0, 18.6, 19.0, and 21.0 L/hr-kg for days 3, 8, 13, and 23 of lactation were used in the model as a table function to describe the change in cardiac output over time (Clewell, 2001b). Additionally, the blood flow to the mammary tissue was also found to increase during lactation. Reported fractional blood flows to the mammary tissue of 9, 10, 11, 14, 14, and 15% of the cardiac output on PNDs 1, 5, 10, 15, 17, and 21, again from Hanwell and Linzell (1973), were used.

6.4.2.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities and clearance rate (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the lactating and neonatal rat model were derived. The values can be found in Tables 6-7 and 6-8. Details on the derivation can be found in Clewell (2001b).

6.4.2.2.1 Affinity Constants and Maximum Velocities for Active Uptake Processes

Whenever possible, chemical specific parameters were kept the same in human and in male, female, neonatal, and fetal rats. However, it was necessary to change a few of the parameters, including the maximum velocity capacity (Vmaxc). The Km values were similar between tissues and between female and male rat and human models. However, the maximum velocity capacity differs between tissues (Wolff and Maurey, 1961). Since values for the tissue maximum velocity capacity for perchlorate (Vmaxc-*p*) were not given in literature, the values were estimated with the model. In order to determine Vmax with the model, the simulation for the tissue of interest was compared to various data sets with several different perchlorate dose levels. The value for Vmaxc within a given compartment was then determined by the best fit of the simulation to the data.

6.4.2.2.2 Effective Partitions, Permeability Area Cross Products and Clearance Values

Anbar et al. (1959) measured the mammary gland:blood ratios in the rat four hours after an intra-peritoneal injection of 100 mg radiolabeled perchlorate ($^{36}ClO_4^-$) as potassium perchlorate. They reported an effective partition of 0.66 for the rat mammary gland. Clewell (2001b) used this value in the model. Since the partition for iodide into the mammary gland was not available in the literature, Clewell (2001b) assigned the same effective partition coefficient as used for perchlorate.

When available, iodide partition coefficients were calculated from the tissue:blood ratios measured during the clearance phase of iodide data in the tissue of interest. For example, GI tract and contents were determined from the clearance portion of the data from the iodide kinetic study in the lactating rat.

For tissues in which a clearance was described (urinary clearance and dissociation of perchlorate from the binding sites), a clearance value was determined by fitting the model simulation to the appropriate tissue data. Since perchlorate is quickly excreted in urine and binding has little effect on serum levels at high doses, the simulation for the 10 mg/kg-day dose group was primarily dependent on the urinary clearance value (ClUc_*p*). The urinary clearance value for perchlorate was therefore based on the fit of the model to the serum data at the high dose. The value obtained in this manner was similar to that determined by fitting the male rat PBPK simulation to urinary perchlorate at several doses (Merrill, 2001a) and to the high dose in the pregnant rat (Clewell, 2001a). The rate of dissociation of perchlorate from the binding sites was fit to the serum data across doses.

6.4.2.3 Lactating Rat and Neonate Model Parameterization and Validation

This section summarizes how Clewell (2001b) used the various data sets to parameterize the model and how the validation exercises were performed.

6.4.2.3.1 Perchlorate Model Parameterization

Clewell (2001b) performed model parameterization for perchlorate using the data obtained for the tissues from the AFRL/HEST drinking water studies on PND5 and PND10. Optimized kinetic parameters (Vmaxc and permeability area) were determined by visually fitting the model simulation to the experimental data. As for the previous model structures (adult male rat, human,

pregnant rat and fetus), it was necessary to account for the serum binding of perchlorate in order to adequately describe the serum perchlorate concentrations at the lower doses (0.01 and 0.1 mg/kg-day). Figure 6-38 illustrates the importance of binding in the model simulations in the dam on these days.

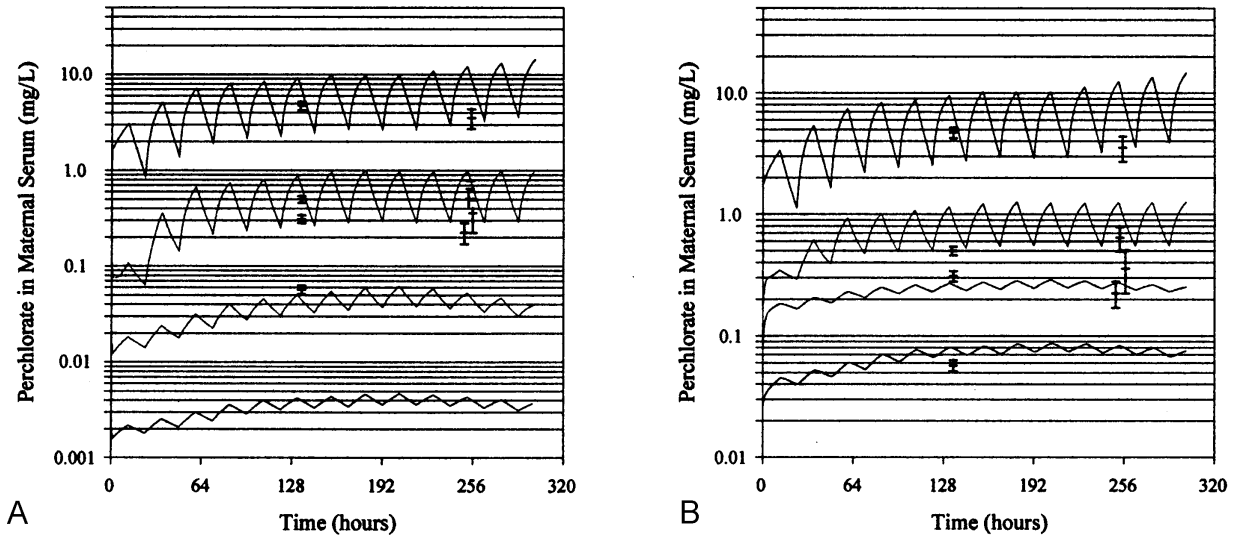

Figure 6-38. Simulations illustrating the necessity of including plasma binding in the lactating dam and neonatal rat PBPK model structure (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) for maternal serum perchlorate concentrations (mg/L) on PND5 and PND10 at doses to the dam of 10.0, 1.0, 0.1, and 0.01 mg/kg-day are shown with (A) and without (B) plasma binding.

Figure 6-39 shows the perchlorate tissue concentrations (mg/L) in the lactating dam thyroid (A) and in maternal milk (B) at PND5 and PND10 for the 0.01, 0.1, 1.0 and 10.0 mg/kg-day doses. It was noticed that during the drinking water studies, the daily dose to the dams varied somewhat due to their changing water intake. Therefore, all of the model simulations of the drinking water studies reflect the actual daily dose to the dam, which Clewell (2001b) calculated from the daily water consumption and body weight measurements.

Figure 6-40 shows the model simulations of the male and female neonate plasma levels compared to the data obtained in the AFRL/HEST drinking water study. Plasma concentrations

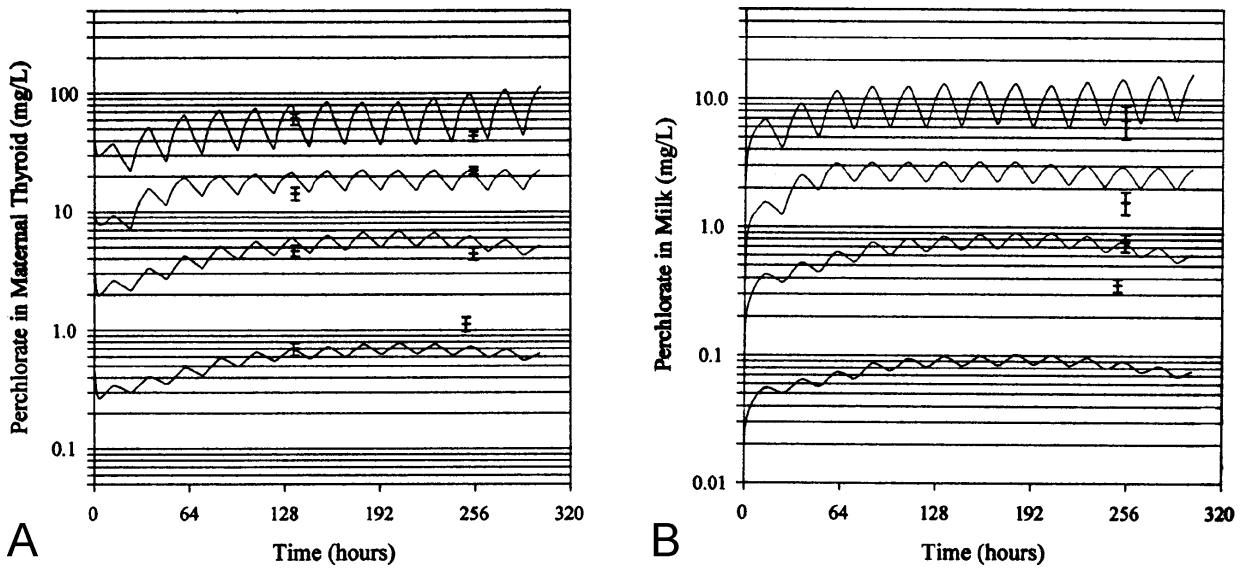

Figure 6-39. Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in the maternal thyroid (A) and milk (B) on PND5 and PND10 at doses in drinking water to the dam of 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate (Clewell, 2001b).

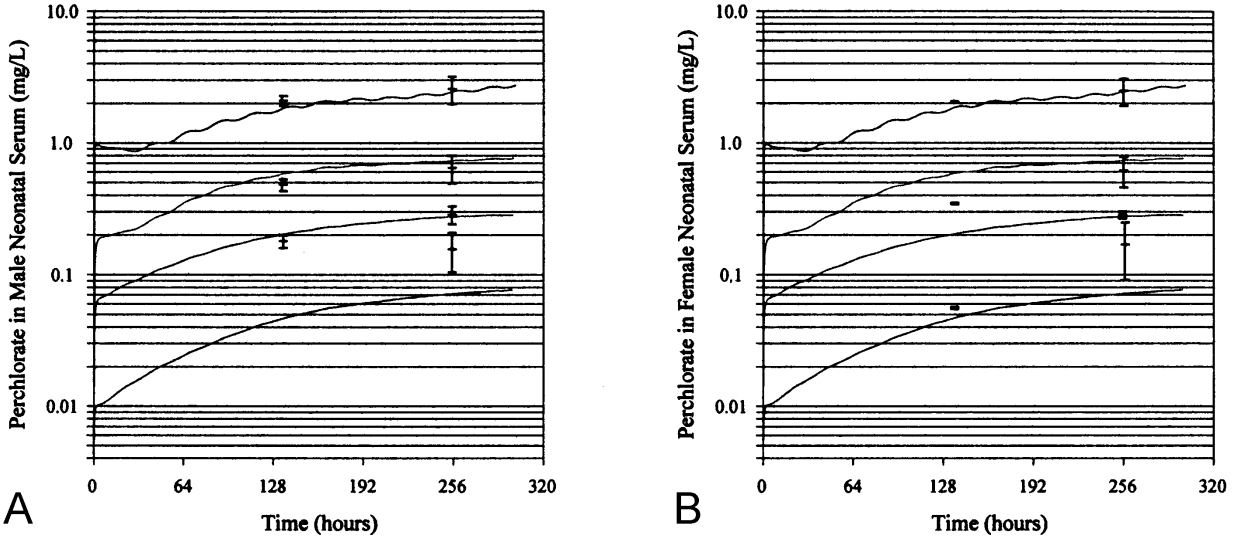

Figure 6-40. Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in the serum of male (A) and female (B) neonates on PND5 and PND10 at doses in drinking water to the dam of 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate (Clewell, 2001b).

varied significantly between the male and female neonates, and Clewell (2001b) noted that the difference appears to be a function of age. At PND5, the male neonatal plasma concentrations were nearly 4 times higher than those of the female neonates in the 0.1 mg/kg-day dose group. By PND10, however, no significant sex difference was found in the plasma perchlorate concentrations at the same dose.

Clewell (2001b) fit the male neonatal serum data because the male pups showed higher perchlorate concentrations in the serum than the female pups (Yu, 2000). The neonatal serum was under-predicted by the model in the 0.01 mg/kg-day dose group. Clewell (2001b) strongly asserts that this was due to the fact that the milk concentration was also under-predicted in that same dose group. The three higher doses are well described in the male neonate. The female pups also show acceptable fits at PND10. However, since the PND5 data were much lower in the female than male neonates, the model over-predicts the PND5 time-points in the 0.1 and 1.0 mg/kg-day doses. Fits of the model to neonatal skin and GI tract are discussed in Clewell (2001b).

As in the maternal model, the clearance value for urinary excretion was determined by the fit of the model to the serum from the 10 mg/kg-day dose, while the lower doses were used to determine the kinetic parameters for the binding in the neonate. Both binding and urinary clearance were considerably lower in the pup than in the dam (Table 6-7).

6.4.2.3.2 Iodide Model Parameterization

Clewell (2001b) developed the iodide aspect of the model by visually fitting the model to measured tissue concentrations in the dam and neonate from the control group of the inhibition kinetic study. Only the values for Vmax and permeability area needed to be fit with the model. As shown in Figure 6-41, the model simulations of iodide concentrations (ng/L) after an iv injection of 2.10 ng/kg radioalabeled iodide ($^{125}I^-$) on PND10 versus the experimental data in the lactating dam are shown in the dam serum (A) and thyroid (B) and in male (C) and fetal (D) neonatal serum.

The model simulations describe the data well with the exception of the longest time point in the neonates. The clearance value for urinary excretion was determined by fitting the maternal serum prediction to the above data while keeping good fits in the other tissues, such as maternal skin, GI, and mammary gland (Clewell, 2001b). Permeability area values were adjusted to

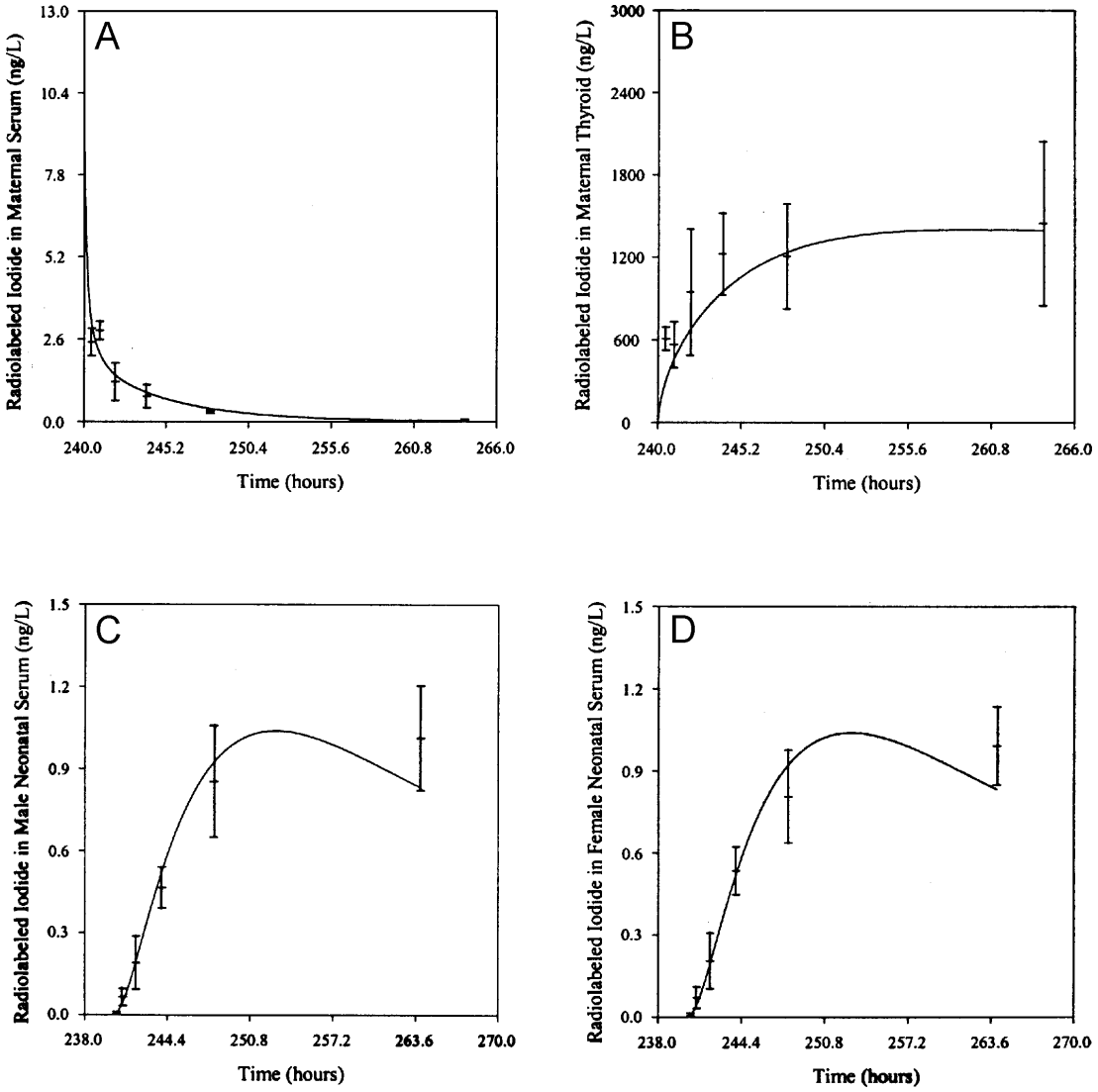

Figure 6-41. Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean ± SD) of iodide concentrations (mg/L) in the maternal serum (A) or thyroid (B) and in male (C) or female (D) neonatal pups on PND10 after an iv dose to the lactating dams of 2.10 ng/kg $^{125}I^-$ (Clewell, 2001b). Data of Yu (2000, 2002).

describe the behavior of the iodide data; varying the permeability area values toward 1.0 L/hr-kg generally increased the rate at which uptake and clearance in a particular tissue occurred; decreasing permeability area slowed the uptake and clearance.

The behavior of the iodide in the neonatal skin and GI tract and contents appeared to be different from the dam. The iodide tended to stay in the tissue of the neonate longer, requiring a slower clearance in the fetal tissues than was used in the corresponding maternal tissue. As a result, permeability area values used for the GI and skin in the neonate were lower than those used in the dam (Table 6-8). For example, the permeability area value in the skin was determined to be 0.5 L/hr-kg in the dam, but was decreased to 0.02 L/hr-kg in the neonate. However, these values correspond well to the values used for the fetus in the pregnancy model (Clewell, 2001a).

The neonatal urinary clearance value was determined to be 0.02 L/hr-kg in the neonate, which is very similar to the maternal value (0.03 L/hr-kg of the dam). This was a surprise, because the neonate was expected to have a much lower rate of excretion than the more mature dam; however, Clewell (2001b) notes that this trend is supported in the literature. Capek and Jelinek (1956) measured the amount excreted by pups at various ages. The neonates required external stimulation by the mother in order to release the urine from their bladders. However, when that stimulation was supplied, the neonates were able to excrete urine at the same rate as an adult rat. Therefore, it is reasonable that the urinary excretion rate is similar between the pup and adult. The amount of iodide lost to urine is then dependent on both the urinary clearance value and the concentration of the ion in the kidney (Clewell, 2001b).

6.4.3 Model Validation

The ability of the model to simulate the kinetics of perchlorate in the lactating dam and neonate was tested against the perchlorate time course data collected *in vivo* by AFRL/HEST. Since the study was performed with an acute perchlorate dose, it was necessary to make minor changes in the thyroid perchlorate parameters. The long-term exposure to perchlorate in the drinking water studies that were used to determine the perchlorate parameters is sufficient to induce up-regulation in the thyroid (Yu, 2000). Therefore, the thyroid parameters in the dam at this point would be different from those seen in an acute situation. Clewell (2001b) achieved the model fits to the acute data by altering the partition coefficient (from 2.25 in the drinking water to 0.13 in the acute exposure) and permeability area value (from 6.0E-4 to 4.0E-5) into the thyroid at the basolateral membrane (thyroid follicle). The value for the partitioning into the follicle in a naïve thyroid was calculated as described previously from Chow and Woodbury

(1970). The permeability area value in the naïve thyroid follicle was determined by fitting the model prediction to the thyroid data, while keeping good fits in the serum and other tissues. Figure 6-42 shows the model predictions versus the data time course of perchlorate concentrations in maternal serum (A), thyroid (B), or mammary gland (C) and in neonatal serum.

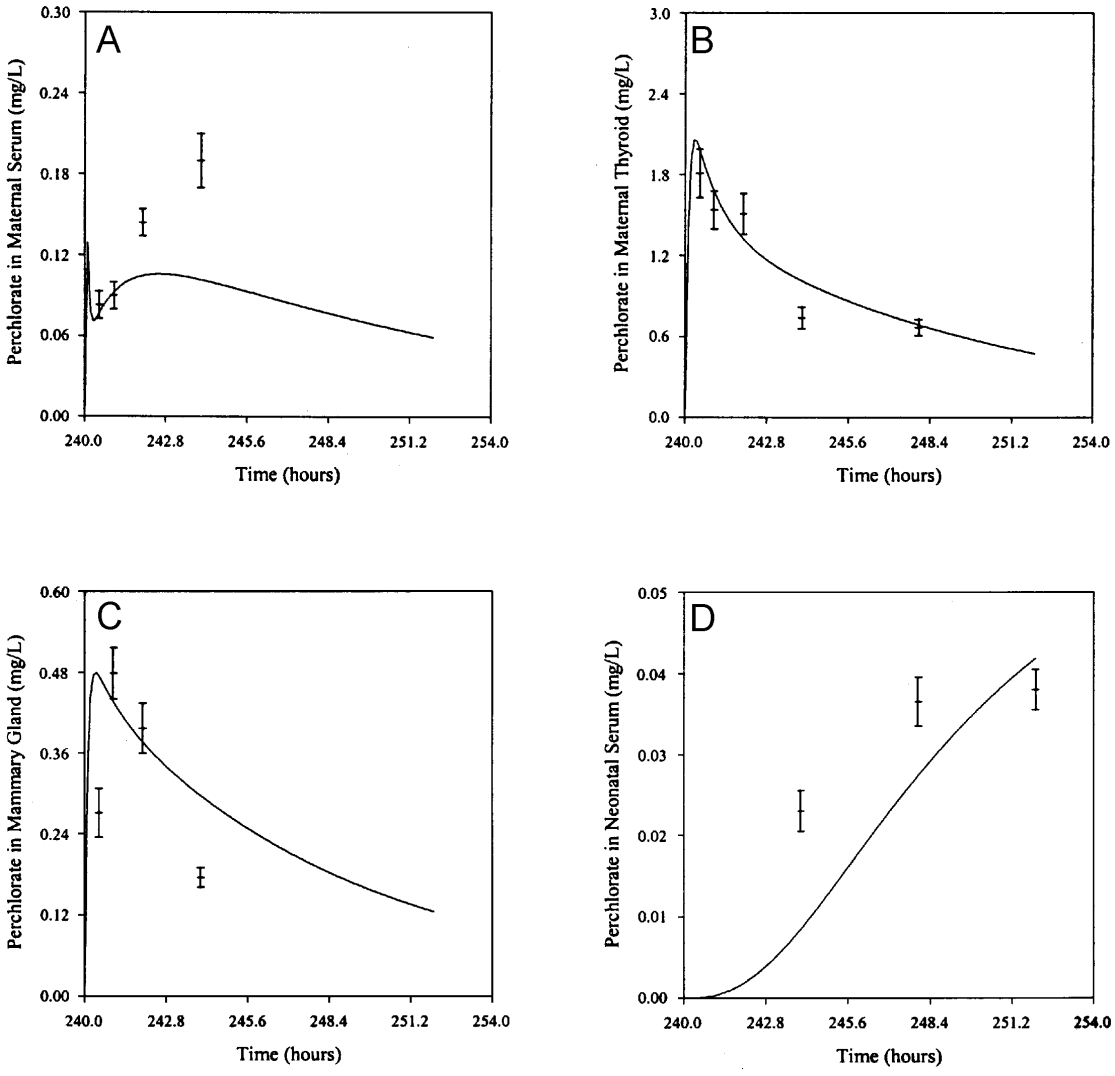

Figure 6-42. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of perchlorate in the maternal serum (A), thyroid (B), or mammary gland (C) and in neonatal serum (D) after an iv dose of 1.0×10^6 mg/kg perchlorate on PND10. Data of Yu (2000, 2002) and Yu et al. (2000).

The maternal serum is not fit particularly well and the neonatal serum fit could also be improved. Clewell (2001b) notes the difficulties may be due to the use of the iv kinetic data as well as some additional challenges not yet met by the model with respect to the mammary gland. Clewell (2001b) increased the transfer of perchlorate through the milk in the acute studies in order to fit the model derived on drinking water studies to these acute (iv) data. That is, the value for the Vmax into the mammary tissue was increased in order to allow more perchlorate into the mammary compartment, and the permeability area into the milk was decreased in order to minimize the back flow of perchlorate into the mammary from the milk. This essentially forced the perchlorate in the milk to be passed to the neonate rather than return to the mammary tissue of the mother. The Vmaxc for the binding in the neonate was decreased slightly from the value used in the drinking water simulations. This may have been due to increased transfer of iodide in the acute simulations. When the same parameters were used in the mammary compartment that were determined with the drinking water studies, the amount in the mammary tissue was low and the clearance of the mammary was too slow. As a result, acute neonatal serum levels were under-predicted. By adjusting the Vmaxc, the model was able to achieve reasonable fits to the available data in the maternal and neonatal tissues. Clewell (2001b) suggests that different fractions of the dose are transferred through the milk during an acute (iv) exposure versus a drinking water scenario.

Figure 6-43 shows the model predictions against the data obtained in the AFRL/HEST cross-fostering study described in Section 6.4.1.1.2. Perchlorate concentrations (mg/L) in the maternal thyroid of dams exposed during gestation (A) or only during lactation (B) show similar results. Perchlorate concentrations (mg/L) in neonatal serum exposed only during gestation (C) or only during lactation (D) also contained similar levels. Because the data were taken on PND10, the sex difference seen at the earlier time points was not present and the simulation is shown for the average of all pups.

The model is able to predict the data from the cross-fostering study very well. It is apparent from the data and from the model prediction of the cross-fostering data that the gestational exposure to perchlorate does not affect the perchlorate concentrations of the maternal serum and thyroid or the neonatal serum. This is in agreement with other studies that indicate the rapid clearance of perchlorate in the urine (Yu et al., 2000), but not in agreement with the toxicological

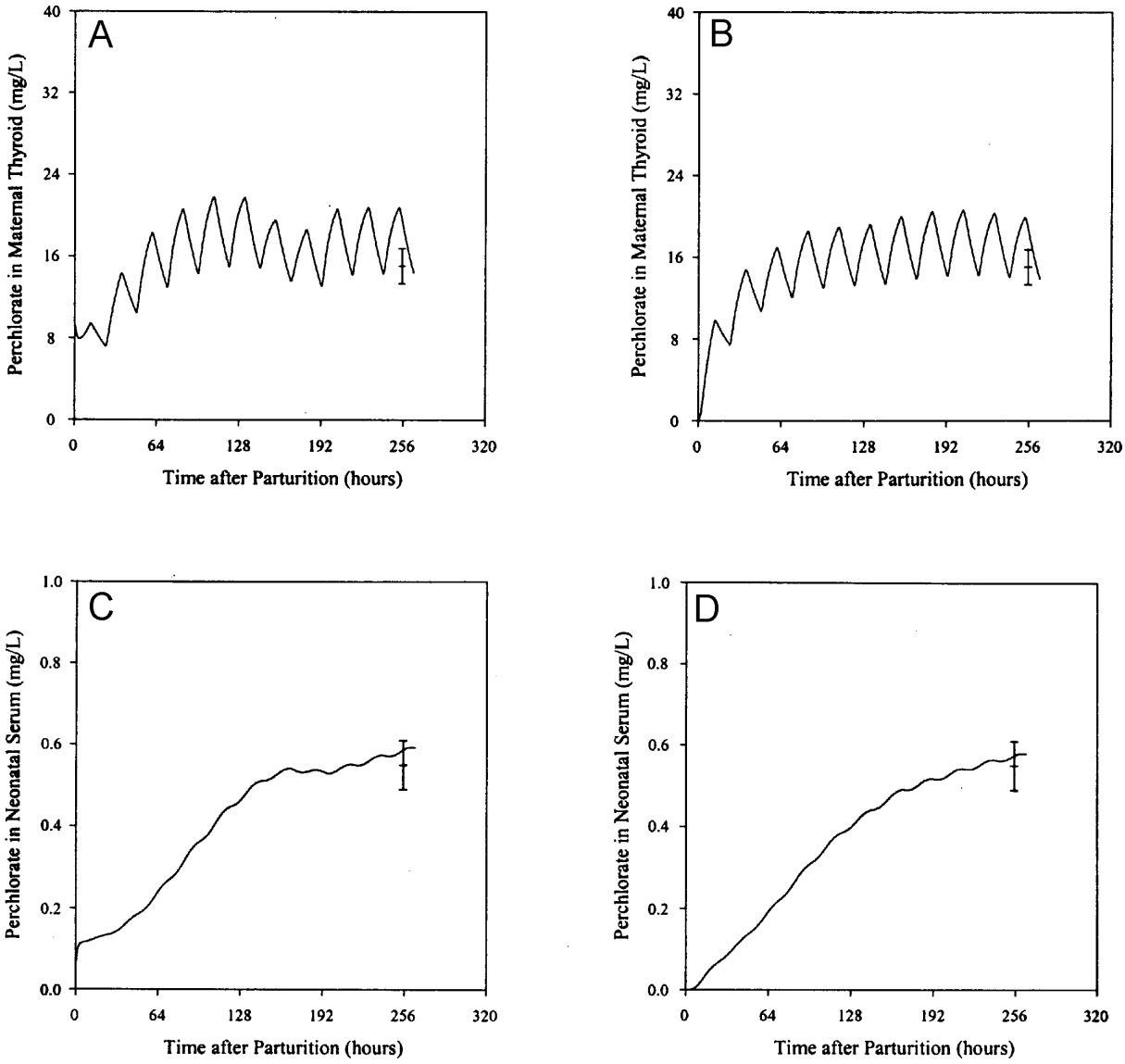

Figure 6-43. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of perchlorate in the maternal thyroid during gestation (A) or during lactation only (B) and in the neonatal serum during gestation (C) or during lactation only (D) after an iv dose of 1.0×10^6 mg/kg perchlorate on PND10. Data of Mahle (2001).

observations between the 1998 and 2001 developmental neurotoxicological studies performed by Argus Research Laboratories, Inc. (1998; 2001). Differences in the hormone data are discussed in Clewell (2001c) and other differences may be due to strain differences (Fail et al., 1999). From the model, even though the neonatal urinary excretion is much lower than that of the dam

(0.005 vs. 0.07 L/hr-kg), the prenatal exposure does not affect the serum levels of the neonate past PND2. This is in accord with the observations made of the BMDL estimates for the post-natal thyroid discussed in Chapter 5.

Additional validation exercises were performed by Clewell (2001b), showing reasonably adequate model fits to the data of Potter et al. (1959) and that of Sztanyik and Turai (1988) as shown in Clewell (2001b). Maternal radiolabeled iodide concentrations were overpredicted in the thyroid on PND18. The maternal milk concentrations were also overpredicted for the earlier time point, but were within the range at the later. The model predicted the radiolabeled iodide data obtained in the litters of Sztanyik and Turai (1988) quite well. This indicates that the lactation and neonatal kinetics are characterized accurately.

Figure 6-44 shows that the Clewell (2001b) model is able to predict the radiolabeled iodide ($^{125}I^-$) uptake-inhibition data in maternal thyroids on PND10 from the AFRL/HEST "acute" (iv) studies with perchlorate. The inhibition was described well by the model across the range of time points from 0.5 to 24 hours postdosing. The top line indicates the prediction for the control thyroid, and the bottom line shows the effect of perchlorate. The model is able to describe the kinetics of iodide under both conditions.

The Clewell (2001b) model is also able to predict the radiolabeled iodide uptake inhibition data from AFRL/HEST obtained after "chronic" drinking water exposures. Figure 6-45 shows the radiolabeled iodide ($^{125}I^-$) concentrations (mg/L) in the maternal thyroids at PND5 after 23 days of dosing with perchlorate at 0.0, 0.01, 1.0, and 10.0 mg/kg-day.

6.4.4 Summary

Clewell (2001b) highlights some important differences in the lactating dam and neonatal rat model structure that were necessary in order to adequately describe the distribution kinetics of perchlorate and iodide. The loss of iodide and perchlorate in the milk results in much faster clearance rates of the anions from the dam. Studies also suggest that the loss of iodide to the mammary gland and milk decreases the iodide available for the maternal thyroid (Brown-Grant, 1961; Yu, 2000; Yu et al., 2000). The thyroidal maximum capacities are lower in the lactating and pregnant dam than in the male rat. Model parameterization in the male rat indicated the need for Vmaxc values for uptake into the follicle of the thyroid of 2.2×10^3 L/hr-kg for perchlorate and 5.5×10^4 L/hr-kg for iodide while the gestation model required values of 1.5×10^3 L/hr-kg

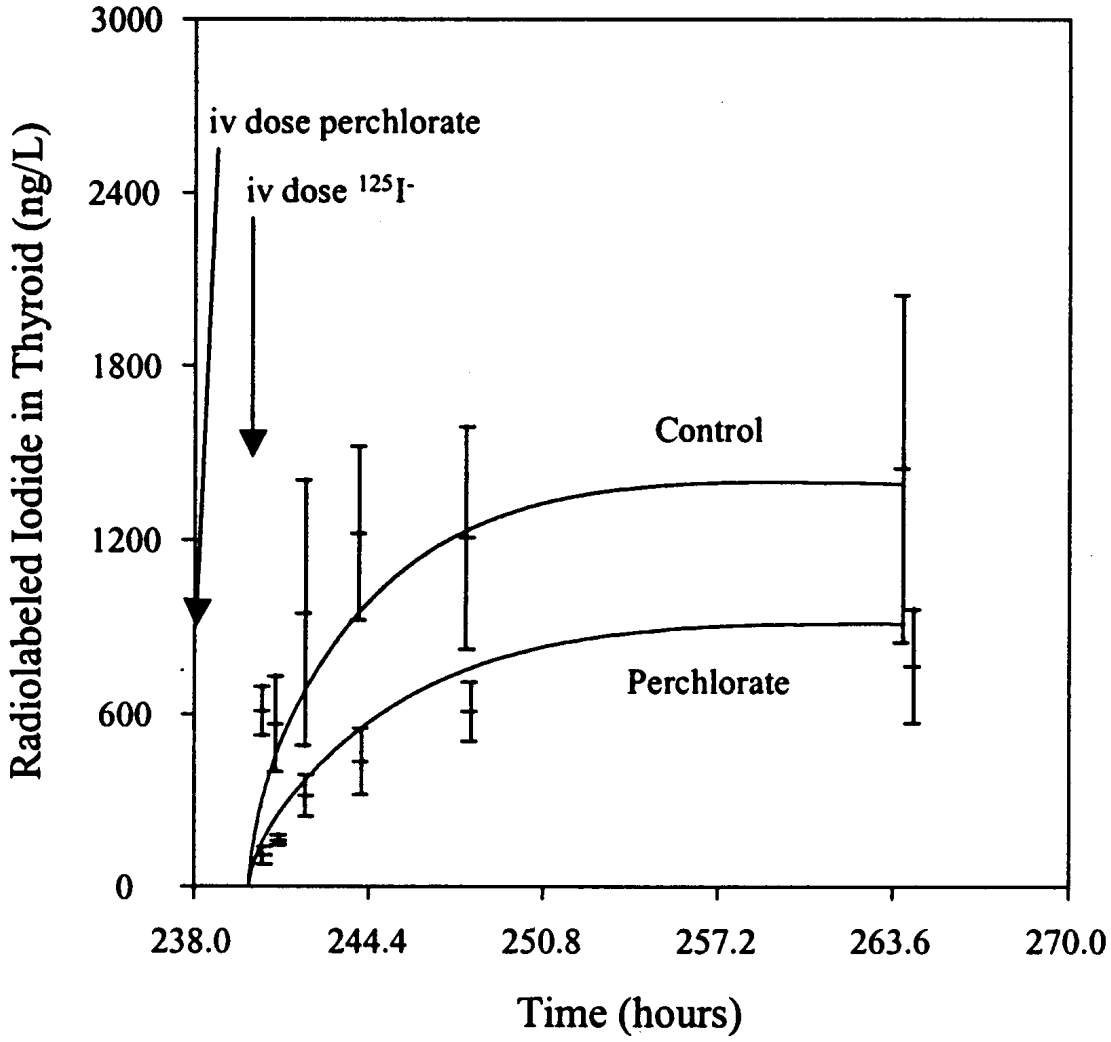

Figure 6-44. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide in the maternal thyroid with and without an iv dose of perchlorate at 1.0 mg/kg perchlorate 2 hours prior to an iv dose of 2.10 ng/kg $^{125}I^-$ to the dam on PND10. The top simulation indicates the control thyroid and the lower indicates the inhibited thyroid. Data of Yu (2000) and Yu et al. (2000).

and 4.0 x 10^4 L/hr-kg for the same parameters. This difference is supported in the literature. Versloot et al. (1997) suggest that the pregnant rat may have a lowered reserve of iodide in the thyroid toward the end of pregnancy, causing increased activity in the thyroid. This may also be true in the lactating rat. The skin of the lactating dam also required a smaller value for Vmaxc

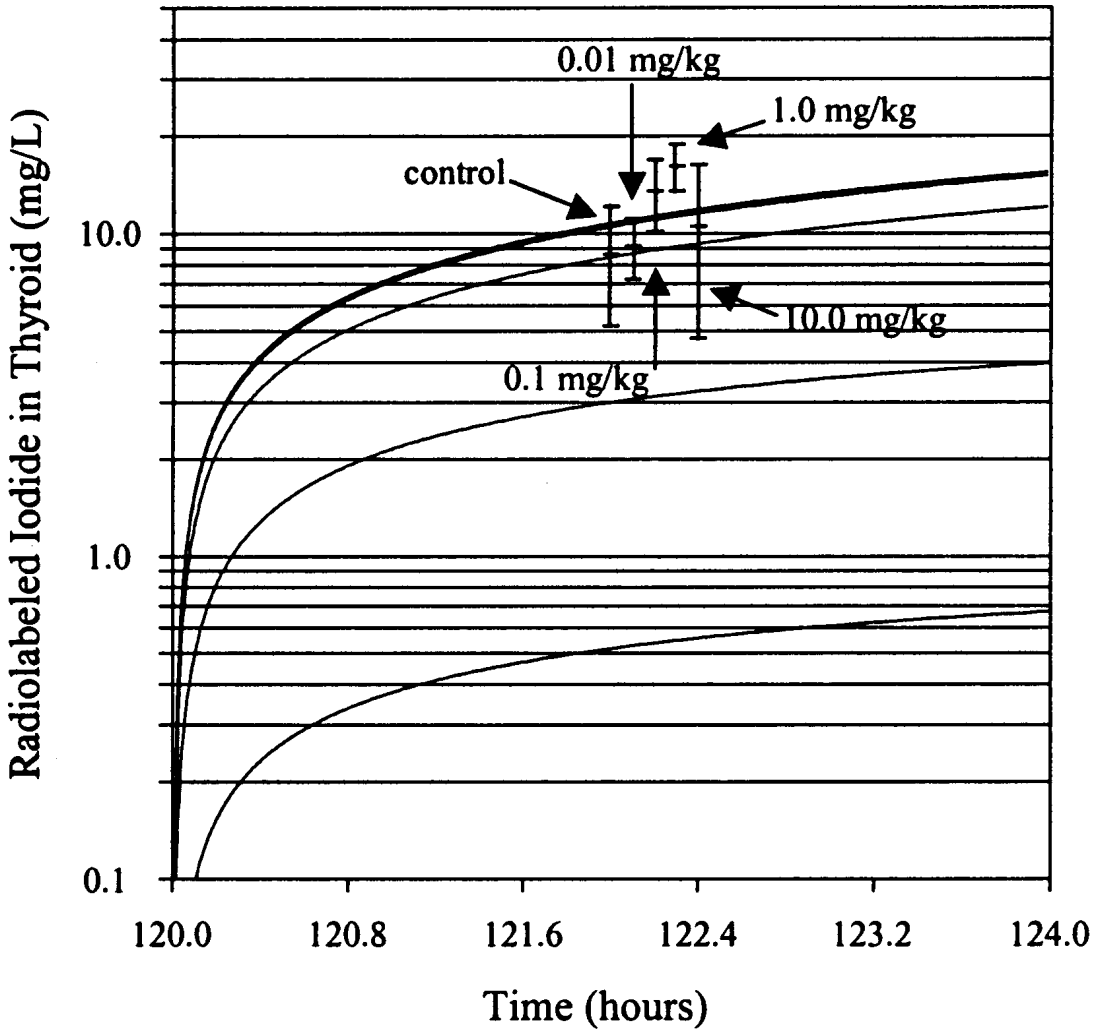

Figure 6-45. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide in the maternal thyroid on PND5 after 23 days dosing with perchlorate in drinking water at 0.0, 0.1, 1.0, and 10.0 mg/kg-day. All experimental data were taken two hours post-dosing. Data of Yu et al. (2000).

than the male rat. This is supported by the work of Brown-Grant and Pethes (1959), who reported higher levels of iodide in the skin of male rats than in female rats. Skin, therefore, appears to be a more important iodide reserve in the male rat than the female.

The described PBPK lactation model is able to predict the distribution of perchlorate in the tissues of active uptake and serum of the lactating dam and neonate on PND5 and PND10 after exposure to perchlorate in drinking water. Perchlorate distribution in this dynamic system is

described utilizing a pharmacokinetic approach to the modeling and accounting mathematically or physiological changes, such as changing tissue volumes and maternal and neonatal growth. The model predicts the transfer of perchlorate to the neonate and is also able to describe the uptake into tissues of interest in the neonate, such as the GI contents and skin; however, the EPA believes that both the maternal and neonatal serum fits could be improved. This may already be accomplished with the additional data to which Clewell (2001b) alludes or, as noted previously, the radionuclide modeling efforts of the ICRP (2001, 1989) may be informative.

The kinetic behavior of iodide is well described with the existing model, in spite of the physiological complexity of the described system. The dam and neonate were accurately simulated at a range of doses that spans four orders of magnitude (2.10 to 33,000 ng/kg) between days 1 and 18 of lactation. The active sequestration of iodide in maternal and neonatal tissues and the transfer of iodide between mother and neonate was described kinetically with the model; data have been simulated at a variety of doses and at various time points up to 14 days after exposure. The fact that the model was able to simulate data from other laboratories under a variety of different conditions attests to the validity of the model structure and its applicability to other studies. This also provides greater confidence in the model structure.

The clear differences between the perchlorate data from iv and drinking water studies draw attention to unresolved issues in the transfer kinetics of perchlorate. Although lactational transfer has long been studied, the transport mechanisms of this ion have yet to be elucidated in the literature. A second transporter has been identified in the mammary gland, which actively transports anions against the chemical gradient. However, the relationship of this transporter and the anion concentration resulting from prolonged exposure to the high doses of perchlorate used in these studies is not known. Clewell (2001b) suggests that it is possible that the high anion load resulting from the long-term exposure to perchlorate may have resulted in decreased transport of the ion. It is feasible that the movement of iodide may be regulated in the mammary tissue, because the ion is vital to the development of the newborn. The data obtained between the acute and drinking water studies suggest that a feedback mechanism is in place, because the model over-predicts the milk transfer in the drinking water data when the acute parameters are used. Clewell (2001b) notes in-house experiments that may help resolve these issues are currently underway. Additional data were provided by Yu (2002), but is not clear that all these data have been provided to the Agency or how these will be used to improve the modeling effort.

6.5 APPLICATION OF PBPK MODEL STRUCTURES TO INTERSPECIES EXTRAPOLATION

As discussed in the introduction to this chapter, the purpose of developing the proposed PBPK model structures was to aid interspecies extrapolation. All of the proposed model structures adequately describe both perchlorate and iodide distributions as evidenced by the fit of the model predictions against the experimental data shown in the preceding sections of this chapter. The degree of confidence in the model descriptions differed for the acute (iv) versus chronic (drinking water) data to some degree in the laboratory animals. A rather large degree of intersubject variability was evident among the human subjects, but in general the structures are accepted as quite sound and informative to the task.

The models do not link the perchlorate and iodide kinetics to perturbations in thyroid hormone. The existing data and current structures were not designed to address the complex issues involved with hormone homeostasis of the hypothalamic-pituitary-thyroid feedback axis as described in Chapter 3 or illustrated in the beginning of this chapter. Such a model would need to incorporate the hormone levels in tissues and serum and processes such as hormone production, storage, and secretion in the thyroid; conversion of T4 to T3 in the tissues; deiodination of T4 and T3 to less active forms and a feedback mechanism between the hormone levels, TSH, and the thyroid NIS. Kohn et al. (1996) developed a PBPK model that attempts to describe the effect of dioxin on thyroid hormones. Although perchlorate and dioxin act on the endocrine system through different modes of action, it is likely that a similar approach to that of Kohn et al. (1996) would be required to begin to address the hormone feedback system in the case of perchlorate. Parameterization and validation of such a model system would take a significant number of additional studies.

Nevertheless, the model structures as they exist currently are useful, particularly when employed in the conceptual framework proposed in Section 6.1. Because the models predict perchlorate and iodide kinetics, two relevant dose metrics to the mode of action can be evaluated: (1) the area under the curve (AUC) of perchlorate in the serum and (2) the degree (expressed as a % of baseline) of iodide uptake inhibition in the thyroid.

Because developmental effects are of concern, an argument could be made that peak and not AUC is the appropriate dose metric–the rationale being that any transient dose could be responsible for permanent deficits. However, the AUC values, as opposed to peak

concentrations, were used based on the assumption that these dose metrics would represent an averaging of the serum and thyroid perchlorate concentrations and would be better correlated with the inhibition effect on iodide uptake. The correlation was shown to be good between the AUC and the degree of inhibition (see Section 6.5.2). Further, due to the rapid phase of distribution after an iv dose, measurements of concentrations are very difficult to attain experimentally and are more variable. Using simulated peak concentrations after iv injections is potentially problematic due to the inexact modeling of the actual distribution of dose in the tail-vein volume and the exact time of mixing in the whole blood compartment (Merrill, 2001e). It was also observed by EPA that the ratios for peak perchlorate serum values (Merrill, 2001e; Table 6) were in good agreement with those for the perchlorate serum AUC and that the serum AUC were slightly more conservative if really different at all at the lower doses of concern to the risk assessment.

The perchlorate AUC concentration in the thyroid was also considered, but the EPA and AFRL/HEST agreed that this was a less satisfactory dose metric based on a number of considerations. These included the following: that the thyroid Vmaxc estimates had to be adjusted to account for upregulation of the NIS, but that this adjustment was more an empirical exercise than a true biological model (since the hormone changes discussed above regulate the NIS); that the thyroid concentrations were not actually measured in the fetus and neonate so that verification of the parameters was not possible; and that the effects of perchlorate are related to its effects on the NIS and secondary impact on thyroid hormone economy rather than to the concentrations in the gland itself. Results of a sensitivity analysis on the adult male rat model structure supported these conclusions (Merrill, 2001e). The results of the sensitivity analysis will be discussed in Section 6.5.1. Thus, the models were exercised to develop human equivalent exposure (HEE) estimates based on internal perchlorate concentration and iodide uptake inhibition, both components of exposure in the proposed EPA model (Merrill, 2001e). The purpose of Section 6.5.2 is to describe the modeling exercises underlying the HEE estimates that are used in Chapter 7.

6.5.1 Sensitivity Analysis of Proposed Adult Male Rat Model

A sensitivity analysis was performed on the adult male rat model of Merrill (2001c) in order to determine which parameters had the most significant impact on serum and thyroid AUC

perchlorate concentrations. All chemical specific kinetic parameters were increased individually by 1% from the original, optimized values. The model-predicted dosimetrics were recalculated after each change to determine the effect on the AUC estimates. This exercise was performed at the four-hour time point after iv dosing for the 0.1 and 1.0 mg/kg-day doses. The equation describing the calculation of the Sensitivity Coefficient value for each PBPK perchlorate parameter tested is (Merrill, 2001e):

$$\text{Sensitivity Coefficient} = \frac{(A - B)/B}{(C - D)/D}, \qquad (6\text{-}2)$$

where:

A = AUC for either serum or thyroid perchlorate with 1% increased parameter value,
B = AUC for either serum or thyroid perchlorate at initial parameter value,
C = Parameter value increase 1% over initial parameter value, and
D = Original initial starting parameter value.

Results are presented for the physiological parameters and chemical specific parameters separately. Tables 6-9 and 6-10 provide the results for the 0.1 mg/kg-day dose, and Tables 6-11 and 6-12 provide the results for the 1.0 mg/kg-day dose. The sensitivity coefficients for the AUC estimates in both the thyroid and serum are provided and the changes in predicted AUC estimates for the thyroid and serum are presented in the final two columns (Merrill, 2001e).

The sensitivity of serum and thyroid concentrations to model parameters is not linear. At an iv dose level of 1.0 mg/kg, the model prediction of the AUC for serum ClO_4^- concentration is most sensitive to urinary clearance (ClUc_*p*). A one percent increase in this value, from 0.07 to 0.0707 ng/hr-kg, causes a decrease in AUC serum ClO_4^- concentration from 4.69×10^5 to 4.63×10^5 ng, with a sensitivity coefficient of −1.271 (Table 6-12). Serum concentration is next most sensitive to the rate ClO_4^- unbinds from plasma proteins (Clunbc_*p*), with a sensitivity coefficient of -0.869 (Table 6-12).

TABLE 6-9. SENSITIVITY ANALYSIS FOR PHYSIOLOGICAL PARAMETERS IN THE ADULT MALE RAT MODEL AT 0.1 mg/kg PERCHLORATE (ClO_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
BW	3.00E-01	3.03E-01	0.315	0.182	1.88E+06	9.95E+04
Blood Flows (fraction of cardiac output, QCc [L/hr])						
QCc	1.40E+01	1.41E+01	-0.005	-0.006	1.88E+06	9.94E+04
QTc	1.60E-02	1.62E-02	NS[d]	NS	1.88E+06	9.94E+04
QSKc	5.80E-02	5.86E-02	NS	-0.003	1.88E+06	9.94E+04
QGc	1.60E-02	1.62E-02	0.011	0.008	1.88E+06	9.94E+04
QLc	1.70E-01	1.72E-01	NS	NS	1.88E+06	9.94E+04
QKc	1.40E-01	1.41E-01	-0.016	-0.010	1.88E+06	9.93E+04
QFc	6.90E-02	6.97E-02	NS	NS	1.88E+06	9.94E+04
Tissue Volumes (fraction of body weight)						
Vplasc	4.10E-02	4.14E-02	0.155	0.079	1.88E+06	9.94E+04
VRBCc	3.30E-02	3.33E-02	0.192	0.109	1.88E+06	9.95E+04
Vttotc	7.70E-05	7.78E-05	0.187	0.113	1.88E+06	9.95E+04
VDTc	2.44E-01	2.46E-01	0.928	0.114	1.89E+06	9.95E+04
VTBc	1.57E-01	1.58E-01	0.203	0.114	1.88E+06	9.95E+04
VTc	6.00E-01	6.05E-01	0.453	0.114	1.88E+06	9.95E+04
VGc	5.40E-03	5.45E-03	0.197	0.112	1.88E+06	9.95E+04
VGJc	1.68E-02	1.70E-02	0.165	0.091	1.88E+06	9.94E+04
VGBc	4.10E-02	4.14E-02	0.197	0.114	1.88E+06	9.95E+04
VSkc	1.90E-01	1.92E-01	-0.053	-0.023	1.87E+06	9.93E+04
VSkBc	2.00E-02	2.02E-02	0.203	0.117	1.88E+06	9.95E+04
VLc	5.50E-02	5.56E-02	0.197	0.114	1.88E+06	9.95E+04
VKc	1.70E-02	1.72E-02	0.197	0.113	1.88E+06	9.95E+04
VFc	7.40E-02	7.47E-02	0.208	0.118	1.88E+06	9.95E+04

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]AUC Thyroid Concentration using original parameters = 1.88E+06 ng ClO_4^-.
[c]AUC Serum Concentration using original parameters = 9.94E+04 ng ClO.
[d]NS = sensitivity coefficient less than 0.001.

TABLE 6-10. SENSITIVITY ANALYSIS FOR CHEMICAL SPECIFIC PARAMETERS IN THE ADULT MALE RAT MODEL AT 0.1 mg/kg PERCHLORATE (ClO_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
Iodide Tissue/Blood Partition Coefficients						
PS_*p*	3.10E-01	3.13E-01	0.149	0.085	1.88E+06	9.94E+04
PR_*p*	5.60E-01	5.66E-01	0.192	0.111	1.88E+06	9.95E+04
PK_*P*	9.90E-01	1.00E+00	0.192	0.111	1.88E+06	9.95E+04
PL_*p*	5.60E-01	5.66E-01	0.187	0.108	1.88E+06	9.95E+04
PG_*p*	1.80E+00	1.82E+00	0.160	0.088	1.88E+06	9.94E+04
PGJ_*p*	2.30E+00	2.32E+00	0.165	0.090	1.88E+06	9.94E+04
PT_*p*	1.30E-01	1.31E-01	1.184	0.113	1.90E+06	9.95E+04
PDT_*p*	7.00E+00	7.07E+00	0.928	0.114	1.89E+06	9.95E+04
PF_*p*	5.00E-02	5.05E-02	0.197	0.114	1.88E+06	9.95E+04
PSk_*p*	7.00E-01	7.07E-01	11.154	6.024	2.08E+06	1.05E+05
PRBC_*p*	8.00E-01	8.08E-01	11.324	6.112	2.09E+06	1.05E+05
PS_*p*	3.10E-01	3.13E-01	0.149	0.085	1.88E+06	9.94E+04
PR_*p*	5.60E-01	5.66E-01	0.192	0.111	1.88E+06	9.95E+04
PK_*P*	9.90E-01	1.00E+00	0.192	0.111	1.88E+06	9.95E+04
PL_*p*	5.60E-01	5.66E-01	0.187	0.108	1.88E+06	9.95E+04
PG_*p*	1.80E+00	1.82E+00	0.160	0.088	1.88E+06	9.94E+04
PGJ_*p*	2.30E+00	2.32E+00	0.165	0.090	1.88E+06	9.94E+04
PT_*p*	1.30E-01	1.31E-01	1.184	0.113	1.90E+06	9.95E+04
PDT_*p*	7.00E+00	7.07E+00	0.928	0.114	1.89E+06	9.95E+04
PF_*p*	5.00E-02	5.05E-02	0.197	0.114	1.88E+06	9.95E+04
PSk_*p*	7.00E-01	7.07E-01	11.154	6.024	2.08E+06	1.05E+05
PRBC_*p*	8.00E-01	8.08E-01	11.324	6.112	2.09E+06	1.05E+05
Perchlorate Active Uptake Parameters - Vmaxc (ng/hr-kg BW) Km (ng/L)						
Vmaxc_T*p*	2.90E+03	2.93E+03	47.830	6.088	2.77E+06	1.05E+05
Km_T*p*	2.50E+05	2.53E+05	45.154	6.090	2.72E+06	1.05E+05
Vmaxc_DT*p*	1.00E+05	1.01E+05	55.875	6.081	2.92E+06	1.05E+05
Km_DT*p*	1.00E+08	1.01E+08	55.673	6.081	2.92E+06	1.05E+05
Vmaxc_G*p*	1.00E+04	1.01E+04	55.769	6.080	2.92E+06	1.05E+05
Km_G*p*	2.00E+05	2.02E+05	55.774	6.081	2.92E+06	1.05E+05
Vmaxc_S*p*	6.50E+05	6.57E+05	54.713	5.678	2.90E+06	1.05E+05
Km_S*p*	2.00E+05	2.02E+05	55.060	5.811	2.91E+06	1.05E+05

TABLE 6-10 (cont'd). SENSITIVITY ANALYSIS FOR CHEMICAL SPECIFIC PARAMETERS IN THE ADULT MALE RAT MODEL AT 0.1 mg/kg PERCHLORATE (ClO_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
Perchlorate Plasma Binding Parameters						
Vmaxc_B*p*	9.50E+03	9.60E+03	54.857	6.417	2.90E+06	1.06E+05
km_B*p*	1.10E+04	1.11E+04	54.916	5.590	2.91E+06	1.05E+05
Kunbc_*p*	1.00E-01	1.01E-01	54.948	5.096	2.91E+06	1.04E+05
Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)						
ClUc_*p*	7.00E-02	7.07E-02	54.047	5.399	2.89E+06	1.05E+05
PAGc_*p*	8.00E-01	8.08E-01	54.905	5.752	2.91E+06	1.05E+05
PAGJc_*p*	8.00E-01	8.08E-01	54.905	5.752	2.91E+06	1.05E+05
PATc_*p*	5.00E-05	5.05E-05	23.273	5.776	2.31E+06	1.05E+05
PADTc_*p*	1.00E-02	1.01E-02	24.398	5.775	2.33E+06	1.05E+05
PASKc_*p*	4.00E-01	4.04E-01	3.759	-4.354	1.95E+06	9.50E+04
PARBCc_*p*	1.00E-01	1.01E-01	3.455	-4.508	1.94E+06	9.49E+04

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]AUC Thyroid concentration using original parameters = 1.88E+06 ng ClO_4^-.
[c]AUC Serum concentration using original parameters = 9.94E+04 ng ClO_4^-.

The predicted AUC for total thyroid concentration at a dose level of 1.0 mg/kg-day is most sensitive to changes in the maximum capacity of the thyroid colloid (Vmaxc_DTp). A one percent increase in this value from 1.00×10^5 to 1.0110^5 ng/hr-kg results in an increase in AUC thyroid concentration from 9.84×10^6 to 1.04×10^7 ng (Table 6-12). However, the AUC thyroid concentration is almost equally sensitive to other parameters of saturable processes, including Vmaxc, Km, and the permeability area cross product values of other saturable tissues.

With a lower iv dose of 0.1 mg/kg, the blood serum concentration remains sensitive to changes in urinary clearance, but demonstrates increased sensitivity to the parameters of saturable compartments and effective partitioning with skin (PSk_*p*) and red blood cells (PRBC_*p*). Serum concentration is most sensitive to the maximum capacity for plasma binding (Vmaxc_B*p*) at this dose level (Table 6-10).

At the lower dose level of 0.1 mg/kg, thyroid concentrations show a similar sensitivity to parameters of saturable processes, including plasma binding, permeability area cross products,

TABLE 6-11. SENSITIVITY ANALYSIS FOR PHYSIOLOGICAL PARAMETERS IN THE ADULT MALE RAT MODEL AT 1.0 mg/kg PERCHLORATE (ClO_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
BW	3.00E-01	3.03E-01	−5.944	−0.534	9.81E+06	4.67E+05
Blood Flows [fraction of cardiac output, QCc (L/hr)]						
QCc	1.40E+01	1.41E+01	−0.192	−0.014	9.84E+06	4.69E+05
QTc	1.60E-02	1.62E-02	0.021	NS[b]	9.84E+06	4.69E+05
QSKc	5.80E-02	5.86E-02	−0.085	0.001	9.84E+06	4.69E+05
QGc	1.60E-02	1.62E-02	0.128	0.005	9.84E+06	4.69E+05
QLc	1.70E-01	1.72E-01	0.021	NS	9.84E+06	4.69E+05
QKc	1.40E-01	1.41E-01	−0.234	−0.021	9.84E+06	4.69E+05
QFc	6.90E-02	6.97E-02	0.021	NS	9.84E+06	4.69E+05
Tissue Volumes (fraction of bodyweight)						
Vplasc	4.10E-02	4.14E-02	−7.734	−0.701	9.80E+06	4.66E+05
VRBCc	3.30E-02	3.33E-02	−7.649	−0.691	9.80E+06	4.66E+05
VTtotc	7.70E-05	7.78E-05	−7.841	−0.683	9.80E+06	4.66E+05
VDTc	2.44E-01	2.46E-01	7.606	−0.683	9.87E+06	4.66E+05
VTBc	1.57E-01	1.58E-01	−7.500	−0.682	9.80E+06	4.66E+05
VTc	6.00E-01	6.05E-01	−2.322	−0.683	9.83E+06	4.66E+05
VGc	5.40E-03	5.45E-03	−7.649	−0.685	9.80E+06	4.66E+05
VGJc	1.68E-02	1.70E-02	−7.883	−0.710	9.80E+06	4.66E+05
VGBc	4.10E-02	4.14E-02	−7.628	−0.682	9.80E+06	4.66E+05
VSkc	1.90E-01	1.92E-01	−8.799	−0.829	9.80E+06	4.65E+05
VSkBc	2.00E-02	2.02E-02	−7.606	−0.680	9.80E+06	4.66E+05
VLc	5.50E-02	5.56E-02	−7.628	−0.683	9.80E+06	4.66E+05
VKc	1.70E-02	1.72E-02	−7.628	−0.685	9.80E+06	4.66E+05
VFc	7.40E-02	7.47E-02	−7.585	−0.676	9.80E+06	4.66E+05

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]Original AUC Thyroid concentration = 9.84E+06 ng ClO_4^-.
[c]Original AUC Serum concentration = 4.69E+05 ng ClO_4^-.
[d]NS = sensitivity coefficient less than 0.001.

TABLE 6-12. SENSITIVITY ANALYSIS FOR CHEMICAL–SPECIFIC PARAMETERS IN THE MALE RAT MODEL AT 1.0 mg/kg PERCHLORATE (ClO_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
Perchlorate Tissue/Blood Partition Coefficients						
PS_*p*	3.10E-01	3.13E-01	−7.862	−0.728	9.80E+06	4.66E+05
PR_*p*	5.60E-01	5.66E-01	−7.649	−0.688	9.80E+06	4.66E+05
PK_*P*	9.90E-01	1.00E+00	−7.649	−0.688	9.80E+06	4.66E+05
PL_*p*	5.60E-01	5.66E-01	−7.670	−0.692	9.80E+06	4.66E+05
PG_*p*	1.80E+00	1.82E+00	−7.905	−0.714	9.80E+06	4.66E+05
PGJ_*p*	2.30E+00	2.32E+00	−7.883	−0.711	9.80E+06	4.66E+05
PT_*p*	1.30E-01	1.31E-01	12.911	−0.683	9.90E+06	4.66E+05
PDT_*p*	7.00E+00	7.07E+00	7.606	−0.683	9.87E+06	4.66E+05
PF_*p*	5.00E-02	5.05E-02	−7.628	−0.684	9.80E+06	4.66E+05
PSk_*p*	7.00E-01	7.07E-01	−8.885	−0.846	9.80E+06	4.65E+05
PRBC_*p*	8.00E-01	8.08E-01	−7.649	−0.691	9.80E+06	4.66E+05
Perchlorate Active Uptake Parameters - Vmaxc (ng/hr-kg BW), Km (ng/L)						
Vmaxc_T*p*	2.90E+03	2.93E+03	12.890	−0.683	9.90E+06	4.66E+05
Km_T*p*	2.50E+05	2.53E+05	−15.745	−0.682	9.76E+06	4.66E+05
Vmaxc_DT*p*	1.00E+05	1.01E+05	123.554	−0.687	1.04E+07	4.66E+05
Km_DT*p*	1.00E+08	1.01E+08	120.784	−0.687	1.04E+07	4.66E+05
Vmaxc_G*p*	1.00E+04	1.01E+04	122.062	−0.687	1.04E+07	4.66E+05
Km_G*p*	2.00E+05	2.02E+05	122.062	−0.687	1.04E+07	4.66E+05
Vmaxc_S*p*	6.50E+05	6.57E+05	120.997	−0.806	1.04E+07	4.66E+05
Km_S*p*	2.00E+05	2.02E+05	122.914	−0.641	1.04E+07	4.66E+05
Perchlorate Plasma Binding Parameters - Vmaxc (ng/hr-kg BW), Km (ng/L)						
Vmaxc_B*p*	9.50E+03	9.60E+03	122.062	−0.500	1.04E+07	4.67E+05
km_B*p*	1.10E+04	1.11E+04	122.062	−0.694	1.04E+07	4.66E+05
Kunbc_*p*	1.00E-01	1.01E-01	122.275	−0.869	1.04E+07	4.65E+05
Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)						
ClUc_*p*	7.00E-02	7.07E-02	115.031	−1.271	1.04E+07	4.63E+05
PAGc_*p*	8.00E-01	8.08E-01	122.275	−0.685	1.04E+07	4.66E+05
PAGJc_*p*	8.00E-01	8.08E-01	122.275	−0.686	1.04E+07	4.66E+05
PATc_*p*	5.00E-05	5.05E-05	100.969	−0.686	1.03E+07	4.66E+05
PADTc_*p*	1.00E-02	1.01E-02	120.784	−0.687	1.04E+07	4.66E+05
PASKc_*p*	4.00E-01	4.04E-01	123.341	−0.567	1.04E+07	4.67E+05
PARBCc_*p*	1.00E-01	1.01E-01	122.062	−0.687	1.04E+07	4.66E+05

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]Original AUC Thyroid concentration = 9.84E+06 ng ClO_4^-.
[c]Original AUC Serum concentration = 4.69E+05 ng ClO_4^-.

and urinary clearance. However, the predicted thyroid concentrations at both dose levels (1.0 and 0.1 mg/kg) are most sensitive to a change in Vmax_DT*p*. The Vmax values of the thyroid were established by empirically fitting thyroid radioiodide and perchlorate uptake from several data sets ranging in three orders of magnitude

6.5.2 Derivation of Human Equivalent Exposure Estimates

As discussed, the following internal dosimetrics were chosen to represent output from each of the PBPK models: area under the curve (AUC) perchlorate concentrations in serum and thyroid; peak serum and thyroid perchlorate concentrations; the total amount of perchlorate excreted in the urine; the AUC for the lactational and placental transfer of perchlorate; and the percent inhibition of iodide uptake into the thyroid. In order to explore the dose-response elationship of these values, the target dosimetrics were evaluated across several doses in both acute and sub-chronic exposure scenarios using previously developed PBPK models at the AFRL/HEST; i.e., the models for the adult male rat (Merrill, 2001c) and human (Merrill, 2001d) described in Section 6.2, the pregnant and fetal rat model (Clewell, 2001a) and the lactating and neonatal rat model (Clewell, 2001b).

Acute (iv) pharmacokinetic studies in the adult male rat were used as the basis for this dose-response analysis because iodide uptake inhibition could be correlated to perchlorate levels. Further, as discussed in Section 6.1, the initial inhibition of iodide is viewed in the conceptual model as the important step in the transient phase (Figure 6-2). Transient decrements in T4 can result in permanent neurodevelopmental sequelae. In drinking water studies, upregulation of NIS in the rat is so rapid that it resulted in no measurable thyroid iodide inhibition, so the iv doses were used to estimate this initial insult. The target internal dosimetrics were first calculated in each of the rat models for acute exposure to perchlorate (single *iv* administration) at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/kg. In order to correlate perchlorate parameters to data-validated inhibition, the 2 to 4 hr time-frame was used for all acute calculations. The AUC for thyroid and serum were calculated by integrating predicted tissue concentrations from 2 to 4 hrs post dosing.

These same dosimetrics calculated for acute exposures were also determined for subchronic (drinking water) perchlorate exposures at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/kg-day. In order to achieve steady state concentrations, the models were run until the

predicted peak and trough heights did not change from one day to the next (Merrill, 2001e). Serum and thyroid perchlorate AUC concentrations were then determined over a 24 hr period (240-264 hrs in male, lactating, and neonatal rats; 480-504 hrs in pregnant and fetal rats). Although the tissues reach steady state perchlorate concentrations within one week, the above time-points were chosen in the lactation and gestation models for their ability to be verified with data (Clewell, 2001a,b). The male rat model was run at the same time as lactation for the sake of consistency with the other models. The total perchlorate AUC in the serum and thyroid were determined from each the models at 240 and 264 hrs (or 480 and 504 hrs). The difference in the two values was then divided by 24 hrs to give the AUC in units of ng/L-hr.

The AFRL/HEST experiments (Yu, 2000; Yu et al., 2000) have shown upregulation of the NIS to be both time and dose-dependent. Thus, at lower doses, the rat thyroid was completely upregulated after only a few days of drinking water exposure. Iodide uptake in the thyroid at higher perchlorate doses ($\geq$10 mg/kg-day) was completely restored by the 18th day of exposure, the time of data collection in the pregnant and fetal rats (Clewell, 2001).

Drinking water studies in the adult male rats showed elevated perchlorate uptake in the thyroid at drinking water doses of 3.0 mg/kg-day and higher (Yu et al., 2000; Merrill et al., 2001c). Increased perchlorate uptake also results from upregulation of NIS. Since perchlorate is transferred into the thyroid via NIS, the inhibiting anion is "upregulated" along with iodide. In order to simulate increased perchlorate concentrations in thyroids of the 3.0, 10.0, and 30.0 mg/kg-day dose groups, the original value for follicular Vmaxc (Vmaxc_T*p*) was adjusted to obtain the best fit of the model simulation to experimental data (Table 6-13). Since there were no pharmacokinetic data available for the 5.0 and 100.0 mg/kg-day dose groups, values for Vmaxc_T*p* were estimated from a Michaelis-Menten fit to the adjusted Vmaxc's at 3.0, 10.0, and 30.0 mg/kg-day doses (Figure 6-46). Target dosimetrics in the male rat were calculated for both originally optimized parameters and these adjusted ("upregulated") parameters.

This process of adjusting ("upregulating") the Vmaxc_T*p* values was not necessary in the gestation, lactation, or human models, as they were able to successfully describe perchlorate concentrations in serum and thyroid at all measured doses (0.01 – 10.0 mg/kg-day in gestation and lactation; 0.02 – 12 mg/kg-day in human) using one set of model parameters (Clewell, 2001a,b; Merrill, 2000). Merrill (2001e) posits that it was not necessary because it is likely that a loss of maternal iodide to the fetus and neonate causes dams to exist in a chronic state of

TABLE 6-13. "UP-REGULATED" VALUES OF VMAXC_T*p*[a] AFTER PERCHLORATE DRINKING WATER EXPOSURE IN THE ADULT MALE RAT MODEL (Merrill, 2001e)

Drinking Water Dose (mg/kg-day)	Adjusted Vmaxc_T*p* (ng/hr-kg)
0.01	2900
0.1	2900
1	2900
3	9000
5	17500[b]
10	32000
30	55000
100	79000[b]

[a]Maximum velocity capacity of active transport in the thyroid follicle.
[b]Data not available for these dose levels.

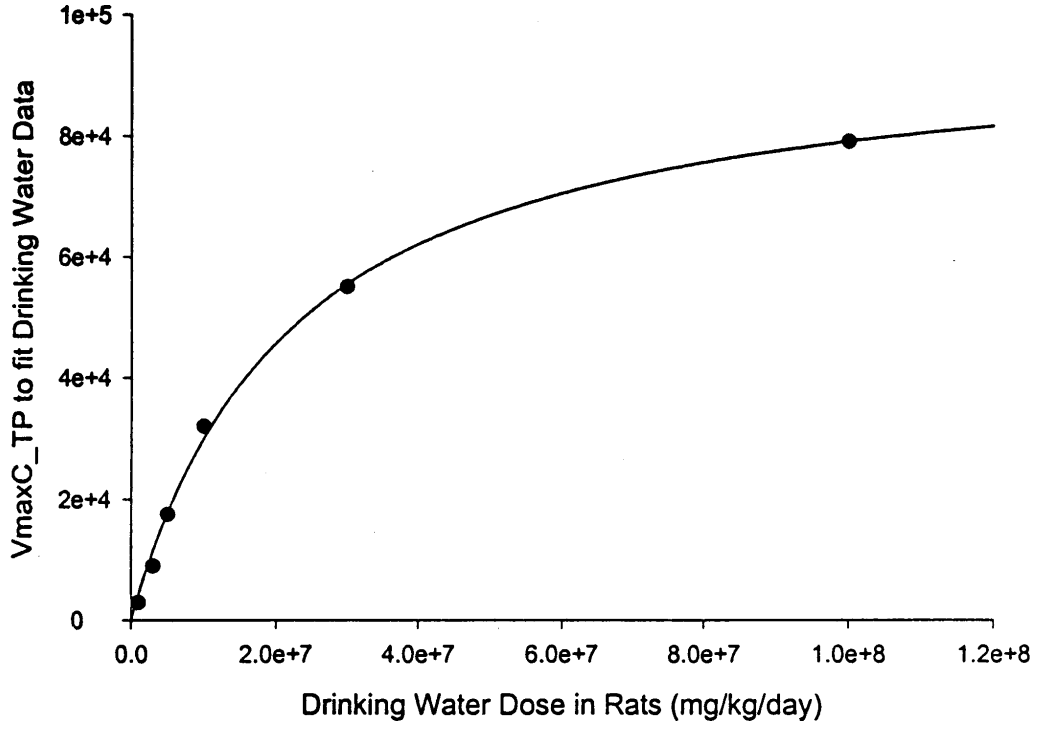

Figure 6-46. Upregulation of maximal capacity (ng/kg-hr) of active transport into the thyroid follicle for perchlorate (Vmaxc_T*p*) optimized by fitting to drinking water data in the rat. Upregulation is first noted in the 3.0 mg/kg-day dose group.

thyroidal up-regulation. As a result, the effect of perchlorate on the thyroid was less dramatic than in the male rat where a completely naïve system is perturbed by an inhibiting chemical. Thus, the PBPK models for gestation and lactation were able to describe thyroid perchlorate levels at drinking water doses from 0.01 to 10.0 mg/kg-day without adjusting the follicular Vmaxc (Vmaxc_T*p*) values with dose.

Increased follicular Vmaxc values were not needed to fit the human data likely due to the larger size of the human thyroid colloid versus that of the rat and to the differences in plasma protein binding discussed in Chapter 3.

The human PBPK model (Merrill, 2001d) was used to calculate all target dose metrics in both acute and two-week drinking water perchlorate exposures in a 70 kg adult at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/kg-day. Acute serum and thyroid perchlorate AUC concentration estimates were calculated with the model over an eight hr time period (from 24 to 32 hrs post-exposure) in order to correlate perchlorate parameters to data-validated iodide inhibition. For two-week drinking water exposures, the thyroid and serum perchlorate AUC concentration estimates were calculated over a 24 hr period after serum and thyroid concentrations reached steady state. The 240 to 264 hr time period was chosen for consistency with the male rat model (Merrill, 2001c).

The adult human model (Merrill, 2001d) was also used to predict dosimetry in a 15 kg child. The same dosimetrics were run in the model for the child and adult. However, since an average child drinks less water than an adult (approximately 1 L/d as opposed to 2 L/d in the adult), the actual exposures of a child and adult from the same water source would be different. For example, a 15kg child consuming 1 L of contaminated water would receive a daily dose (per kg bodyweight) that was 2.3 times that of a 70 kg adult consuming 2 L of water. Table 2 shows the concentration of the drinking water required to deliver the same dose to a 15 kg child and a 70 kg adult. For the purpose of this paper, dosimetric comparisons were calculated using the same dose (mg/kg-day) in the adult and child.

Figure 6-47 shows the curve generated from plotting the experimentally-determined percent inhibition versus the corresponding PBPK-derived serum (A) and thyroid (B) perchlorate AUC concentration estimates after acute (iv) exposure in rats. Thyroidal radiolabeled iodide ($^{125}I^-$) uptake measurements were taken two hours after iv administration of perchlorate. The solid line

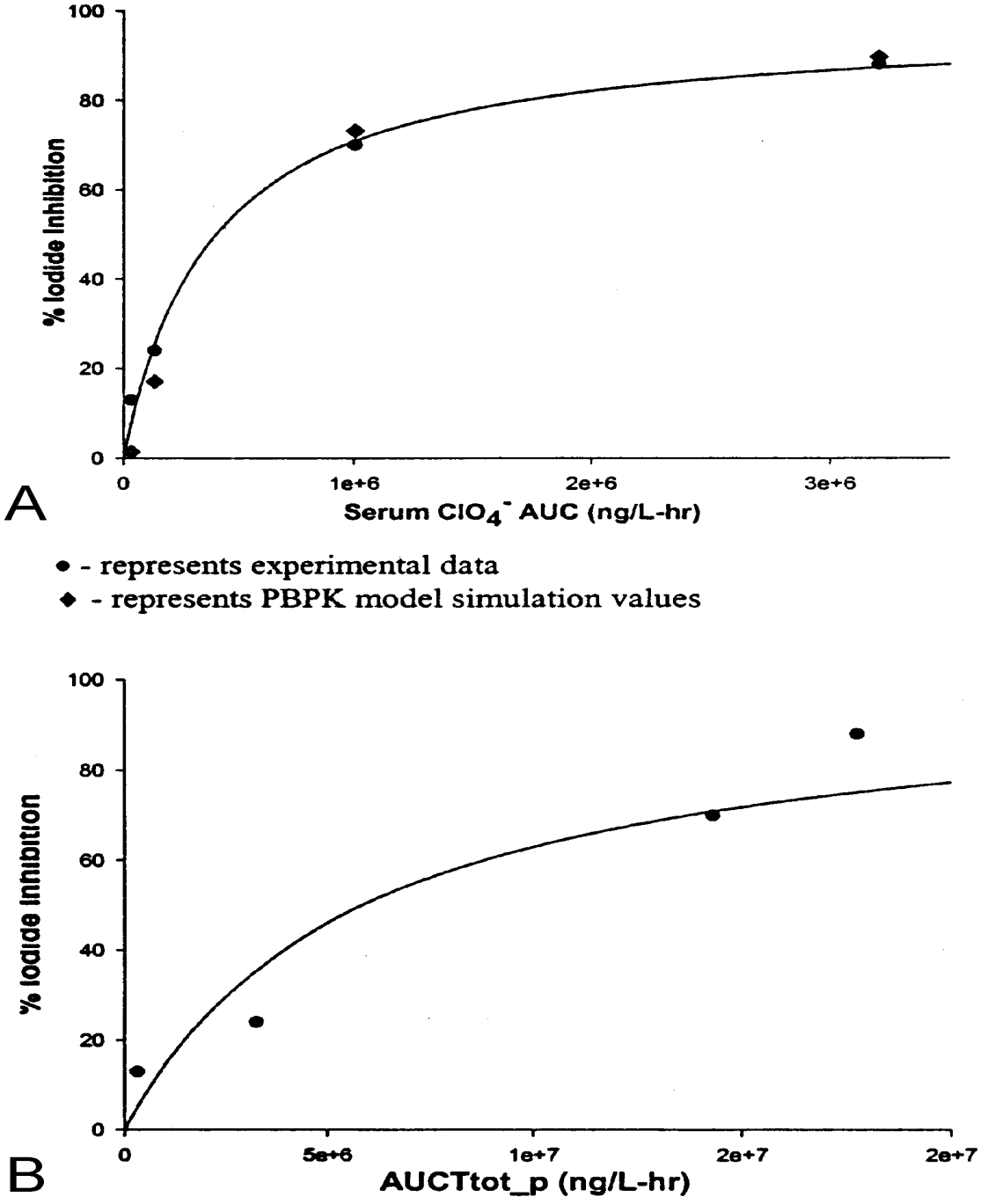

Figure 6-47. Michaelis-Menten fit of the "acute" male rat area under the curve (AUC) for serum (A) and thyroidal perchlorate (AUCTtot_*p*) in ng/L-hr. Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after iv injection of perchlorate.

represents a fit (not a PBPK model simulation) using the Michaelis-Menten type equation given below:

$$Y = (A \times AUC_{dose})/(AUC_{dose} + B) \quad (6\text{-}3)$$

Where 'Y' represents the predicted percent inhibition of radioiodide uptake, 'A' represents the maximal percent inhibition of radioiodide uptake, 'B' is related to the affinity of iodide uptake based on serum concentration, and AUC_{dose} represents the AUC at each specific dose of perchlorate. The above equation was also used to derive the dose-response relationship in subsequent figures. The correlation coefficient (r^2) greater than 0.91 in all cases indicated excellent fit for all (see Merrill, 2001e; Table 3).

Figure 6-48 shows the PBPK-derived AUC perchlorate concentration estimates for drinking water exposure to the adult male rat versus the calculated percent inhibition of radioiodide in the serum (A) and thyroid (B). The values for AUC of perchlorate concentration in the serum were determined by running the adult male rat model (Merrill, 2001c) across doses. Corresponding percent inhibitions were calculated by putting serum AUC perchlorate concentration values into the equation from Figure 6-47. Human response (thyroid inhibition) to subchronic exposure is similar to that of an acute exposure in the rat. This approach allows the sub-chronic serum levels in the rat be related to iodide uptake in the native thyroid. The values for AUC of thyroid perchlorate concentration (B) were determined by running the male rat model (Merrill, 2001c) at steady state (between 240 and 264 hours of drinking water exposure) across the doses shown. Corresponding percent inhibitions were calculated by putting thyroid AUC values in the equation from Figure 6-47.

The actual human iodide inhibition data (Greer et al., 2000) were plotted as a function of the perchlorate AUC concentration estimates for serum and thyroid calculated with the PBPK model in Figure 6-49. The measured percent inhibition of radiolabeled iodide uptake in the serum and thyroid on Day 2 of drinking water exposure to perchlorate is shown versus the PBPK-derived estimates for human volunteers (both male and female). Inhibition data from time points earlier than Day 2 of perchlorate in the human drinking water (Greer et al., 2000) and inhibition data from acute perchlorate dosing in humans were not available. Therefore, the inhibition measurements on Day 2 of perchlorate drinking water exposure were the closest-

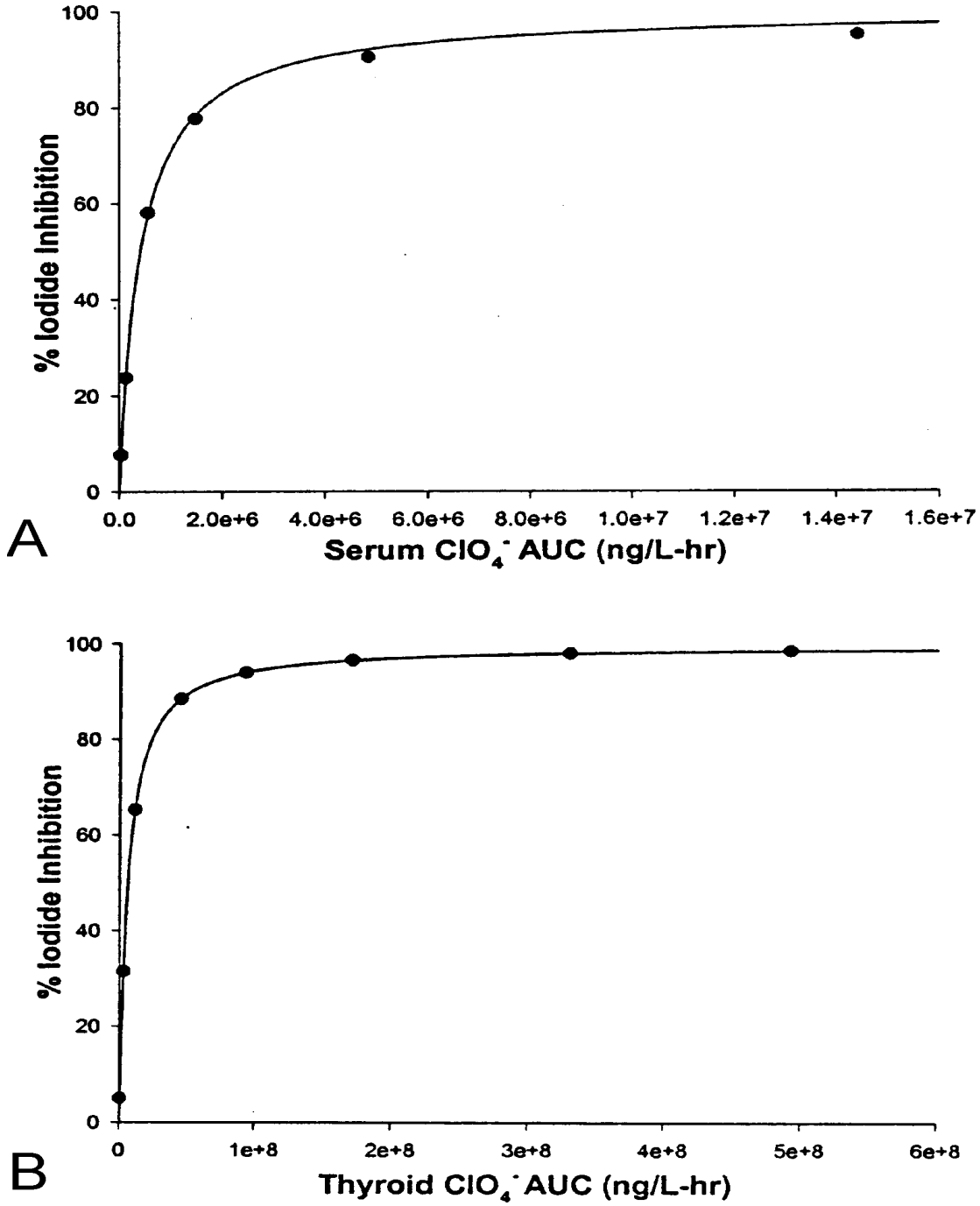

Figure 6-48. Michaelis-Menten fit of the "chronic" male rat area under the curve (AUC) for serum (A) and thyroidal (B) perchlorate (ng/L-hr). Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after drinking water exposure of perchlorate. Fit for serum calculated percent inhibition of radioiodide uptake calculated from equation used in Figure 6-47 (A) and for thyroid from Figure 6-47(B).

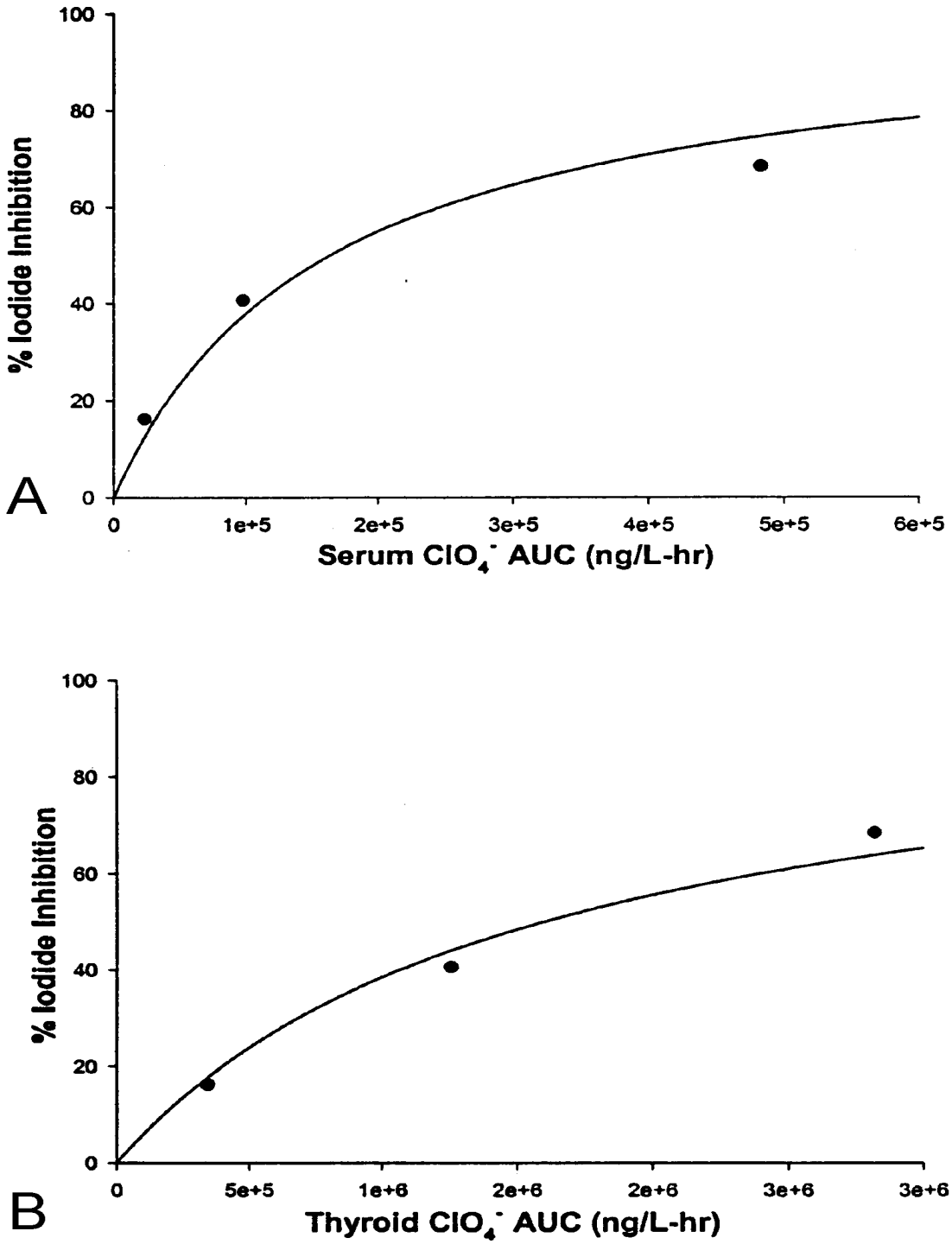

Figure 6-49. Michaelis-Menten fit of the human area under the curve (AUC) for serum (A) and thyroidal (B) perchlorate (ng/L-hr) on exposure Day 2. Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after drinking water exposure of perchlorate.

available representation of an acute human dose. Measured serum TSH and thyroid hormones indicated that thyroids were in normal homoeostatic state in human volunteers during the entire two week study (Merrill, 2001d).

The HEE estimates were calculated using the models as described in Section 6.1 (Figure 6-4). The HEE that would result in the same perchlorate AUC concentration estimates for serum (A) and thyroid (B) in the human and rat and the corresponding percent inhibition of iodide uptake is presented in Figure 6-50. Values for percent inhibition were determined from the rat serum AUC during drinking water exposures to perchlorate using the Michaelis-Menten equations from Figure 6-47. The correlation coefficient for both the serum and thyroid AUC versus percent iodide uptake inhibition relationship was 0.99.

6.5.3 Summary

The correlation coefficients for the dose-response relationships using the PBPK-model generated HEE estimates between serum and thyroid perchlorate AUC concentration versus iodide inhibition indicated good fits. Tables of the actual estimates and their ratios can be found in Merrill (2001e).

The rat serum ratios (AUC and peak concentrations) change significantly between 0.1 and 3.0 mg/kg-day due to binding of perchlorate by plasma proteins. Plasma binding is saturated at doses greater than 1.0 mg/kg-day. Male rat to human ratios are notably lower than those ratios between rats, as plasma binding of perchlorate occurs to a much lesser extent in humans.

HEE estimates were calculated for both a 15 and 70 kg human. The differences between the 15 and 70 kg human HEE estimates were never greater than 75%, indicating that body weight doesn't significantly affect the target dose metrics. Interestingly, the HEE estimates were greater in the 15 kg child. One might expect the adult and child HEE estimates to be nearly equal, given no parameters were changed in the human model except body weight. However, physiological parameters within the model are linearly scaled by body weight; whereas, chemical-specific parameters are scaled nonlinearly (e.g., as a multiple of body weight to a power of ¾). As indicated later in the sensitivity analysis, the internal dose metrics presented are more sensitive to chemical-specific parameters, especially those describing saturable kinetics. Therefore, the chemical-specific parameter values for the 15 kg child are proportionally greater

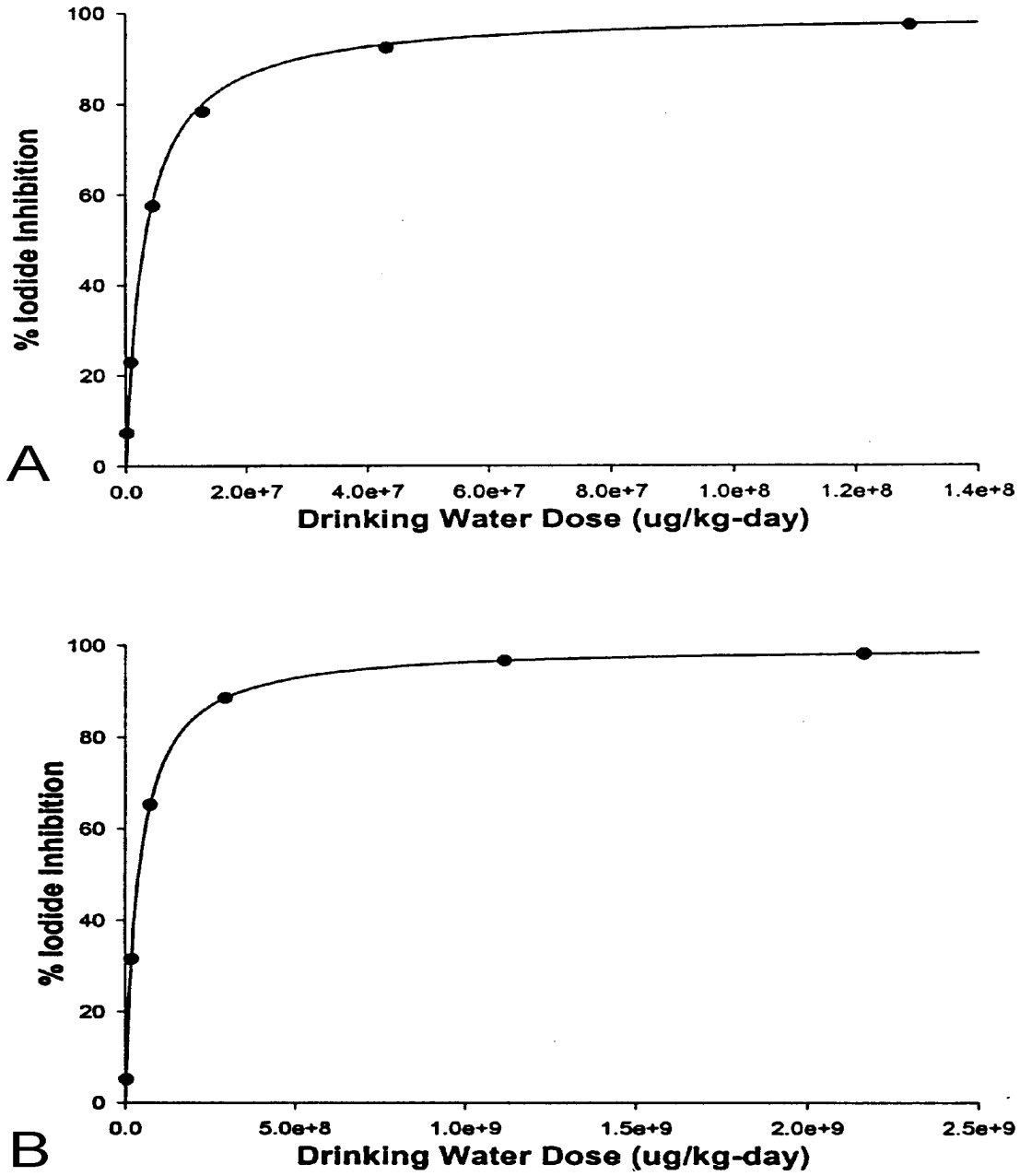

Figure 6-50. Michaelis-Menten fit of the human equivalent exposure (HEE) of perchlorate in drinking water derived from the area under the curve (AUC) for serum (A) or thyroid (B) versus percent predicted inhibition in the rat after an "acute" (iv) dose.

1 (in terms of body weight) than those of the adult. As a result, a slightly higher dose is required to
2 saturate these tissues in a child.

When comparing the dose metrics for serum versus thyroid, the HEE estimates calculated from the thyroid were less than the HEE estimates calculated from the serum by a factor of 100 at a 0.01 mg/kg-day dose level. This difference became a factor of 10 starting at the 5.0 mg/kg-day concentration for the 15 kg child and at 10.0 mg/kg-day for the adult.

These considerations will be explored in Chapter 7 to develop dosimetric adjustment factors for the observed effect levels.

7. DOSE-RESPONSE ASSESSMENTS FOR HUMAN HEALTH

The available database prior to initiation of the perchlorate testing strategy in 1997 (see Chapter 3) on the health effects and toxicology of perchlorate or its salts was very limited. The majority of human data were clinical reports of patients treated with potassium perchlorate for hyperthyroidism resulting from an autoimmune condition known as Graves' disease. Potassium perchlorate still is used diagnostically to test TSH, T3, and T4 production in some clinical settings. The primary effect of perchlorate is to decrease the production of thyroid hormones by competitively inhibiting iodide anion uptake into the thyroid at the *sodium (Na^+)-iodide (I^-) symporter (NIS)* and by causing a discharge of stored iodide from the thyroid gland.

It was difficult to establish a dose-response for the effects on thyroid function from daily or repeated exposures in healthy humans based on the data in patients with Graves' disease because of a variety of confounding factors, including that the disease itself has effects; that often only a single exposure and not repeated exposures were tested; that only one or two doses were employed; and that often the only effect monitored was iodide release from the thyroid or control of the hyperthyroid state. There were limited data in normal human subjects and laboratory animals that support the effect of perchlorate on thyroid hormones, but the majority of these studies suffer from the same limitations as those with the Graves' disease patients, with respect to the number of doses and exposures. These limitations prevent establishment of a quantitative dose-response estimate for the effects on thyroid hormones after long-term repeated exposures to perchlorate in healthy human subjects.

In addition, on December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. Environmental Protection Agency, 2001c). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a

NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Some of the clinical studies contained in this database fall in this category of studies not to be considered. However, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. Therefore, because of the scientific shortcomings of these studies, they will not be used as "principal studies" in the derivation of an RfD. The ethical issues surrounding the conduct of these studies or their use for regulatory purposes in light of the Agency's interim policy will not be discussed in this document. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies which intentionally dose human subjects with toxicants to identify or quantify their effects.

Thyroid hormone deficiencies, such as those induced by perchlorate, can affect normal metabolism, growth, and development. However, no robust data existed previously with which to evaluate potential target tissues or effects other than those in the thyroid. The data on the thyroid effects were also insufficient for quantitative dose-response assessment. Additionally, there were no data with which to evaluate the effects of perchlorate in potentially susceptible populations, such as developing fetuses; nor were there data on the effects of perchlorate on the reproductive capacity of male or female laboratory animals.

Benign tumors had been reported in the thyroids of male Wistar rats and female BALB/c mice treated with repeated, high-dose exposures (2 years at 1,339 mg/kg-day and 46 weeks at 2,147 mg/kg-day, respectively) of potassium perchlorate in drinking water, establishing perchlorate as a carcinogen. Benign tumors in the thyroid have been established to be the result of a series of progressive changes that occur in the thyroid in response to interference with thyroid-pituitary homeostasis (i.e., perturbation of the normal stable state of the hormones and functions shared between these two related glands). This progression is similar regardless of the cause of the thyroid hormone interference (Hill et al., 1989; Capen, 1997; Hurley et al., 1998). EPA has adopted the policy that for the dose-response of chemicals that cause disruption in the thyroid but that do not have genotoxic activity (i.e., cause damage to DNA or show other genetic disruption) a threshold for carcinogenicity is to be based on precursor lesions (U.S. Environmental Protection Agency, 1998e).

In the case of perchlorate, an overall model based on its mode of action has been developed as shown in Figure 7-1. The model supports iodide inhibition as the key event that precedes the hormone and thyroid changes with subsequent neurodevelopmental and neoplastic sequelae. Focusing on the key event of iodide uptake inhibition allows a harmonized approach to both the "noncancer" and "cancer" toxicity that occurs downstream along the continuum. Thus, one harmonized risk estimate is derived for both sequelae based on their common mode of action.

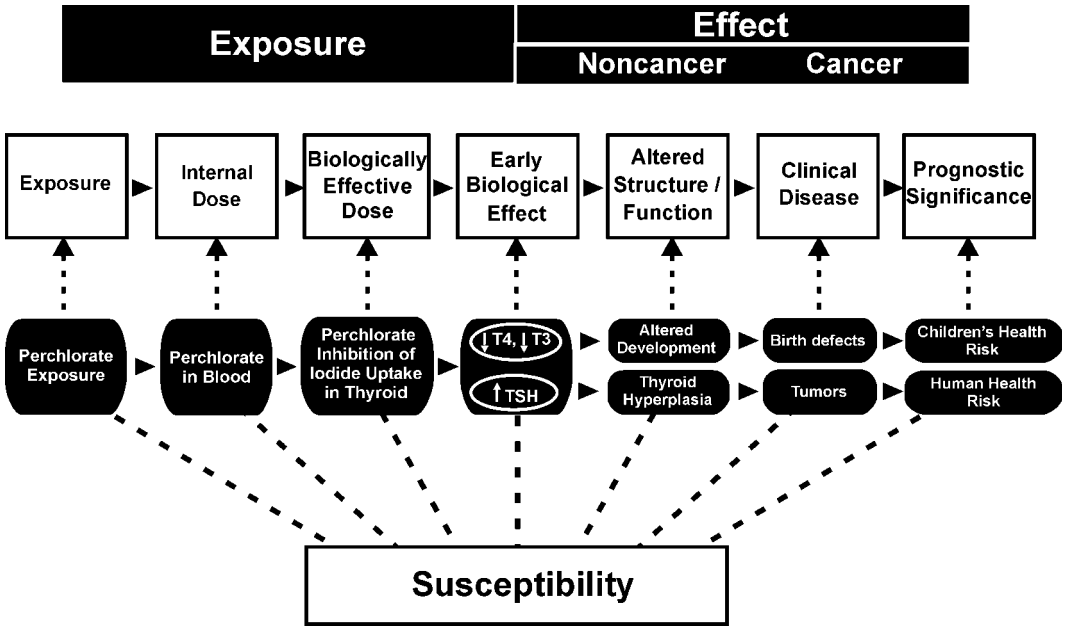

Figure 7-1. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U. S. Environmental Protection Agency, 1994a; Schulte, 1989). The model maps the toxicity of perchlorate on this basis by establishing casual linkage or prognostic correlations of precursor lesions.

This chapter presents the synthesis of the most relevant data for deriving a revised quantitative assessment of human health risk for perchlorate. The new data were consistent with the limited historical characterization and the 1998 EPA assessment in that the anti-thyroid

effects remain the focus of concern and the key event of its mode of action remained identified as the inhibition of iodide uptake at the NIS. However, data from the testing strategy allowed a more comprehensive evaluation of the possible sequelae of the iodide uptake inhibition and its thyroid-pituitary axis perturbations with respect to other endpoints, notably effects in dams and their offspring and on nerurodevelopmental, reproductive, and immunotoxicity parameters.

The key event is defined as an empirically observable precursor step that is a necessary element of the mode of action or is a marker for such an element. This will be discussed in Section 7.1.1. Section 7.1.2 discusses dosimetric adjustment of effect levels observed in the laboratory animals to human equivalent exposures (HEE). Choice of the point of departure for the assessment based on a quantitative consideration of the key event, observed effects, and weight of the evidence is discussed in Section 7.1.3. Application of factors to account for uncertainty and variability in the extrapolations required to use the data is discussed in Section 7.1.4. The overall operational derivation is presented in Section 7.1.5, and the assignment of confidence levels is discussed in Section 7.1.6. Section 7.1.5 also presents a discussion of the cancer assessment in the context of the RfD. Section 7.2 discusses the inhalation reference concentration. Susceptible population considerations are discussed in Section 7.1.5.3. Section 7.3 presents a brief summary of the findings.

7.1.1 Key Events and Weight of the Evidence

Results of the testing strategy have established that the critical target tissue for perchlorate is the thyroid gland, with some remaining concern for adequate characterization of its potential for immunotoxicity, notably contact hypersensitivity. Changes in thyroid weights, three response indices of thyroid histopathology (colloid depletion, hypertrophy and hyperplasia), and thyroid and pituitary hormones were consistently altered across the array of experimental designs represented by the data base. The developmental and reproductive NOAEL and LOAEL values were higher than those associated with thyroid toxicity per se.

Figure 7-2 highlights the temporal considerations that have to be superimposed on evaluation of the data from the various studies in laboratory animals and humans in order to characterize the anti-thyroid effects from perchlorate exposure. Conceptually, competitive inhibition of iodide uptake at the NIS by perchlorate is the key event leading to both potential neurodevelopmental and neoplastic sequelae. The decrement in iodide uptake leads to

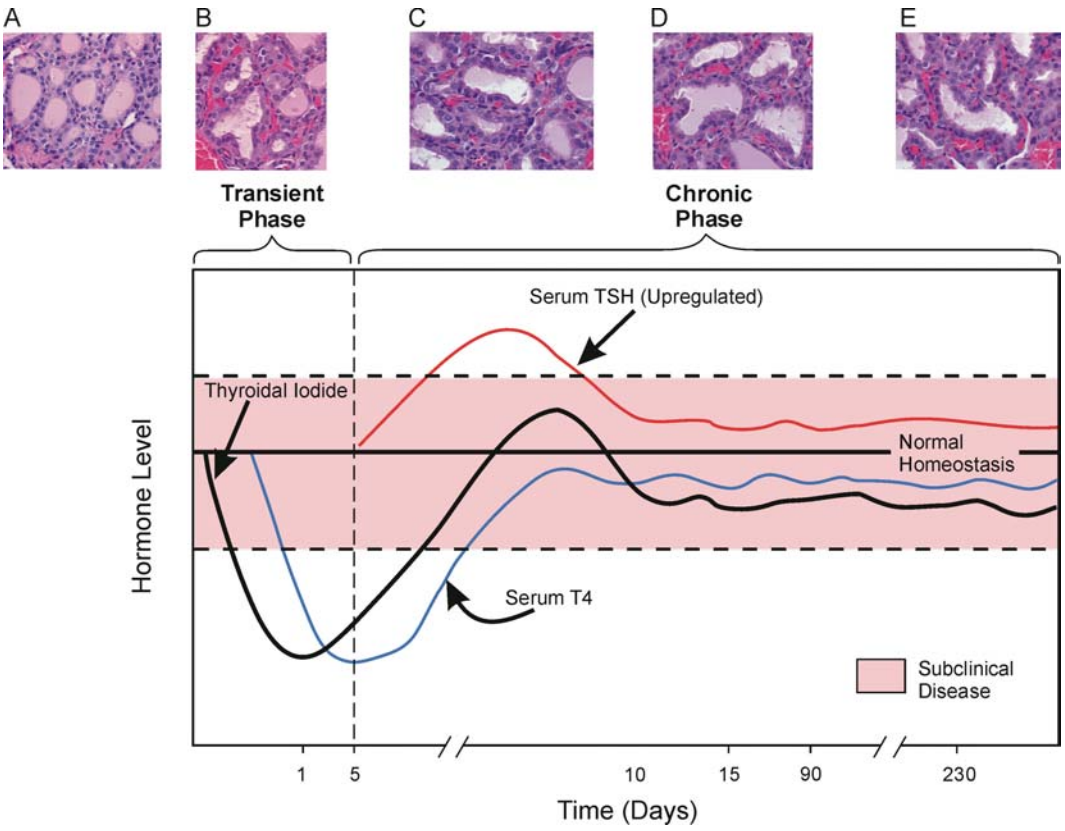

Figure 7-2. Schematic of thyroid and pituitary hormone levels with associated pathology after acute versus chronic dosing with perchlorate. The transient phase is represented by decreases in thyroidal iodide due to the inhibition by perchlorate at the NIS with subsequent drop in T4. The transient drops in T4 can lead to permanent neurodevelopmental sequelae. Once TSH is upregulated via the hypothalamic-pituitary-thyroid feedback, T4 appears to be in normal homeostasis but actually can represent subclinical or undiagnosed disease (hypothyroxinemia). The upregulation of TSH can result in neoplasia. Normal thyroid tissue is represented in Panel A. Panel B shows lace-like colloid depletion which is more pronounced in subsequent panels C, D and E. Panels D and E represent hypertrophy and hyperplasia.

subsequent drops in T4 (and T3) that can lead to permanent neurodevelopmental deficits. Corroborating evidence for this likely outcome given the mode of action of perchlorate comes from the iodide deficiency literature and recent studies showing that maternal hypothyroxinemia (i.e., decrements in T4 with or without concomitant increases in TSH) is linked to poor developmental, neuropsychological and cognitive outcomes (Haddow, et al., 1999; Pop et al.,

1999; Morreale de Escobar, et al., 2000). It should be noted that medical concern for hypothyroxinemia remains in the "chronic phase"; i.e., once TSH upregulates to attempt to regulate the hypothalamic-pituitary-thyroid feedback system back to an apparent homeostasis, because this stress on the system essentially represents a "subclinical" disease state. Indeed, adverse outcome in women with hypothyroxinemia per se has been demonstrated because adversity includes the inability of an organism to respond to additional stressors. The system in this case, particularly when considered on a population level, would present a diminished capacity to compensate for other anti-thyroid insults. Since a large percentage of women are believed to already be hypothyroid, the importance of this effect to women in general, pregnant women, and fetuses on a population level can not be discounted. Weiss (2000) has noted that even if the magnitude of effect may be relatively small for most environmental levels, such neurotoxicity is extremely significant for public health.

Of notable concern, as previously discussed in Chapter 3, is that the developing fetus is dependent on the mother for its T4 and T3 through parturition, as illustrated in Figure 7-3 for humans with a similar pattern in rats. During the period illustrated in Figure 7-3, a number of critical stages in neural development take place, some of which depend on thyroid hormones. The cell precursors of the brain and spinal cord which compose the central nervous system (CNS) begin to develop early in embryogenesis through the process called neurulation. Beginning early in the second week of gestation in rodents (GD9.5 in rats) and the first month of gestation in humans, specific areas of the CNS begin to form with the neurogenesis and migration of cells in the forebrain, midbrain, and hindbrain. This sequence of developmental processes includes proliferation, migration, differentiation, synaptogenesis, apoptosis, and myelination (Rice and Barone, 2000). As discussed in Chapter 3, thyroid hormones play a role throughout this process, regulating proliferation, migration, and differentiation. Alterations in these processes can result in abnormalities of the brain and developmental delays.

The upregulation in TSH in the "chronic phase" (see Figure 6-2) also presents an increased potential for neoplasia because stimulation of the thyroid to produce more T4 and T3 can result in hyperplasia. Both the decrement in T4 and T3 and increase in TSH is shown in Figure 7-1 at the same step along the continuum. Which of these thyroid responses is the most sensitive to hormone changes has not specifically been studied in the perchlorate testing strategy. As noted in the analyses of the studies in Chapter 5, there is a considerable degree of overlap among the

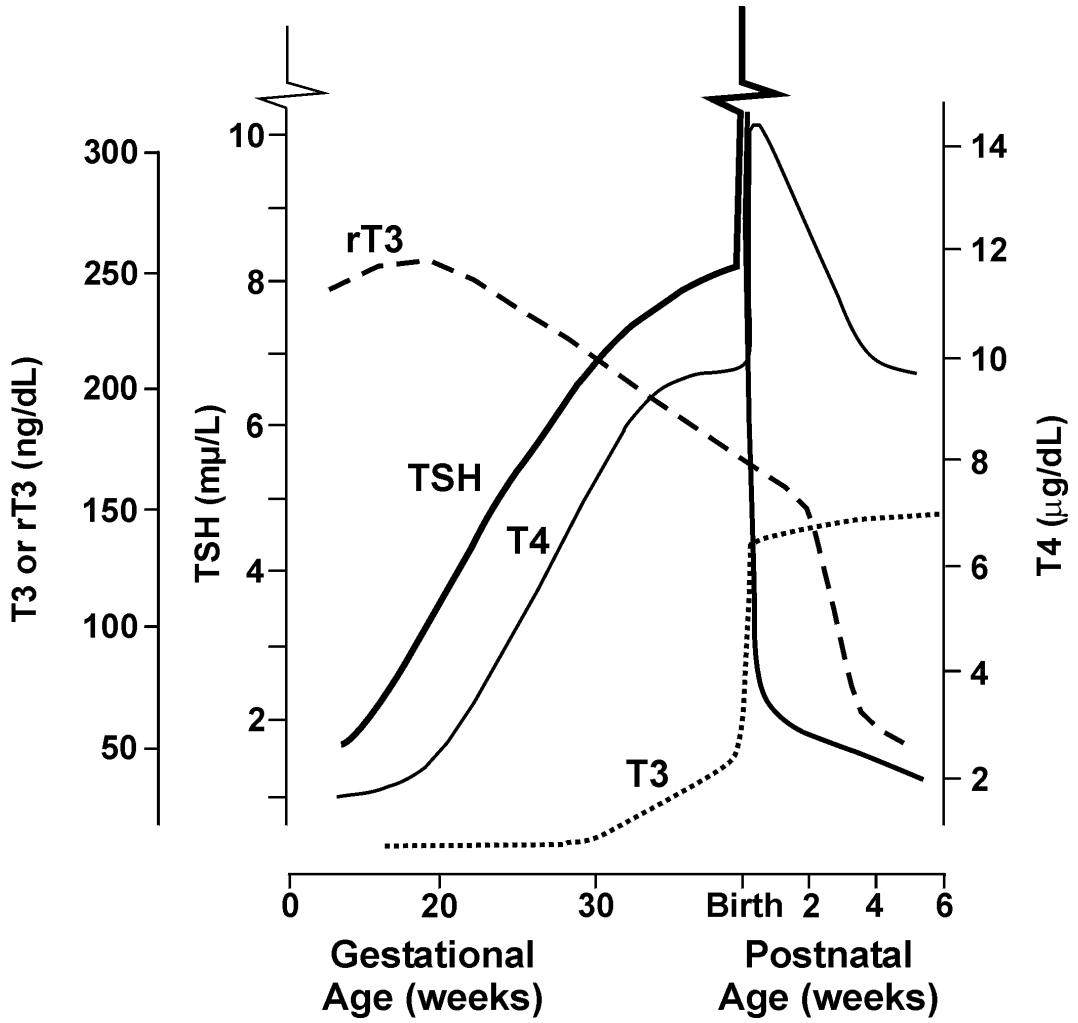

Figure 7-3. Pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation in the human (from Fisher, 1996). A similar pattern is thought to exist in the rat (see text for further details).

three different diagnoses of thyroid histopathology: colloid depletion, hypertrophy, and hyperplasia.

Colloid depletion does appear to be slightly more sensitive across the perchlorate studies. The fact that thyroid follicular colloid depletion is a consistent finding not only across this study, but in rodents in general, would suggest that it is a good indicator of sufficient exposure to inhibit thyroid hormone synthesis. From a physiologic point of view this may be logical and supports the mode-of-action model. If there is any reserve thyroid hormone in the colloid, it is depleted

before serum hormones are altered. Once serum levels are altered, TSH is upregulated and hypertrophy and hyperplasia are initiated in an attempt by the gland to restore circulating levels of T4 and T3. The diagnosis of colloid depletion has been reported with a similar compound, sodium chlorate, in the rat (Hooth et al., 2001), with many other chemicals in the rat, and with numerous goitrogens and pharmaceutical agents in the mouse. Colloid depletion in association with hypertrophy and hyperplasia suggests sufficient dose of the compound to inhibit colloid synthesis and decreases of circulating serum thyroid hormone levels sufficient to stimulate TSH.

Colloid depletion as the most sensitive indicator is most notable in the pups of the 2001 "Effects Study" on GD21 and then immediately post parturition on PND4. Alternatively, as discussed in Chapter 5, it may have been harder to diagnose hypertrophy and hyperplasia in the younger (smaller) and growing glands. The BMDL for colloid depletion increased with post-natal age and by PND21, hyperplasia was also present. In contrast, all three thyroid indices were present in the PND4 pups of the previous Argus Laboratories, Inc. (1998a) study. This may be due to the difference in dosing of the dams. The dams in the 1998 study were only dosed during gestation and, therefore, likely had a greater decrement in thyroid hormones. The dams in the 2001 study were dosed for two weeks during cohabitation, sufficient time as evidenced in the data described in Chapter 6, for upregulation of NIS to compensate.

Other studies indicate that whichever index is most sensitive could be dependent on dose spacing in the study, age of animals on test, and sacrifice time point. For example, hyperplasia was the most sensitive of the three in the P2-generation adults (19 week F1-generation pups) and these same pups developed thyroid adenomas.

The proposed mode of action mapped in Figure 7-1 is supported by correlations between thyroid hormones and TSH and between thyroid hormones or TSH and an objective measure of lumen size from laboratory animals exposed to ammonium perchlorate. There were positive correlations between T3 and T4, and negative correlations between either T3 and T4 and TSH, as expected based on the mode of action model (Appendix 7A). The positive correlation between TSH and decreased follicular lumen size and negative correlation between T4 or T3 and decreased follicular lumen size similarly support the proposed model (Appendix 7A). Some of the correlations used in the 1998 assessment were precluded due to the limited severity scoring system used by the PWG.

Additional support for the mode of action comes from data that now allow the linkage of both neurodevelopmental and neoplastic sequelae into the model. These definitive data were not available prior to the 1997 perchlorate testing strategy and especially not before the most recent studies recommended by the 1999 external peer review. The repeat of observed effects on the motor activity and brain morphometry results by new studies allowed definitive determination that perchlorate exposure poses a neurodevelopmental hazard.

Repeatability and variability in statistics, sometimes a concern for evaluation of behavioral assays (Cory-Slechta et al., 2001) were addressed by the Bayesian approach employed for the motor activity analysis (Dunson, 2001a) that showed remarkable reproducibility between the two studies despite the deficits previously noted for the Argus Research Laboratories, Inc. (1998a) study. The effects on the size of the corpus callosum measurements were also repeated, and effects on additional brain regions identified. The new data were subject to a more rigorous statistical analysis than in 1998. The profile analysis described in Chapter 5 required that all areas of the brain measured were altered in a dose-dependent fashion and effects were again demonstrated not only in the corpus callosum but other brain regions as well (Geller, 2001d).

Likewise the neoplastic potential for perchlorate that had been demonstrated only at high doses in historical studies was confirmed at lower doses by the thyroid adenomas reported by the PWG (Wolf, 2000; 2001) for the F1-generation pups at 19 weeks (P2 parents) from the two-generation reproductive study (Argus Laboratories, Inc., 1999). Consistent with the proposed mode-of-action model, the anti-thyroid effects leading to neoplasia are likely to be via the non-linear mechanism described above. The genotoxicity battery established that perchlorate is not directly damaging to DNA.

Thus, the key event for the anti-thyroid effects of perchlorate is its perturbation of the hypothalamic-pituitary-thyroid axis by competitive inhibition of iodide uptake at the NIS. The evidence for this effect is built upon the observation of consistent changes across a range of experimental designs, including various species. These changes demonstrate effects on thyroid and pituitary hormones, increases in thyroid weight, and increases in three different diagnoses of thyroid histopathology (colloid depletion, hypertrophy, and hyperplasia). In addition, corresponding neurodevelopmental (motor activity and brain morphometry) and neoplastic outcomes were observed in special assays; these outcomes are also consistent with the proposed

mode of action and provide further evidence to confirm that the perturbation of the thyroid hormone economy should be viewed as adverse.

Due to the age and time-dependent nature of the critical effect, no one principal study is being chosen for this derivation. Instead, a weight-of-the-evidence approach will be taken to arrive at a point of departure in Section 7.1.3.

7.1.2 Dosimetric Adjustment of Exposures Associated with Effect Levels

Adjustments for interspecies differences in the internal dose delivered to target tissues should be made before an evaluation of the data array for valid comparisons across endpoints (U.S. Environmental Protection Agency, 1994). Based on the mode of action and the available PBPK model structures, two dose metrics were considered to describe the biologically effective dose for perchlorate: (1) the area under the curve (AUC) for perchlorate in the serum associated with drinking water exposures and (2) the percent of iodide uptake inhibition in the thyroid. These correspond to the different exposure components along the exposure-dose-response continuum in the mode-of-action model (Figure 7-1).

As described in Chapter 6, the serum perchlorate AUC was developed as the first dose metric based on data in rats and humans after drinking water exposures. To predict the "transient" phase of initial iodide inhibition in the rat, i.e., before upregulation of the NIS or increases in TSH, the second dose metric was based on RAIU measurements made in adult male rats dosed with perchlorate by iv two hours prior to an iv dose of radiolabled iodide. Table 7-1 presents the human equivalent exposures (HEE) estimates calculated using the PBPK models for serum perchlorate AUC as the dose metric. Table 7-2 shows the ratios for this same dose metric that can be applied in the parallelogram approach to arrive at estimates for different life stages used to observe effects in the different experimental endpoints. Fetal rat predictions were based on data developed for GD21. Neonatal rat predictions were based on data for PND10. This approach was taken since PBPK models for human pregnancy and lactation do not exist for perchlorate distribution. The calculation using the ratios approach is described in Chapter 6. The resultant adult HEE values for the different life stages of the rat experiments are shown in Table 7-3.

It can be observed in the tables in Merrill (2001e) that the pregnant and lactating rats have significantly higher average serum perchlorate concentrations at the lowest drinking water dose

TABLE 7-1. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL DOSES IN THE MALE RAT FOR 15 AND 70 KG HUMAN BASED ON PERCHLORATE AREA UNDER THE CURVE (AUC) IN SERUM OR THYROID AS THE DOSE METRIC (Merrill, 2001e)

Adult Male Rat DW[a] Dose (mg/kg-day)	Human 15 kg HEE (mg/kg-day) based on serum[b] AUC	Human 70 kg HEE (mg/kg-day) based on serum[b] AUC	Human 15 kg HEE (mg/kg-day) based thyroid[b] AUC	Human 70 kg HEE (mg/kg-day) based on thyroid[b] AUC
0.010	0.030	0.021	0.0002	0.0001
0.1	0.145	0.100	0.002	0.001
1.0	0.745	0.505	0.008	0.006
3.0	2.05	1.35	0.052	0.035
5.0	3.35	2.25	0.145	0.098
10.0	6.75	4.45	0.725	0.460
30.0	20.3	13.2	163.0	110.0
100.0	65.0	43.8	490.0	330.0

[a]DW = drinking water.
[b]Calculated from PBPK-derived rat AUC(s) at steady state between 240 and 264 hrs during DW exposure, using upregulated Vmaxv_T_P values from (Merrill, 2001e: Table 1).

TABLE 7-2. RATIO OF PBPK-DERIVED PERCHLORATE AREA UNDER THE CURVE (AUC) SERUM CONCENTRATIONS IN DRINKING WATER FOR VARIOUS EXPERIMENTAL LIFE STAGES (Merrill, 2001e)

Rat DW[a] Dose (mg/kg-day)	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
0.01	0.63	0.58	1.44	1.16	2.28	1.99
0.1	0.73	0.54	1.06	0.85	1.46	1.56
1.0	0.90	0.84	1.44	1.01	1.61	1.20
3.0	0.94	0.95	1.67	1.71	1.77	1.80
5.0	0.95	0.98	1.74	2.14	1.82	2.18
10.0	0.96	1.01	1.80	2.70	1.87	2.69
30.0	0.97	1.02	1.84	3.33	1.90	3.26
100.0	0.97	1.03	1.85	3.65	1.92	3.55

[a]DW = drinking water.

TABLE 7-3. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL LIFE STAGES IN THE RAT USING SERUM PERCHLORATE AREA UNDER THE CURVE (AUC) AS THE DOSE METRIC

Dose (mg/kg-day)	Human Equivalent Exposure[a] (mg/kg-day)				
	Adult Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	0.02	0.01	0.03	0.01	0.02
0.1	0.10	0.07	0.10	0.05	0.08
1.0	0.51	0.46	0.73	0.43	0.52
3.0	1.35	1.3	2.3	1.3	2.4
5.0	2.25	2.14	3.92	2.20	4.82
10.0	4.4	4.22	7.9	4.4	11.9
30.0	13.2	12.8	24.3	13.5	43.95
100.0	43.8	42.5	81.0	45.11	160.0

[a]Based on predicting the area under the curve in the blood (AUCB) using the human PBPK model that achieves an equivalent degree to that simulated for the rat experimental regimen associated at different life stages. See Tables 7-1and 7-2 and Chapter 6 for explanation of calculation.

(0.01 mg/kg-day). This is likely due to increased binding in the serum (Merrill, 2001e). It has been shown that the estrus cycle affects the concentration of binding proteins within the blood. Thyroxine, which is displaced from plasma proteins by perchlorate, is bound to a greater extent in the pregnant rat (Iino and Greer, 1960). It follows then that perchlorate would also be bound to a greater extent during pregnancy and possibly lactation. Since serum binding affects only the low doses, it is reasonable that the higher doses (1.0 through 100 mg/kg-day) would be similar across the male, pregnant and lactating rats (Merrill, 2001e).

Tables 7-4 through 7-7 are a comparable set of tables but are based on using thyroid uptake inhibition as the dose metric. Table 7-5 shows the percent of iodide uptake inhibition predicted at each dose for the various life stages used in the various laboratory rat experiments.

7.1.2.1 Choice of Dose Metric

Because developmental effects are of concern, an argument could be made that peak—and not AUC—is the appropriate dose metric with the rationale that any transient dose could be responsible for permanent deficits. However, the AUC values, as opposed to peak

TABLE 7-4. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL DOSES IN THE ADULT MALE RAT FOR 15 AND 70 KG HUMAN BASED ON % IODIDE UPTAKE INHIBITION IN THE THYROID

Rat iv Dose (mg/kg)	Adult male rat inhibition at 2-hr post iv dose	Human 15 kg HEE (mg/kg-day)	Human 70 kg HEE (mg/kg-day)
0.01	1.5%	0.006	0.004
0.1	16.3%	0.075	0.048
1.0	74.5%	1.5	0.9
3.0	90.0%	4.8	2.7
5.0	93.5%	8.0	4.9
10.0	96.2%	16.0	9.0
30.0	98.1%	35.0	19.3
100.0	98.7%	50.0	33.0

TABLE 7-5. PBPK-MODEL PREDICTED % INHIBITION OF IODIDE UPTAKE IN THE THYROID[a]

Rat DW [b] Dose (mg/kg-day)	Adult Male Rat	Pregnant Rat	Fetal Rat[c]	Lactating Rat[d]	Neonate Rat[c,d]	70 kg Human
0.01	1.5%	3.2%	-129.1%	0.5%	0.4%	2.8%
0.1	16.3%	30.1%	27.9%	5.3%	1.3%	23.7%
1.0	74.5%	88.7%	81.2%	62.9%	3.0%	80.2%
3.0	90.0%	93.8%	90.3%	92.8%	3.3%	92.3%
5.0	93.5%	97.0%	90.4%	95.8%	3.1%	95.2%
10.0	96.2%	97.9%	97.9%	97.6%	3.8%	97.4%
30.0	98.1%	98.6%	98.9%	98.5%	6.1%	98.9%
100.0	98.7%	98.8%	99.2%	98.8%	13.4%	99.4%

[a]Based on iv administration to rat and drinking water in human.
[b]DW = drinking water
[c]Values for these tissues not validated versus data.
[d]All calculations are for PND10 in lactating and neonatal rat.

TABLE 7-6. RATIOS OF PBPK-DERIVED % IODIDE UPTAKE INHIBITION IN DRINKING WATER FOR VARIOUS EXPERIMENTAL LIFE STAGES[a]

Rat DW[b] Dose (mg/kg-day)	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat[c]	Male Rat: Neonate Rat[c]	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat[c,d]
0.01	0.48	3.24	−0.01	4.02	−0.02	1.2
0.1	0.54	3.06	0.59	12.75	1.08	4.2
1.0	0.84	1.18	0.92	24.53	1.09	20.7
3.0	0.96	0.97	1.00	27.49	1.04	28.4
5.0	0.96	0.98	1.03	30.45	1.07	31.2
10.0	0.98	0.99	0.98	25.61	1.00	26.0
30.0	0.99	1.00	0.99	16.06	1.00	16.1
100.0	1.00	1.00	1.00	1.37	1.00	7.4

[a]Inhibition in human was PBPK-derived from 2 wks ClO_4^--exposure in drinking water (DW); all rat values simulated from an iv dose.
[b]DW = drinking water
[c]Model predicted in fetal and neonate rats not validated with data.
[d]All calculations are for PND10 in lactating and neonatal rat.

TABLE 7-7. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL LIFE STAGES IN THE RAT USING % IODIDE UPTAKE INHIBITION IN THE THYROID AS THE DOSE METRIC

Dose (mg/kg-day)	Human Equivalent Exposure[a] (mg/kg-day)				
	Adult Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	0.004	0.002	—	0.01	0.02
0.1	0.048	0.026	0.03	0.15	0.61
1.0	0.90	0.756	0.83	1.06	22.05
3.0	2.7	0.259	2.70	2.62	74.2
5.0	4.9	4.70	5.05	4.80	149.2
10.0	9.0	8.82	8.82	8.91	230.5
30.0	19.3	19.1	19.1	19.3	309.96
100.0	33.0	33.0	33.0	33.0	33.0

[a]Based on predicting the % iodide uptake in the thyroid using the human PBPK model that achieves an equivalent degree to that simulated for the rat experimental regimen associated at different life stages. See Tables 7-4 and 7-6 and text for explanation of calculation.

concentrations, were used based on the assumption that these dose metrics would represent an averaging of the serum and thyroid perchlorate concentrations and would be better correlated with the inhibition effect on iodide uptake. The correlation was shown to be good between the AUC and the degree of inhibition (Figures 6-47 through 6-50). Further, due to the rapid phase of distribution after an iv dose, measurement of concentrations are very difficult to attain experimentally and are more variable. Using simulated peak concentrations after iv injections is potentially problematic due to the inexact modeling of the actual distribution of dose in the tail-vein volume and the exact time of mixing in the whole blood compartment (Merrill, 2001e). It was also observed by EPA that the ratios for peak perchlorate serum values (Merrill, 2001e: Table 6) were in good agreement with those for the perchlorate serum AUC and that the serum AUC were slightly more conservative if different at all.

Merrill (2001e) expressed concern regarding the thyroid values in neonates and fetuses because these values were not validated against experimental data. Fetal and neonatal thyroid were never actually analyzed for perchlorate concentration. In the case of the fetus, kinetic parameters were determined by fitting model simulations of fetal thyroid concentration to available iodide data and assuming that the perchlorate:iodide ratio would be similar to that of the mother. In the case of the neonatal rat, no data were available for thyroid concentrations for either perchlorate or iodide. Thus, model predictions were based on allometrically scaling maternal parameters for thyroid uptake. It was the opinion of the AFRL/HEST authors that while the thyroid parameters in the fetus and neonatal rat were highly informative, they should not be used in the formal risk assessment (Merrill, 2001e). EPA concurs with these considerations and recommendation.

In general, the models were believed to provide a good description of perchlorate and iodide disposition in the blood. Using the models to describe dose metrics in the thyroid was viewed as less reliable due to assumptions regarding parameters and the lack of experimental data for validation. The models were able to successfully describe serum perchlorate and iodide concentrations for both acute (based on iv doses) and chronic drinking water in the adult male, pregnant, neonatal and fetal rat, and greater confidence can be afforded these predictions (Merrill, 2001e).

Tables 7-3 and 7-7 demonstrate good correspondence in the HEE estimates predicted for both dose metrics at the lower doses for the lactating and neonatal rats, but not for the male adult,

pregnant or fetal rats where there is an order of magnitude difference. The iodide inhibition metric predicts a 10-fold lower HEE in both the adult male and pregnant dam when compared to the HEE estimated based on the serum AUC. The fetal rat value for iodide inhibition was viewed as unreliable for the reasons stated above. All of the factors influencing this disparity are not fully appreciated at this time but can reasonably be ascribed to uncertainty in the thyroid descriptions that were not validated with experimental data, and will require additional studies to characterize accurately. For these reasons, the adjustment factor to arrive at an HEE estimate was based on perchlorate serum AUC as the dose metric.

7.1.3 Point-of-Departure Analysis

Various statistical procedures were used for each of the different outcome measures for the various endpoints described in Chapter 5. The weight-of-evidence approach herein relies on the results, and the details on the statistical analyses are provided in Chapter 5 and associated memoranda from EPA and NIEHS scientists. In general, benchmark dose analysis was used for the thyroid histopathology because the EPA advocates the use of quantitative dose-response modeling to diminish the influence of dose-spacing, sample size, and variability on the NOAEL designation (Crump et al., 1995). Likewise, ANOVA was used to evaluate the thyroid and pituitary hormone data (Crofton and Marcus, 2001) although benchmark analyses were also performed as a comparison (Geller, 2001c). The 1998 benchmark analyses for the hormone data from the previous set of studies (Geller, 1998a) is provided in Appendix 7B.

Specific Bayesian statistical analyses were employed for the motor activity data and for evaluating the significance of the tumors in the 19-week old F1-generation adult rats (Dunson, 2001a,b). Another specific statistical approach, profile analysis, was used to evaluate the brain morphometry effects (Geller, 2001d).

Several studies suggest 0.01 mg/kg-day as the exposure dose that is a level of concern for the adverse effects of perchlorate. The first is the profile analysis on brain morphometry effects in PND21 pups in the "Effects Study" (Argus Research Laboratories, Inc., 2001) which demonstrated a dose-dependent and significant effect on the size of the corpus callosum and other brain regions. Statistically significant changes were also demonstrated in the PND9 pups. This effect repeated effects on brain morphometry observed in the previous neurodevelopmental study (Argus Research Laboratories, Inc. 1998a) that were a noted concern to the EPA in the

1998 risk assessment. Changes in the corpus callosum at a later time point on PND82 were also observed in that previous study.

An increase in the corpus callosum plausibly represents a delay in developing brain structures since this area is known to increase in size and then decrease later during development. Neurodevelopmental toxicity suggestive of delays was also demonstrated by effects on motor activity in both the Argus Research Laboratories, Inc. (1998a) and repeated in the Bekkedal et al. (2000) study. The increases in motor activity represent activity that should have subsided by these test dates. A type of hyperactivity has been noted in monkeys exposed to PCBs (Rice, 2000).

These effects on brain morphometry and motor activity are of particular concern because the relative sensitivity of laboratory animal assays to adequately characterize the types of deficits related to maternal hypothyroxinemia in large population studies is unknown (Morreale de Escobar, 2000; Haddow et al., 1999; Pop, 1999). Screening neurodevelopmental studies may not have the power to ascertain neurological effects that might result from small changes in the thyroid-pituitary hormone economy. As pointed out by Crofton (1998j), the sensitivity of animal models used to explore the role of thyroid hormones in neural development is currently equivocal. Most of the data collected and published to date were with high doses of thyrotoxic chemicals (e.a., methimazole, propylthiouracil) or with thyroidectomy. It is not known whether the available tests are capable of detecting more subtle changes in nervous system development. An analysis presented by Crofton (1998j) suggested that measurements of nervous system development are less sensitive than measurements of T4. Two reasons for this relationship were presented. First, the brain may be protected from perturbations in circulating concentrations of T4, as demonstrated by upregulation of deiodinases in brain tissue that compensate for very large decreases in circulating T4. The second reason, and one for concern in the context development of this model, is that currently available testing methods, particularly screening methods, may not be sufficiently sensitive. Recent data suggest that the battery is insensitive to alterations in thyroid hormones during development (Goldey, 1995a,b).

The 0.01 mg/kg-day dosage as a level for concern was also supported by thyroid histopathology in the database. Changes in colloid depletion observed on PND4 in both the 1998 neurodevelopmental study (Argus Research Laboratories, Inc., 1998a) and the newer 2001 "Effects Study" (Argus Research Laboratories, Inc. 2001) were demonstrated. The BMDL

estimated for those studies on PND4 was 0.33 mg/kg-day, but an estimate of 0.009 mg/kg-day is also obtained with a model demonstrating adequate fit to the data. The BMDL for colloid depletion in pups on GD21 was 0.12 mg/kg-day, but for female pups alone on GD21 was 0.04 mg/kg-day. The BMDL estimated for thyroid hypertrophy in weanling pups from the two-generation study (Argus Research Laboratories, Inc., 1999) was 0.06 mg/kg-day. Of notable concern to this consideration was that the BMDL estimates decreased with duration in the 90-day study. The BMDL estimates for colloid depletion were 0.28 and 0.03 mg/kg-day at the 14-day and 90-day time points in the Springborn Laboratories, Inc. (1998) study. The BMDL estimates for hypertrophy were 0.017 and 0.008 mg/kg-day at the 14-day and 90-day time points. This effect of duration was of concern as it was also evident by the observation of tumors in the F1-generation adults at 19 weeks. Both observations suggest concern that duration may recalibrate either the homeostatic interactions of the hypothalamic-pituitary-feedback system or the cellular sensitivity and demand for the thyroid hormones.

The thyroid hormone data in a number of studies also designated 0.01 mg/kg-day as a LOAEL. Levels of T4 were significantly decreased and TSH levels statistically increased at this dosage in the dams on GD21 in the same study as the significant brain morphometry measurements in the PND21 pups (Argus Research Laboratories, Inc. 2001), revealing no NOAEL for hypothyroidism in the dams. The pups in that study were also affected at 0.01 mg/kg-day. Effects on T3 occurred at GD21, PND5, and PND9 at this dosage. The 0.01 mg/kg-day dose was the LOAEL for effects on T4 and TSH at PND21 in the male pups and for TSH in both sexes at PND9 as well. This same dose (0.01 mg/kg-day) was also the LOAEL for decreases in T4 and increases in TSH at the 14-day and 90-day time points in the 90-day study (Springborn Laboratories, Inc., 1998).

The ANOVA estimates for hormone data were used to characterize this effect after serious consideration. While in clinical studies a normal range typically is defined by a control healthy population, the ANOVA approach is an equally valid approach in that a statistically significant value represents a shift in the mean for the population. The control group defines the range for the unexposed, presumably healthy population, and statistically significant differences indicate that the mean for an exposed group is outside of that normal range. Circadian fluctuations are addressed because the same fluctuations in the control population occur as in the exposed population. A small shift in the mean of a population can have significant consequences to

individuals in the tails of the distributions of those populations. Indeed, such an evaluation underlies the basis for the blood lead level used in the National Ambient Air Quality Standard (Davis and Elias, 1996) and has been noted as an important consideration for neurotoxicity (Weiss, 2000).

The notion that continuous data should be considered in the context of the specific dose-response rather than to *a prioro* categories defined outside of the data under analysis is supported in the benchmark dose literature. Murrell et al. (1998) point out that a continuous quantity measurement such as the hormone data should be scaled by the range from background response level to maximum response level (for increasing response functions). The authors go on to note that it is a biological reality that, whatever the mechanism of effect of the toxicant, there is some dose level beyond which no further change in response is seen or is theoretically feasible. In general, there is some type of limitation or saturation phenomenon that occurs at high enough doses (e.g., in the saturation of the symporter capacity, as suggested by the modeling effort in Chapter 6 and the data of Chow and Woodbury [1970] and of Meyer [1998]).

An analogy to the case of quantal data for which an effect is defined as a probability metric in which the response reaches a maximum at one, is, that for continuous measures, the extra effect can be defined as the change in effect from background standardized by the total range of response (Murrell et al., 1998). The total response range is not necessarily the response range of the observed responses in a study; rather, it is defined by a determination of the minimum and maximum possible responses according to, for example, a model equation fitted to the data as in the case of benchmark analyses. In all BMD analyses, however, the hormone BMDL estimates were shown to be extremely low (Geller, 1998a; Geller, 2001c). This may not necessarily be surprising given that hormones are operative at low doses by definition, but corresponding changes in thyroid histopathology were more consistent with the ANOVA estimates.

Finally, the NOAEL for immunotoxicity suggested by the dermal contact hypersensitivity assay at 0.02 mg/kg-day can be viewed as supportive, especially since deficiencies in this study raise concern for the characterization and because a LOAEL for the effect was demonstrated at 0.06 mg/kg-day.

7.1.4 Application of Uncertainty Factors

The types of uncertainty factors (UF) applied for various extrapolations required to arrive at a reference dose were discussed in Chapter 3. Figure 7-4 illustrates schematically that the interspecies and intraspecies UFs embody attributes of both uncertainty and variability. A factor for variability across humans typically is applied to account for potentially susceptible portions of the population. As shown in Figure 7-5 (Jarabek, 1995b), both of these factors typically are broken into components of approximately three each for pharmacokinetics (toxicokinetics) and pharmacodynamic (toxicodynamic) processes. This scheme is consistent with that used by the World Health Organization (WHO) (Jarabek, 1995b).

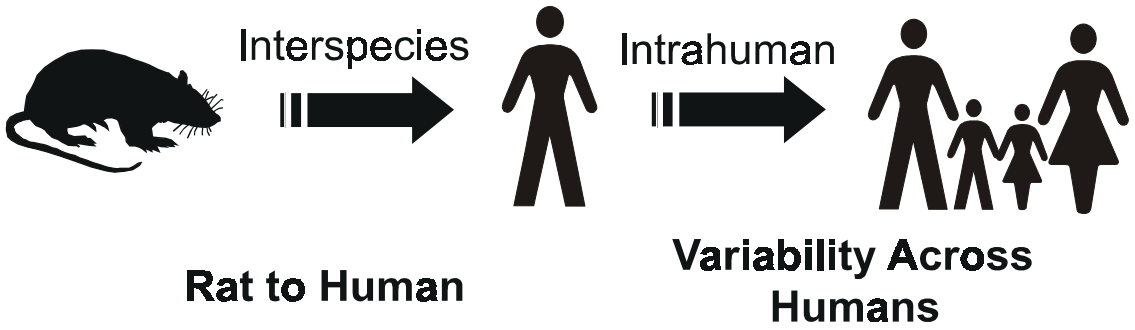

Figure 7-4. Consideration of uncertainty and variability influence interspecies and intrahuman extrapolation.

There were a total of four (4) uncertainty factors applied in this derivation, resulting in a composite factor of 300. The partial factors of 3 represent "halving" of each UF that is believed to be an upper bound on a lognormal distribution; i.e., $10^{0.5}$, so that multiplication of the various partial factors results in a composite of 100 (U.S. Environmental Protection Agency, 1994).

A 3-fold factor for intraspecies variability was retained due to the variability observed in the data and PBPK modeling for the adult humans and because these subjects do not represent kinetic data for the potentially susceptible populations of the hypothyroid or hypothyroxinemic pregnant women and their fetuses. There was also uncertainty in the parallelogram approach to extending the adult structure to address different life stages. These uncertainties might be

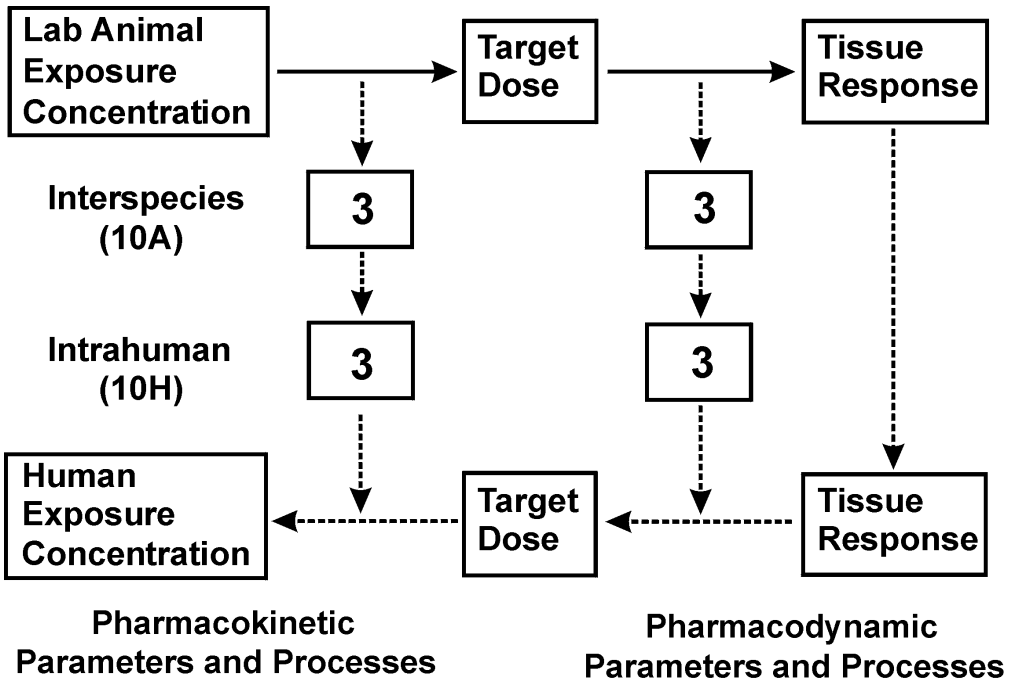

Figure 7-5. Schematic of uncertainty factor components incorporated into exposure-dose-response characterization for interspecies and intrahuman extrapolations (Jarabek, 1995b).

mitigated by further development of pregnancy and lactation models or the models might be further validated with radionucleide data using a parallelogram approach between perchlorate and iodide as described in Chapter 6. This reduced factor was a point of considerable debate, especially given the concern over the animal neurodevelopmental assays for adequately characterizing neuropsychological development deficits in susceptible populations. However, it was also discussed that the UF values are not entirely independent; e.g., aspects underlying the duration extrapolation also might underlay the intrahuman UF (Jarabek, 1995b).

The interspecies factor was omitted due to general confidence that the extrapolation based on perchlorate distribution (and on iodide inhibition by perchlorate at lower doses) was accurately characterized by the PBPK modeling effort described in Chapter 6. Concern for eliminating this factor was again considered in the context of the lack of independence with other applied UF. The concern that the HEE was not based on iodide inhibition but rather the serum

perchlorate AUC was assuaged somewhat by the correlations that demonstrated a close relationship between these two measures.

A full 10-fold factor was applied to extrapolate the LOAEL for the brain morphometry, thyroid histopathology, and hormone changes observed at the 0.01 mg/kg-day level. Designating these changes to be adverse is consistent with the proposed mode of action and existing Agency guidance and procedures. The neurotoxicity assessment guidelines (U.S. EPA, 1998a) specify changes in brain structure as adverse. The OPPTS has used thyroid hormone changes to designate effect levels. Finally, the shallow slope of the response curve at these lower levels suggested that a full factor should be applied.

A 3-fold factor for duration was applied due to the concern for the biological importance of the statistically significant increase in tumors in the F1-generation pups at 19 weeks (P2, second parental generation). The occurrence of these tumors with a dramatically reduced latency and with a significance in incidence greater than the NTP historical data (Dunson, 2001b) for thyroid tumors in this strain of rat was reason for concern. As discussed earlier, the concerns were that this observation represented the potential for *in utero* programming; and that the decrease in the NOAEL/LOAEL estimates for hormone perturbations and histopathology between the 14-day and 90-day time points represented a recalibration of the regulatory feedback system or changes in cellular sensitivity and demand for thyroid hormones with extended exposures. This factor can also be viewed as part of a data base deficiency because there are no long-term bioassays of perchlorate with contemporary design and data quality. While the original strategy aimed at determining a NOAEL for thyroid histopathology as a precursor lesion to tumors in the 90-day study, this finding in the F1-generation cannot be ignored, especially in light of an emerging appreciation of findings suggesting a phenomenon known as *in utero* imprinting with endocrine disruption (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997). Thus, *in utero* disruption of thyroid hormones in the developing fetus may predispose the developing neonate and adult to future environmental insults to the thyroid gland by making the fetus more sensitive. Weiss (2000) has noted that changes in brain functions occur throughout life and some consequences of early damage may not even emerge until advanced age. This could be exacerbated if environmental insults to the thyroid were to be continued throughout life.

The potential for perchlorate to cause immunotoxicity remains a concern so that a 3-fold factor was applied for the database insufficiency. New studies based on recommendations at the

1999 external peer review had some deficiencies and reinforced concern about the lack of an accurate characterization of this endpoint.

7.1.5 Operational Derivation of the Reference Dose

The HEE for the neonatal rat corresponding to brain morphometry and hormone changes observed in the PND21 pups (also the PND9 pups) at the 0.01 mg/kg-day dosage would be 0.02 mg/kg-day (Table 7-3). However, because the dams on GD21 were shown to be hypothyroid (with statistically-significant decreases in T4 and increases in TSH) at this same dosage, and because the temporal windows underlying the neonatal brain morphometry effects are unknown, and because the brain morphometry effects may have occurred *in utero* due to the dams' hormone deficiency, the HEE estimate for dams of 0.01 mg/kg-day was chosen as the operational derivation. It was noted that this choice was not as conservative as using the HEE for iodide inhibition in the dams (0.002 mg/kg-day), but it was viewed as more accurate given the concerns for the reliability of the thyroid estimates.

According to Dollarhide (1998), who spoke with Argus laboratory on behalf of the sponsor (PSG), the reported doses were of ammonium perchlorate and not the anion itself. Thus, an adjustment for percent of the molecular weight of the salt from ammonium (15.35%) must also be made. Further, because the analytical methods measure the anion concentration in environmental samples, this is the appropriate expression for the RfD to use while making valid comparisons for risk characterization. Thus, the derivation for an RfD for the perchlorate anion as itself is as follows:

$$0.01 \text{ mg/kg-day} \times 0.85 / 300 = 0.00003 \text{ mg/kg-day}. \qquad (7\text{-}1)$$

Note that the appropriate adjustment for any salt of perchlorate (e.g., adjustment by a factor of 0.72 for potassium perchlorate) should be made when evaluating toxicity data for similar assessment activities.

It is critically important to distinguish the proposed RfD from any guidance value that may result. An RfD would be only one step in the future regulatory process of determining, based on a variety of elements, whether a drinking water standard for perchlorate is appropriate. As with any draft EPA assessment containing a quantitative risk value, that risk estimate is also draft and

should be construed at this stage to represent Agency policy. The units for an RfD are mg/kg-day. Conversion of an RfD to a drinking water equivalent level (DWEL) is based on adjusting by body weight (kg) and drinking water consumption (L) to arrive at a level expressed in units of mg/L (ppb). Derivation of a maximum contaminant level goal (MCLG) from a DWEL by the OW typically involves the use of a relative source contribution (RSC) factor to account for non-water sources of exposures such as those discussed in Chapters 8 and 9.

Because the effect is viewed to be the result of neurodevelopmental deficits resulting from the hypothyroid or hypothyroxinemic state induced by the mother's exposure, and because developmental neurotoxicity may emerge later in the life or be exacerbated later in life, conversion factors for the adult of 70 kg body weight and 2 L of water per day are considered appropriate. Recent guidance from the OW in its Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (U.S. Environmental Protection Agency, 2000) provides a decision flow chart for derivation of the RSC and recommends 80% as a ceiling and 20% as the floor for this factor when data are adequate to estimate sources of exposure. When data are not adequate to estimate other anticipated exposures, OW recommends a default RSC of 20%. (U.S. Environmental Protection Agency, 2000: Chapter 4, Section 4.2.2.4 on apportionment decisions). EPA does not recommend that high-end intakes be assumed for every exposure source since the combination may not be representative of any actually exposed population or individual.

A hypothetical adjustment of the 0.00003 mg/kg-day RfD by 70 kg and 2 L would thereby result in a DWEL of 1 ug/L (ppb) and application of an RSC between 0.2 to 0.8 would thereby result in an MCLG in the range of 0.2 to 0.8 ug/L (ppb). These values are in the range of current analytical capabilities. As discussed in Chapter 1, improvements to the analytical methods on the near horizon or expected to be published this spring could result in minimum reporting limits in this range and lower (Yates, 2001).

Concern is often expressed in the regulatory arena for the potential added susceptibility of children in developing DWEL estimates based on different conversion factors (15 kg and 1 L). Consequently, the EPA asked for additional PBPK simulations to help inform this dialogue. As shown in Table 7-1, the HEE estimates for a 15 kg human for serum perchlorate AUC can be as great as two-fold higher than those predicted for the 70 kg human due to differences in distribution volumes and excretion. Thus, if the 15 kg and 1 L values are used to convert this 2-

fold higher HEE value in an analogous derivation to the adult RfD derivation and DWEL calculation above, an estimate of 1 ppb that is equivalent to the adult conversion results.

7.1.5.1 Comparison with Derivation Considering Human Data

It is important to evaluate this derivation in context with the evidence from the available and relevant human data. As described in Chapter 4, the EPA felt that both the observational epidemiological and the human clinical studies have significant scientific and technical limitations that preclude their use as the basis for a quantitative dose-response assessment. The clinical study subject attributes (euthyroid adults) and study design issues (sample size, RAIU time points, etc.) made these data less reliable than the laboratory animal toxicological data to ascertain effect levels for the basis of an RfD derivation. In addition, on December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. Environmental Protection Agency, 2001c). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Some of the clinical studies contained in this database fall in this category of studies not to be considered. However, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. Therefore, because of the scientific shortcomings of these studies, they will not be used as “principal studies” in the derivation of an RfD. The ethical issues surrounding the conduct of these studies or their use for regulatory purposes in light of the Agency’s interim policy will not be discussed in this document. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA’s possible use of third-party studies which intentionally dose human subjects with toxicants to identify or quantify their effects.

These issues not withstanding, a dose of 0.007 mg/kg-day has been suggested by some authors in an abstract (Greer et al., 2000) to be a NOAEL estimate. This was based on an

average 6.2 % decrease relative to baseline of RAIU measured on Day 14 of exposure to seven subjects at the 8-hour time point (unpublished data presented in Merrill, 2001a; Attachment #7). The values for RAIU ranged from a 38.6% decrease in a 34-year old female to a 27.9% increase in a 49-year old female at that dosage.

Prior to the articulation of the Agency's interim policy, the Agency had conducted a comparison of its reference dose derivation considering the results of the study described above, which falls within the category of a "third-party study" described by the authors as demonstrating a NOAEL in humans. If this study were to be considered in lieu of the laboratory animal data and PBPK modeling, the following would need to be considered. The seven subjects (six females and one male) were euthyroid and ranged in age from 18 to 49. Because this is a limited data set (small sample size), with noted variability and because of relevance to the elderly woman, cardiac risk patient, hypothyroid or hypothyroxinemic pregnant woman, or fetus as the susceptible population is difficult to ascertain, an uncertainty factor of 3-fold for this iodide uptake inhibition level as a minimal LOAEL as well as a 3-fold factor for intrahuman variability would be warranted. This is particularly relevant if this value is viewed in context with the neurodevelopmental effects in laboratory animal data. At a minimum each factor should be 3-fold, and discussion with respect to the meaning of these factors with respect to population effects again entertained. None of the human studies of perchlorate reviewed in Chapter 4 have adequately investigated neurodevelopmental outcomes. The concern for duration of exposure was at least a 3-fold factor per the above laboratory animal data discussion and should also be applied, as well as the 3-fold factor for database deficiencies because these considerations and deficiencies are not obviated by the use of human data.

Thus, a derivation based on the available human data would estimate the RfD at a maximum of 0.00007 mg/kg-day, an estimate in rather good agreement with that proposed based on the laboratory animal data (0.00003 mg/kg-day). If a larger UF were to be applied to the human data, as could be justified for the intrahuman factor, the resultant estimate would be essentially equivalent to that proposed using the laboratory animal data.

The consistency between the estimates based on the laboratory animal versus the human data is likely due, at least in apart, to the use of AFRL/HEST PBPK modeling (Merrill, 2001c,d; Clewell, 2001a,b) to perform the interspecies extrapolation rather than the use of default factors. It should be noted that the original motivation for performing these human studies (as discussed

in Chapter 3) in the perchlorate testing strategy was to support such interspecies pharmacokinetic extrapolation and not to derive NOAEL estimates for thyroid effects in the human population. In addition, as noted in Chapter 4, the EPA felt that both the observational epidemiological and the human clinical studies have significant scientific and technical limitations that precluded their use as the basis for a quantitative dose-response assessment. As mentioned previously, under the interim policy articulated on December 14, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Nonetheless, the use of both previously published and newly-derived human data by the Air Force in its modeling efforts was important. The AFRL/HEST PBPK model approach allowed EPA to confirm that humans were as sensitive as rats to the iodide uptake inhibition effects of perchlorate at the NIS, the key event for the proposed mode-of action of perchlorate on the thyroid. In addition, the PBPK models increased the accuracy of interspecies extrapolation by allowing the incorporation and integration of ADME data to describe perchlorate and iodide disposition relative to the key event. These two outcomes from the integration of human and animal data in the AFRL/HEST models provide greater confidence than would the laboratory animal data alone that the reference dose that is derived will be protective of human health.

7.1.5.2 Comparison with Derivation Based on Tumor Data

To address neoplasia as the other potential adverse endpoint, this section will discuss how an estimate could be derived based on the recently acquired tumor data.

7.1.5.2.1 Choice of Dose-Response Procedure

As discussed in Chapter 5, the genotoxicity assays included in the testing strategy determined that perchlorate was not likely to be mutagenic. This was one of the critical determinants in deciding on a dose-response approach for a cancer derivation. The EPA guidance on assessment of thyroid follicular cell tumors (U.S. Environmental Protection Agency, 1998a) sets forth data needs to establish the default dose-response procedure that should be used to establish that a chemical has antithyroid activity (i.e., that it is disrupting the thyroid-pituitary hormone status). Table 7-8 lists the default procedures for thyroid carcinogens that would be

TABLE 7-8. DEFAULT DOSE-RESPONSE PROCEDURES FOR THYROID CARCINOGENS (U.S. Environmental Protection Agency, 1998a)

	Array of Effects		
Example	Mutagenic	Antithyroid	Dose-Response Methodology
1	Either or both unknown		Linear
2	Yes	No	Linear
3	No	Yes	Margin of exposure
4	Yes	Yes	Linear and margin of exposure

used. The thyroid lesions observed (colloid depletion, hypertrophy, and hyperplasia) are among the required lesions to demonstrate antithyroid activity. Table 7-9 shows the types of data required.

TABLE 7-9. DATA DEMONSTRATING ANTITHYROID ACTIVITY (U.S. Environmental Protection Agency (1998a)

Required	Desirable
1. Increases in cellular growth	6. Lesion progression
2. Hormone changes	7. Structure-activity relationships
3. Site of action	8. Other studies
4. Dose correlations	
5. Reversibility	

What has been proposed in this assessment is the harmonization of the "noncancer" and "cancer" assessment approaches because the target tissue is the thyroid and the mode of action is the same for both the neurodevelopmental and neoplastic sequelae. The proposed RfD based on precursor lesions is analogous to a nonlinear approach and viewed as a protective for thyroid tumors.

Perchlorate has clearly demonstrated an effect in both adult, fetal, and neonatal stages in thyroid histopathology, as well as a decrease in lumen size in a dose-dependent fashion. Thyroid and pituitary hormone changes and expected correlations all have been demonstrated for T3, T4,

and TSH across an array of studies at different time points. The site of action has been established as competitive inhibition of the iodide symporter although there remains some uncertainty as to whether that is the only locus for the effect (e.g., evidence for intrathyroidal activity) because of the efflux (discharge) phenomenon. Dose-correlations in this case were not with tumors, but rather for precursor lesions (colloid depletion, hypertrophy, hyperplasia, and decreased follicular lumen size). Reversibility has been demonstrated in thyroid weight, colloid depletion, hypertrophy, hyperplasia, and thyroid and pituitary hormones in the 30-day recovery period after the 90-day study in rats and in T4 levels of the various immunotoxicity experiments in mice.

Lesion progression was difficult to determine because of dose-spacing and differences in sample size and histological methods among the studies. However, there was a progression within the 90-day study between the 14- and 90-day time points.

Analyses of other anions have fairly well established that the mode of action of perchlorate arises from it being an anion that is recognized by the NIS (see Chapter 3).

Thus, the appropriate dose-response procedure for perchlorate would be a nonlinear margin-of exposure approach based on demonstration that it is not genotoxic and that its anti-thyroid effects are consistent with a mode of action leading from inhibition of iodide uptake at the NIS through precursor lesions of perturbation of thyroid hormone economy and resultant histopathological changes in the thyroid gland.

7.1.5.2.2 Dose-response Assessment for Thyroid Neoplasia

Thyroid adenomas were statistically increased in the high dose (30 mg/kg-day) group of F1-generation animals sacrificed as adults (P2-generation) at 19 weeks in the Argus Research Laboratories, Inc. (1999) two-generation reproductive study. Both the latency and incidence of these tumors were remarkable relative to the entirety of the NTP data base for this type of tumor in this strain of rat (Dunson, 2001b). Colloid depletion, hypertrophy, and hyperplasia were all observed at dosages of 0.3 mg/kg-day and above with BMDL estimates of 0.9, 0.15, and 0.0004 mg/kg-day. This last estimate is outside the range of possible dosimetric adjustment so it will not be carried forward, but consideration of the overlap among colloid depletion, hypertrophy, and hyperplasia should be superimposed on the derivation. The HEE values for adult versus neonatal rats are comparable at these dosages. Using the adult male rat dosimetric

adjustment factor to correspond to sacrifice date results in HEE estimates of 0.45 and 0.02 for colloid depletion and hypertrophy.

Using the nonlinear approach and applying a composite factor of 100 to the HEE estimates to account as above for uncertainty in intrahuman variability, duration, and database deficiencies; and with factor for a minimal LOAEL of 3 to account for the fact that hyperplasia occurred at over an order of magnitude lower than these two thyroid histopathology estimates, results in an RfD derivation in the range of 0.005 to 0.0002 mg/kg-day. Applying a larger uncertainty factor for intrahuman variability would result in a range of 0.002 to 0.00007 mg/kg-day. Thus, the derivation based on tumor outcome data supports the mode-of-action concept and corroborates that the proposed RfD that as derived would be protective of both neurodevelopmental and neoplastic sequelae.

7.1.5.3 Possible Susceptibility

Based on the mode-of-action for perchlorate, the competitive inhibition of iodide uptake, and the subsequent perturbation of thyroid hormone homeostasis, a number of factors potentially could cause an increase in susceptibility of a population to perchlorate toxicity. As already indicated by the choice of critical effect, the fetus, and perhaps the developing child, may represent susceptible populations. However, critical data on the steady-state pharmacokinetics and placental dosimetry are lacking to definitively state whether or not there is an inherent pharmacodynamic component to the apparent sensitivity of pups versus dams in the laboratory animal models. Individuals that are iodine deficient may be another susceptible population. The elderly, especially women, and hypothyroid and hypothyroxinemic individuals or those treated with anti-thyroid drugs, may be others more susceptible than the general population to the effects of perchlorate. Patients with cardiac dysfunction or elevated levels of cholesterol may also be at increased risk.

7.1.6 Designation of Confidence Levels

Confidence in the principal study is medium. The dose level of 0.01 mg/kg-day was the lowest tested, and it was determined to be a LOAEL (not NOAEL). The small sample size for the critical effect also reduces confidence in the study. Despite the new data, the confidence in the database at this time remains medium because the sensitivity of these animal assays versus

evaluation of neuropsychological development in human population studies is not known, and because a concern for potential immunotoxicity remains. Based on confidence in the study and on the database together in setting the overall confidence in the RfD, the confidence in the RfD currently is also medium.

7.2 INHALATION REFERENCE CONCENTRATION

Derivation of an inhalation reference concentration is precluded because there are no inhalation data available with which to characterize dose-response or the portal-of-entry modulation of internal dose. However, the EPA has been questioned as to whether the potential for inhalation exposure of perchlorate from showering with contaminated water poses a health risk. Given the low vapor pressure of perchlorate, it is not likely that it would come out of solution. Further, Giardino et al. (1992) characterized shower particle droplet size as ranging from 200 to 3,000 μm. Thus, there is minimal chance for inhalation or deposition of perchlorate-laden droplets in the respiratory tract.

7.3 SUMMARY

The model based on mode of action for perchlorate served as a useful construct for the integration of a diverse set of data. Results of studies in the testing strategy confirmed that the target tissue for perchlorate is the thyroid and that the key event for its antithyroid effects is the inhibition of iodide uptake at the NIS with corresponding perturbations of thyroid hormone economy. Disturbances in thyroid hormone economy were confirmed to result in thyroid histology as diagnosed by decreases in colloid depletion or follicular lumen size and increases in hypertrophy and hyperplasia. Effects on both neurodevelopmental indices (brain morphometry and motor activity) and neoplasia that could be expected based on the mode of action were also demonstrated. Other developmental and reproductive effects were not observed to be as sensitive as the neurodevelopmental and thyroid histopathological changes. Accurate characterization of the immunotoxicity of perchlorate, notably its potential to cause contact

hypersensitivity, either secondarily to these hormone effects or possibly via a direct effect of the anion itself, remains a concern.

APPENDIX 7A

CORRELATION ANALYSES

The correlation analyses were of two types. Hormone levels are continuous, ratio-scaled values, so correlations were computed using the conventional Pearson's r statistic. Correlations between ratio-scaled hormone levels and ordinally-scaled standard histology ratings must be computed using nonparametric correlations. To compare variables from the different scales, it is simplest to recode the data by converting the variable values into rank scores. Spearman's rank order (r_s) was used to compute the correlation between the rankings of two variables. When there were ties in the ranks, as there were in this data set, each value was assigned the mean of the ranks that they would otherwise occupy. A correlation coefficient was then computed for the rankings of the variables of interest.

An alternative statistic used for comparing the data sets was Kendall's tau, best thought of as a measure of agreement or concordance between two sets of ranked data. It searches for the number of inversions in two sets of ranked data (i.e., observations are ranked according to the first variable, then reranked according to the second, and the number of interchanges that occur is used to compute the statistic). The Spearman and Kendall statistics produced nearly identical results. Statistics were computed using SAS® software (PROC RANK and PROC CORR, SAS Institute, Cary, NC). All statistics corresponding to Figures 7A-1 through 7A-7 can be found in Tables 7A-1 through 7A-6.

TABLE 7A-1. PEARSON'S r CORRELATIONS (n = 96) BETWEEN THYROID HORMONES AND TSH IN RATS OF THE CALDWELL et al. (1995) 14-DAY STUDY

	T3	T4	TSH
T3	1.00 p = 0.00	0.81 p = 0.0001	−0.65 p = 0.0001
T4		1.00 p = 0.00	−0.67 p = 0.0001
TSH			1.00 p = 0.00

TABLE 7A-2. SPEARMAN'S r_S CORRELATIONS (n = 95) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND HISTOLOGICAL SEVERITY RATING DECREASE IN FOLLICULAR LUMEN SIZE (LS) IN RATS OF THE CALDWELL et al. (1995) 14-DAY STUDY

	LS
T3	−0.74 p = 0.0001
T4	−0.70 p = 0.0001
TSH	0.79 p = 0.0001
FH	0.75 p = 0.0001

TABLE 7A-3. PEARSON'S r CORRELATIONS (n = 223) BETWEEN THYROID HORMONES AND TSH IN RATS FOR THE COMBINED 14- AND 90-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY

	T3	T4	TSH
T3	1.00 p = 0.00	0.42 p = 0.0001	−0.18 p = 0.007
T4		1.00 p = 0.00	−0.20 p = 0.0027
TSH			1.00 p = 0.00

TABLE 7A-4. PEARSON'S r CORRELATIONS (n = 104) BETWEEN THYROID HORMONES AND TSH FOR THE 14-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY

	T3	T4	TSH
T3	1.00 p = 0.00	0.36 p = 0.0001	−0.11 p = 0.27
T4		1.00 p = 0.00	0.20 p = 0.04
TSH			1.00 p = 0.00

TABLE 7A-5. PEARSON'S r CORRELATIONS (n = 119) BETWEEN THYROID HORMONES AND TSH OF THE 90-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY

	T3	T4	TSH
T3	1.00 p = 0.00	0.66 p = 0.0001	−0.40 p = 0.0001
T4		1.00 p = 0.00	−0.38 p = 0.0001
TSH			1.00 p = 0.00

TABLE 7A-6. PEARSON'S r CORRELATIONS (n = 22 to 27) BETWEEN THYROID HORMONES AND TSH FOR THE F1 RAT PUPS ON PND5 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY (Argus Research Laboratories, Inc., 1998a)

	T3	T4	TSH
T3	1.00 p = 0.00	0.87 p = 0.0001	−0.43 p = 0.03
T4		1.00 p = 0.00	−0.57 p = 0.0046
TSH			1.00 p = 0.00

In general, positive correlations were expected between T3 and T4 and between TSH and the histopathology rating. Negative correlations were expected between T4 and TSH and between T4 and histopathology.

Figure 7A-1 shows the correlations between T3 and T4 and between T4 and TSH levels from the 14-day Caldwell et al. (1995) study in rats. Robust relationships are illustrated: a positive correlation is shown between T3 and T4; whereas, the T4 and TSH varied inversely. Hormone levels also correlated highly with decrease in follicular lumen size. Figure 7A-2 shows the rank of T4 level and TSH level versus the severity rating for follicular lumen size to be highly correlated inversely. Figure 7A-3 shows the correlations for the combined 14-day and 90-day time points (male and female) from the subchronic study performed in rats (Springborn

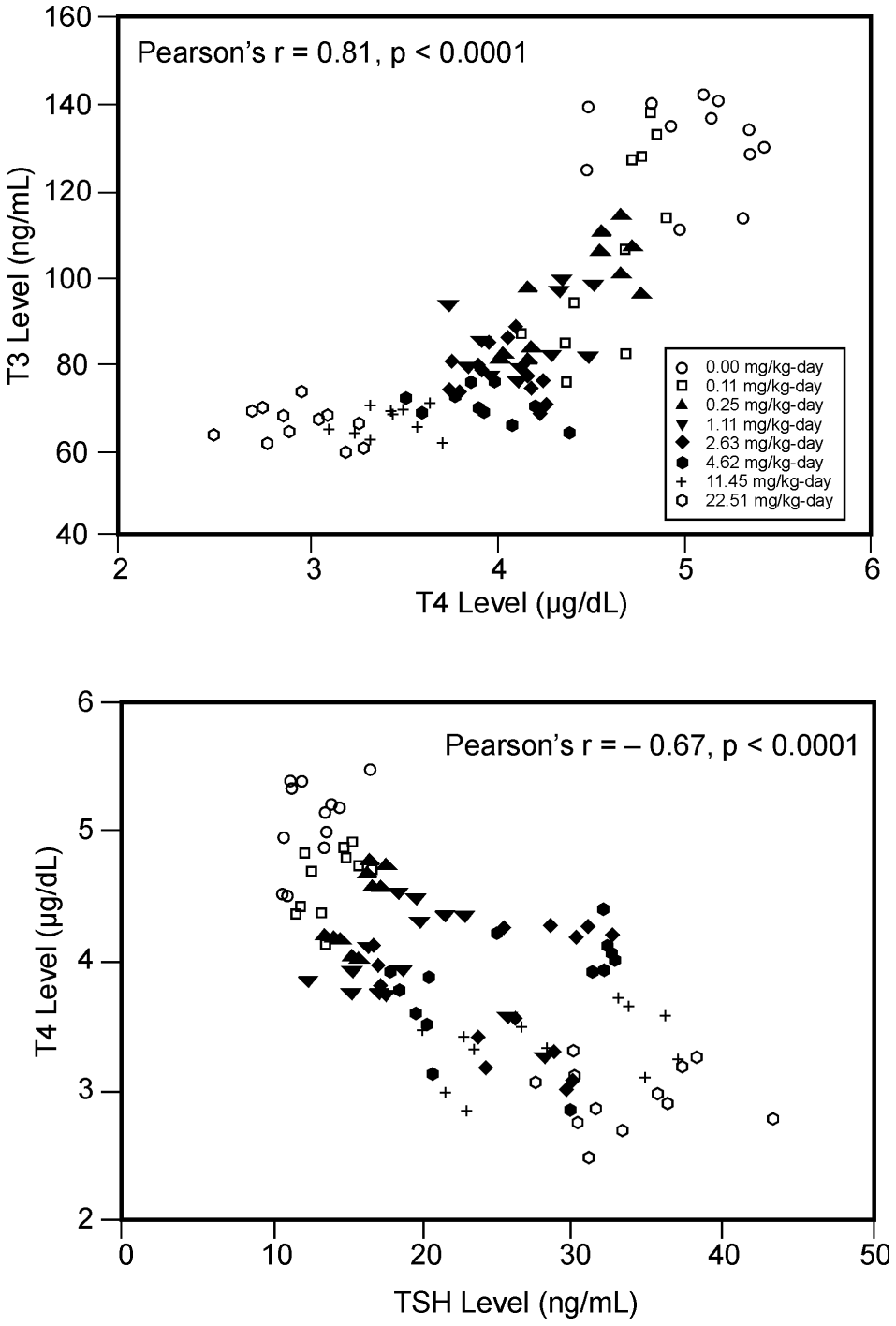

Figure 7A-1. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) in rats of the 14-day Caldwell et al. (1995) study (Geller, 1998a). Data of Channel (1998a) and Crofton (1998a).

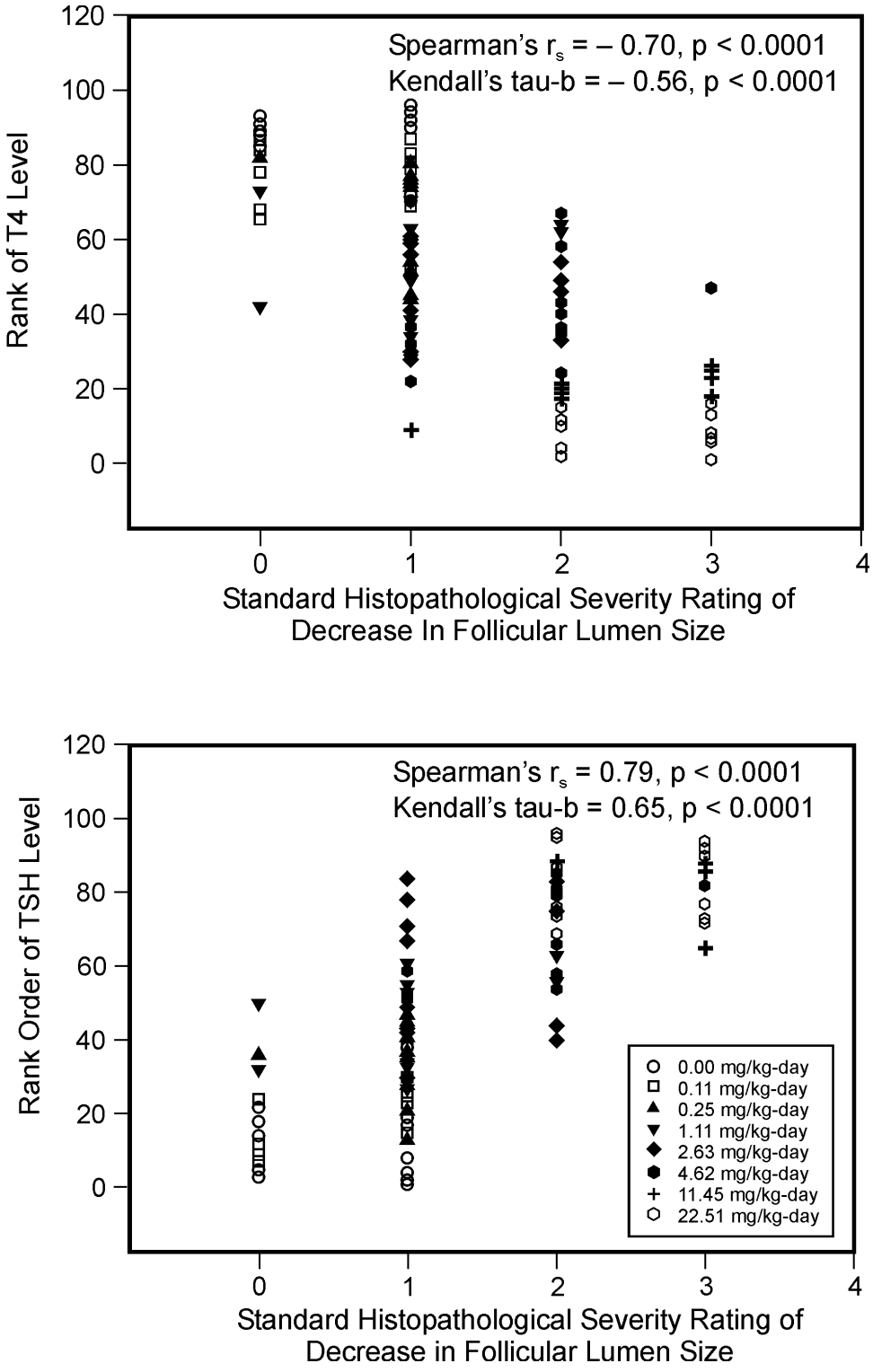

Figure 7A-2. Correlations between the rank order of T4 (top panel) and TSH (bottom panel) versus decrease in follicular lumen size in rats of the 14-day Caldwell et al. (1995) study (Geller, 1998a). Data of Channel (1998a) and Crofton (1998a).

Laboratories, Inc., 1998). As shown in Figure 7A-3 (top panel), T3 and T4 were highly significantly correlated, with low levels of T3 and T4 associated with high doses. Both T4 and TSH were significantly negatively correlated (bottom panel). After 14-days of dosing (Figure 7A-4), T3 and T4 are highly associated (top panel), but there is an unexpected positive relation between T4 and TSH (bottom panel). At the 90-day time point, there are the expected strong correlations between T3 and T4 (Figure 7A-5, top panel) and between T4 and TSH (bottom panel).

Correlations also were performed on the data from the neurodevelopmental study for the PND5 pups (Argus Research Laboratories, Inc., 1998a). T3 and T4 were strongly positively correlated, and T4 and TSH were negatively correlated (Figure 7A-6). Figure 7A-7 (top panel) shows that T4 is negatively associated with a significant decrease in lumen area. Figure 7A-7 (bottom panel) also shows that TSH is positively correlated with a decrease in lumen size.

In total, these correlations lent strong support to the mapping model proposed. Strong correlations were observed between T3 and T4 levels, T3 or T4, and TSH levels, and hormone levels and a decrease in thyroid lumen size. These relationships were most definitive in the Caldwell et al. (1995) study, in which strong correlations existed between the elements of the thyroid hormone homeostasis feedback loop and between hormone levels and severity ratings for lumen size decrease as a measure of thyroid histopathology. In the subchronic (Springborn Laboratories, Inc., 1998) study, correlations were established between hormone levels across both the 14- and 90-day dosing points and for each time point individually. At 14 days of dosing, the expected inverse relationship between T4 and TSH was not found. At the 90-day dosing point, the inverse relationships between T3 or T4 and TSH were found.

Similar relationships were observed in pups on PND5 of the developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a; York, 1998c). The T4 and TSH were significantly correlated negatively, as expected. The T3, T4, and TSH were all significantly correlated with decrease in lumen size. The correlations in the rat studies support the model that manipulations resulting in decreased levels of circulating thyroid hormone are linked to thyroid histopathological changes that are thought to result directly from elevation of TSH.

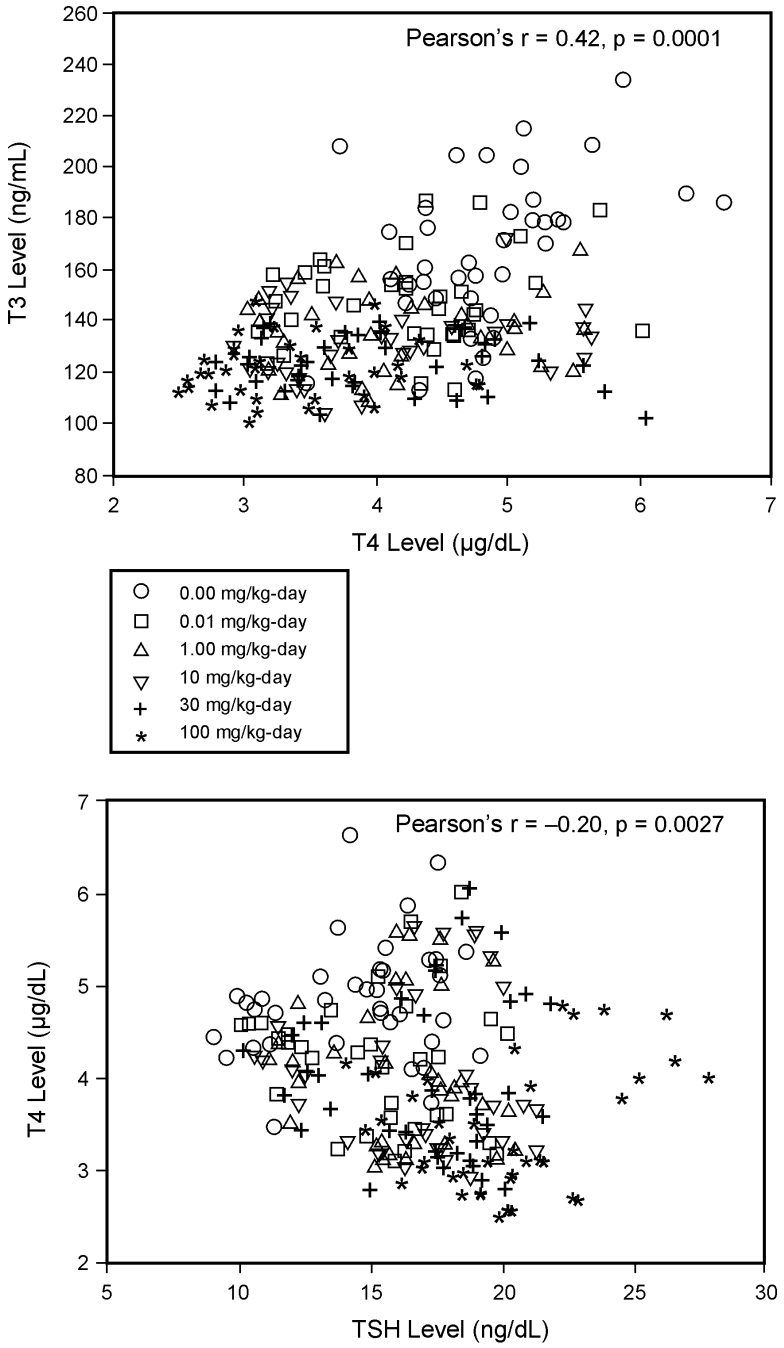

Figure 7A-3. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 14-day and 90-day time points from the Springborn Laboratories Inc. (1998) subchronic study (Geller, 1998a).

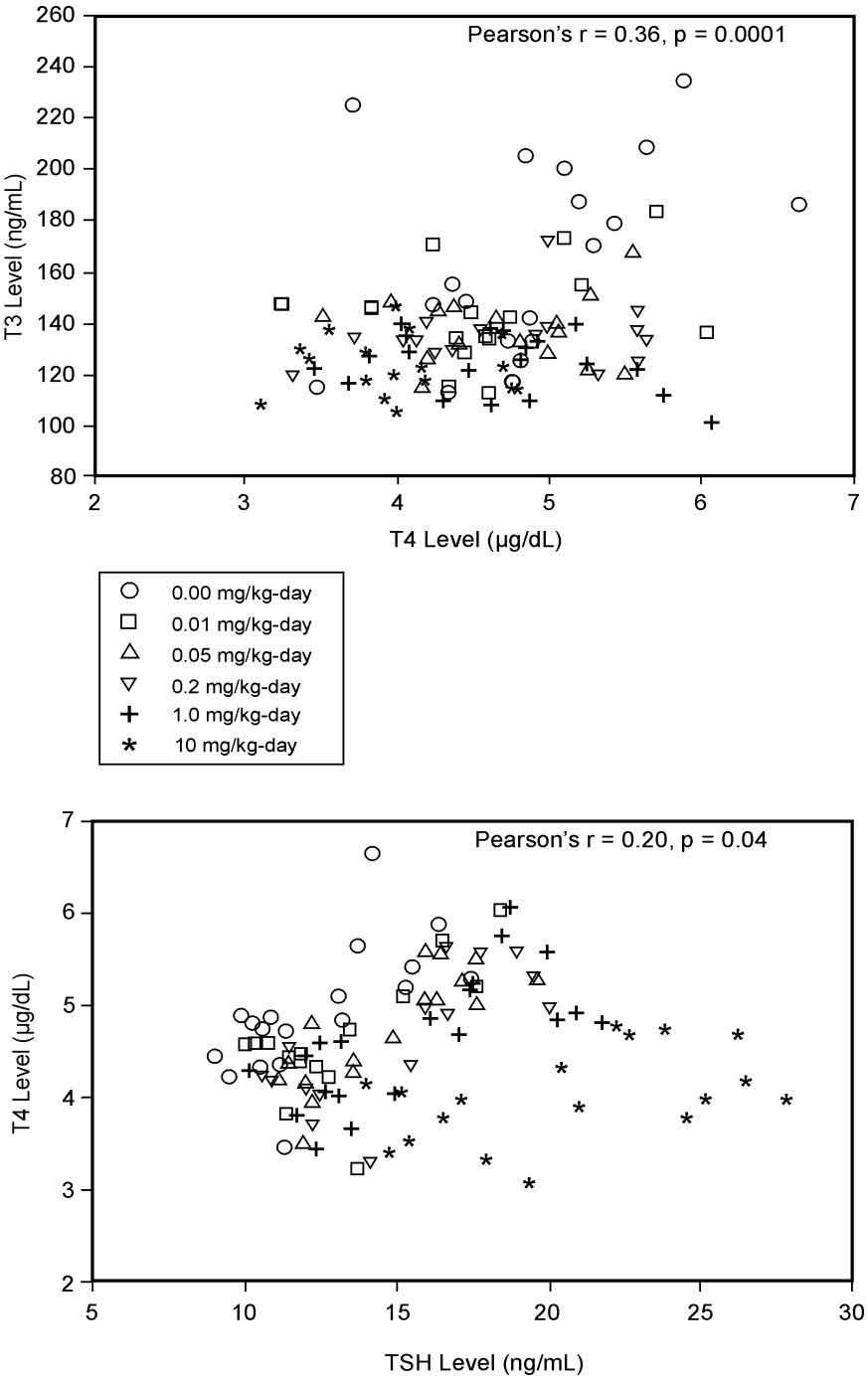

Figure 7A-4. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 14-day time point from the Springborn Laboratories Inc. (1998) subchronic study in rats (Geller, 1998b).

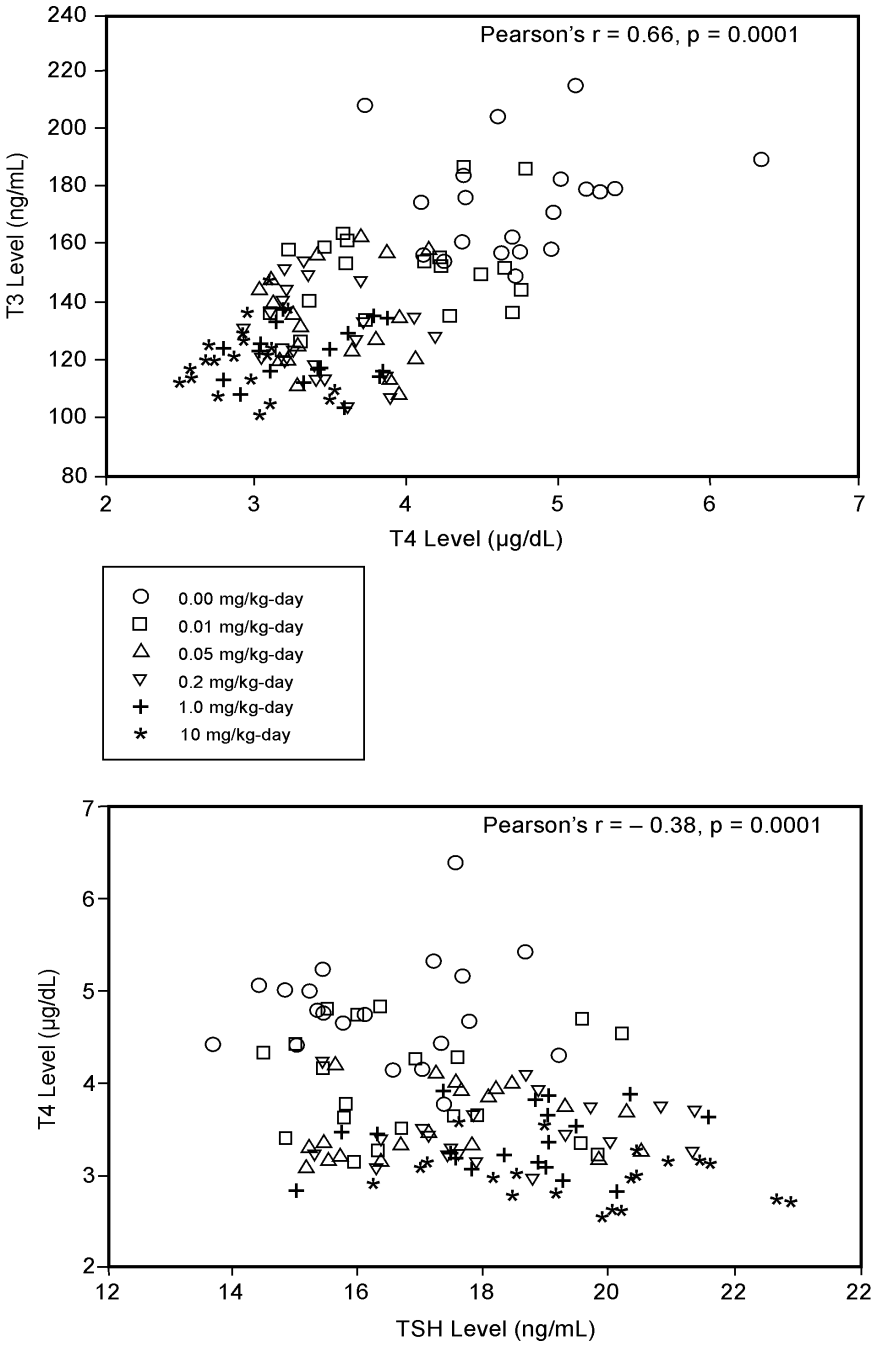

Figure 7A-5. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 90-day time point from the Springborn Laboratories Inc. (1998) subchronic study in rats (Geller, 1998b).

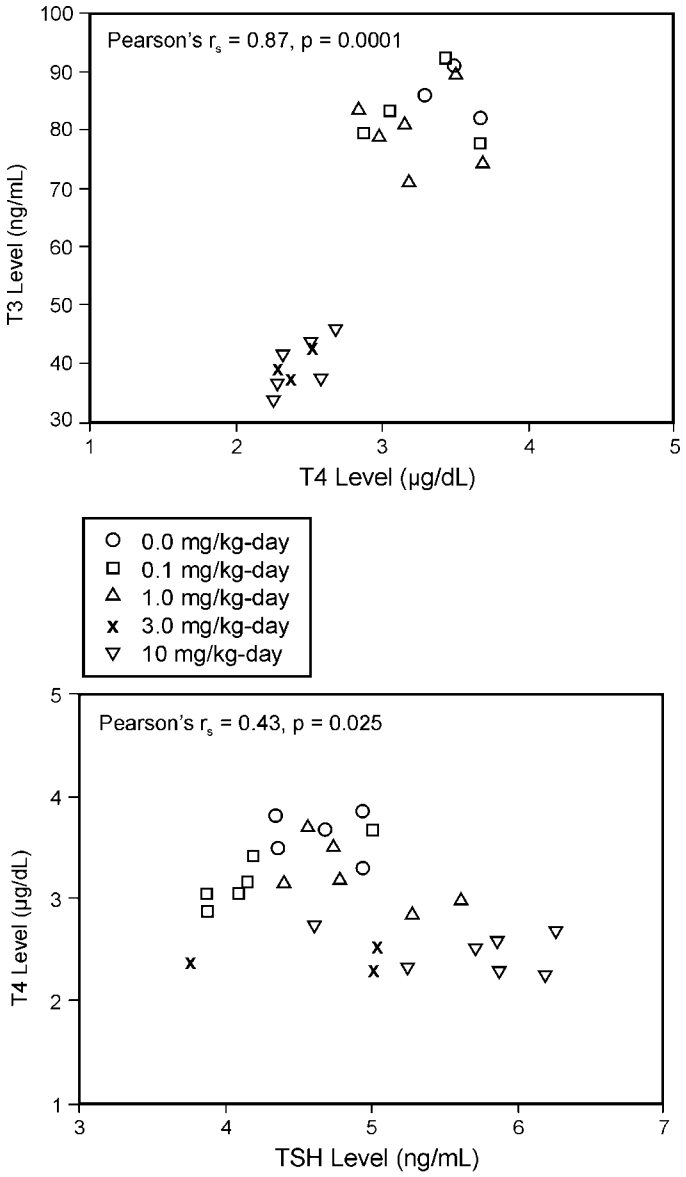

Figure 7A-6. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the F1-generation rat pups on PND5 in the developmental neurotoxicity study (Geller, 1998b). Data of Argus Research Laboratories, Inc. (1998a), York (1998c), Channel (1998c), and Crofton (1998f).

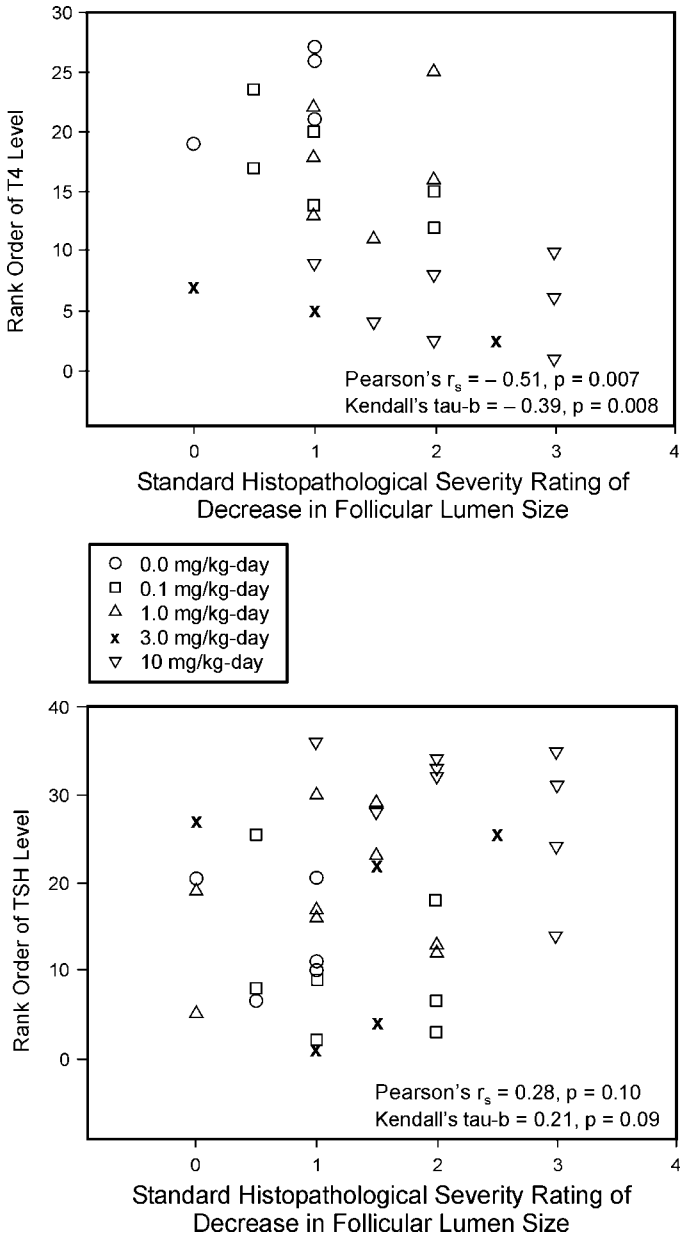

Figure 7A-7. Correlations between the rank order of T4 (top panel) and TSH (bottom panel) versus histopathology severity rating of the decrease in follicular lumen size for the postnatal day 5 (PND5) pups in the 1998 neurodevelopmental study (Geller, 1998b). Data of Argus Research Laboratories, Inc. (1998b), Channel (1998c), and Crofton (1998e, f).

Appendix 7B

Benchmark Dose Statistics for Hormone Analyses

As mentioned in Chapter 5, benchmark dose analyses were performed in addition to the ANOVA for all hormone data. Benchmark analysis of the 2001 "Effects Study" is presented in Geller (2001c). This appendix presents analyses performed on the other data sets provided in the 1998 assessment.

For the continuous hormone data, the BMD and BMDL estimates were calculated using a variety of benchmark response (BMR) values. Generally, the BMR was equal to a response 10% less than the control mean (i.e., 10% of the actual control response was subtracted from the estimate of the control value generated by the fit to the data). This is a less rigorous standard than the (control minus 5% of control) BMR that provided a close match to NOAELs in the evaluation of BMD for developmental toxicity by Kavlock et al. (1995) although this may be warranted because other endpoints (thyroid hormone and histopathology) are being evaluated. For the natural log (ln) transformed data, this means subtracting the constant 0.1053 from the control value, which is equivalent to multiplying the control value by 0.90. The BMD and BMDLs at 20 and 30% less than control and control standard deviations also are provided as a yardstick for evaluating how other clinical criteria may affect the estimates. Hormone data were fit with polynomial (linear or quadratic) or power functions (Table 7B-1).

TABLE 7B-1. CONTINUOUS FUNCTIONS USED IN BENCHMARK DOSE (BMD) MODELING

Power function	$f(dose) = control + slope * dose^{power}$
Polynomial function (includes linear and quadratic)	$f(dose) = \beta 0 + \beta 1 * dose + \beta 2 * dose^2 + ...$

Adequacy of fit for continuous data was evaluated by the statistical goodness-of-fit ($-2 \times$ log likelihood ratio) test provided by the EPA BMD program output, visual comparison,

and whether the fit was biologically plausible. The latter criterion in most cases, non-monotonicities in the function fit to the data, precluded a fit from consideration. In general, the second order quadratic fits suffered from minima or maxima between the data points from the two highest data points in a given experiment. This consideration also precluded the use of polynomials of higher than second order because these higher order polynomials generally had a local maxima or minima between data points (dose levels) and did not model the data plausibly. It should be noted that the interpretation of the test for constant variance included in the output of the version of the BMD software (version 0.96) was not reliable.

7B.1 Benchmark Dose Estimates Submitted to U.S. Environmental Protection Agency

Two sets of BMD calculations were derived from the Caldwell et al. (1995) 14-day study and submitted to the EPA (Dollarhide and Dourson, 1997). One set was calculated for TSH and T4 levels for males and females separately using the THC (polynomial fit) module of the Crump software, and the model coefficients were restricted to be nonnegative to prevent non-monotonicity. This resulted in linear fits to curvilinear data, and the fits were judged to be poor by both visual inspection and statistical goodness-of-fit criteria (Geller, 1998a).

An alternative approach to calculating BMD estimates based on additional risk also was derived using the Kodell-West algorithm (Kodell-West, 1993). This model generates a quadratic fit to the dose-response data using a maximum likelihood estimator, defines an adverse effect level based on the variability present in the data, and then calculates additional risk. The EPA recalculated these fits using Kodell's SAS® program (Geller, 1998a). The EPA estimates correspond to those previously reported, as shown in Table 7B-2 of Appendix 7B. The coefficients of the fits are provided in Table 7B-3. None of the fits to the data reached statistical significance, and all contain minima (T3 and T4) or maxima (TSH) within the dose range tested. Again, the lack of fit raises difficulties with interpretation and suggests that these estimates should not be used as the basis for risk assessment. The EPA also calculated BMD estimates on ln-transformed data because the Kodell-West algorithm assumes constant variance, and the transformed data is more likely to fit this assumption. The BMD estimates calculated with the ln transform, however, were virtually identical to those of the previous estimates.

TABLE 7B-2. BENCHMARK DOSE (BMD) ESTIMATES FOR MALE HORMONE DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY, USING KODELL-WEST ALGORITHM

Responders	BMD Associated with 1% Additional Risk (mg/kg-day)		BMD Associated with 10% Additional Risk (mg/kg-day)		BMD:N(L)OAEL 1%; 10%
TSH	EPA[a]	D&D, 1997[b]	EPA[a]	D&D, 1997[b]	1.11
k = 3	0.832	0.823	2.078	2.074	0.75; 1.87
k = 2	0.176	0.172	0.972	0.970	0.16; 0.88
ln TSH					1.11
k = 3		0.845		2.115	0.76; 1.91
k = 2		0.181		0.987	0.16; 0.89
T3	EPA[a]	D&D, 1997[b]	EPA[a]	D&D, 1997[b]	0.11[c,d]
k = 3	0.980	0.983	2.485	2.495	8.1; 22.59
k = 2	0.209	0.207	1.146	1.151	1.9; 10.42
lnT3					0.11[c,d]
k = 3		0.891		2.244	8.1; 20.4
k = 2		0.190		1.042	1.73; 9.47
T4	EPA[a]	D&D, 1997[b]	EPA[a]	D&D, 1997[b]	0.11[c,d]
k = 3	0.797	0.658	1.969	1.639	7.25; 17.9
k = 2	0.172	0.136	0.927	0.774	1.56; 8.43
ln (T4)					0.11[c,d]
k = 3		1.002		2.490	9.11; 22.64
k = 2		0.215		1.169	1.95; 10.63

[a]EPA refers to BMD estimates calculated using SAS® software received from Dr. Ralph Kodell for Kodell-West calculations (Geller, 1998a).
[b]D&D refers to BMDs included in Dollarhide and Dourson (1997).
[c]LOAEL; otherwise, value indicates NOAEL.
[d]LOAEL from combined male and female.

TABLE 7B-3. COEFFICIENTS AND GOODNESS-OF-FIT STATISTICS OF KODELL-WEST (QUADRATIC POLYNOMIAL) MODEL FITS TO MALE HORMONE DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY[a]

Responders	B0	B1	B2	Dose (mg/kg-day) of Global Max/Min	p of Fit2[b]
TSH	17.182	2.895	-0.0914	max: 15.84	<0.00001
ln TSH	2.825	0.1269	-0.004202	max: 15.11	<0.00001
T3	112.871	-8.987	0.3169	min: 14.18	<0.00001
lnT3	4.7114	-0.09702	0.0034	min: 14.27	<0.00001
T4	4.7712	-0.1791	0.00445	min: 20.11	<0.00001
ln (T4)	1.563	-0.0414	0.0009	min: 23.00	0.00012

[a]Coefficients generated by using SAS software received from Dr. Ralph Kodell (Geller, 1998a). Identical coefficients were generated by using EPA BMD software.
[b]$p > 0.05$ denotes significant fit. Goodness-of-fit derived using -2 log (likelihood ratio) test from EPA BMD software (see Geller, 1998a).

7B.2 U.S. Environmental Protection Agency Benchmark Dose Estimates for Thyroid and Pituitary Hormones

The hormone data from the Caldwell et al. (1995) subchronic (Springborn Laboratories, Inc., 1998) and rabbit developmental studies (Argus Research Laboratories, Inc., 1998c) were best fit by unrestricted power functions. The hormone data from the developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a; York, a,b,c,d,e) and mouse immunotoxicity study (Keil et al., 1998) were fit by either unrestricted power or polynomial functions. It is noted that the unrestricted power function fits generally have an extremely high slope as dose approaches zero. Tables 7B-4 through 7B-14 provide the statistics for each study.

Many of the BMDL estimates derived from these studies were lower than the NOAEL or LOAEL values derived by ANOVA, particularly those derived from power function fits. Murrell et al. (1998) suggested that this occurs when sampling statistics (i.e., small group sample sizes and few dose groups) play a large role in inflating NOAELs while depressing BMDL estimates. This may be the case for some of the data examined herein. Murrell et al. (1995) suggested that under such conditions using the BMD point estimate, rather than the lower confidence limit, would be a more accurate representation of the dose-response behavior.

TABLE 7B-4. BENCHMARK DOSE (BMD) ESTIMATES USING POWER FUNCTION FIT TO COMBINED MALE AND FEMALE HORMONE DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY
(Benchmark response based on 10% change from control value.)

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR: 10% control SD
TSH[a]	0.272	0.014	0.0002	0.44	0.032	4.55e-4	1.29 1.88
ln TSH[a]	0.099	0.017	0.002	0.44	0.039	4.55e-3	−0.1053
Female TSH[b]	0.077	0.19	0.032	0.1	1.90	0.32	1.125 0.48
Female ln(TSH)[a]	0.50	0.078	0.035	0.1	0.78	0.35	−0.1053
Male TSH	No significant fits to male TSH or male ln(TSH) data						
T3[a]	0.107	0.00035	0.00	0.1[c]	0.0035	NA	13.07 10.21
lnT3[a]	0.091	0.0004	2e-6	0.1[c]	0.004	2.00e-5	−0.1053
T4[a]	0.303	0.243	0.096	0.1[c]	2.43	0.96[c]	0.506 0.321
ln (T4)[d]	0.172	0.340	0.0997	0.1[c]	3.40	1.00[c]	−0.1053

[a]Unrestricted quadratic: fit nonmonotonic, not significant. Restricted polynomial (linear): fit not significant.
[b]Unrestricted quadratic: fit monotonic but not significant. Restricted polynomial (linear): fit not significant.
[c]LOAEL; otherwise, value is NOAEL.
[d]Unrestricted quadratic: fit not significant, global minimum at approximate high dose. Restricted polynomial (linear): fit not significant.

The BMD estimates calculated with a benchmark response of 10% less than control on the TSH hormone dose-response data are spread over 2.5 orders of magnitude, a similar range to that seen in the distribution of NOAELs calculated for TSH. The BMDL estimates are distributed more widely, over 5 orders of magnitude. These reflect the steepness of the confidence limits on the slope at low doses.

The T3 BMD estimates are spread over approximately two orders of magnitude, similar to the variability seen across studies in the LOAEL and NOAEL estimates. The T3 BMD estimates are 100-fold lower than the NOAEL/LOAEL estimates, however. A BMDL could be calculated

TABLE 7B-5. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING POWER FUNCTION FIT TO COMBINED MALE AND FEMALE HORMONE DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY (Benchmark response based on 10, 20, and 40% changes from control value.)

Endpoint	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
TSH	0.272	0.014 0.0002	0.083 0.0038	0.507 0.0604	12.861	0.44
ln(TSH)[a]	0.099	0.002	0.043	1.11		0.44
T3	0.0108	0.00035 0.00	0.0338 0.000036	3.27 0.042[c]	130.69	0.10[b]
ln(T3)[a]	0.091	0.000002	0.000642	0.478		0.10[b]
T4	0.303	0.243 0.096	2.28 1.299	21.44 16.78	5.06	0.10[b]
ln(T4)[a]	0.172	0.100	1.213	16.89		0.10[b]

[a]For ln-transformed data, only BMDL estimates are displayed.
[b]LOAEL, not NOAEL.
[c]BMDL calculation failed at some values. This means BMDL value may not be accurate.

for only one of the data sets, and this value was approximately 10,000 times lower than the LOAEL. The BMD estimates comprising the 25th to 75th percentiles for T4 cover the same 2.5 orders of magnitude as those covered by the NOAEL and LOAEL estimates for T4. The BMDL estimates for this same percentile range are distributed a little more widely, but do include the range of T4 NOAEL and LOAEL estimates.

7B.3 Summary of U.S. Environmental Protection Agency Benchmark Dose Analyses

The BMD analyses of previously reported estimates for the hormone data of Caldwell et al., (1995)14-day study in rats (Dollarhide and Dourson, 1997) were shown to be based on inadequate model fits. The EPA was able to successfully model the hormone data. However, these estimates raised a number of issues with respect to approaches for these types of data. An alternative may be to pursue a model form of the Hill equation which recently has been used for endocrine disruption data (Barton et al., 1998).

TABLE 7B-6. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 14-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY (Benchmark response based on 10% change from control value.)

Endpoint	Model	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMDL: N(L)OAEL	BMD: N(L)OAEL	BMR: 10% control SD
TSH	Power	0.45	0.037	0.000075	0.01	0.0075	3.7	1.26 2.52
	Quadratic	0.069	Fit significant, but not monotonic		0.01			
ln TSH	Power	0.43	0.043	Could not calculate	0.01	NA	4.3	−0.1053
	Quadratic	Fit not significant, nonmonotonic			0.01			
T3	Power	0.41	0.000033	Lower limit includes 0	0.01[a]	NA	0.0033	16.65 38.51
	Quadratic	Fit not significant, nonmonotonic			0.01[a]			
lnT3	Power	0.35	0.000168	Lower limit includes 0	0.01[a]	NA	0.0168	−0.1053
	Quadratic	Fit not significant, nonmonotonic			0.01[a]			
T4	Power	0.203	1.16	0.0035	1.0	0.0035	1.16	0.506 0.603
	Quadratic[b]	0.12	3.27	1.09	1.0	1.09	3.27	
ln (T4)	Power	0.22	1.64	0.04	1.0	0.04	1.64	−0.1053
	Quadratic[b]	0.16	3.25	1.06	1.0	1.06	3.25	

[a]LOAEL; otherwise, value is NOAEL.
[b]Global minimum of quadratic function is at dose ≈9.50 mg/kg-day.

TABLE 7B-7. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 14-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY
(Benchmark response based on 10, 20, and 40% changes from control value.)

Endpoint	Model	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	Power	0.203	1.16 0.0035	12.73 1.21	138.94 38.33	5.066	1.0
ln(T4)	Power	0.22	0.037	3.899	36.48		1.0
T3	Power	0.41	0.000033 —	0.207 —	129.39 0.129[a]	166.5	0.01[b]
ln(T3)	Power	0.35	Lower limit includes 0	0.000054[a]	43.16[a]		0.01[b]
TSH	Power	0.45	0.037 0.000076	0.326 0.005	2.89 0.36	12.616	0.01
ln(TSH)	Power	0.43	0.0015	0.098	6.587		0.01

[a]BMDL calculation failed at a number of values. This means BMDL value may not be accurate.
[b]LOAEL, not NOAEL.

TABLE 7B-8. BENCHMARK DOSE (BMD) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 90-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY
(Benchmark response based on 10% change from control value.)

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR: 10% Control SD
TSH[a]	0.42	0.269	0.018	0.05	5.38	0.36	1.633 1.464
ln TSH[a]	0.40	0.492	0.0796	0.05	9.84	1.6	−0.1053
T3[a]	0.01	No fit	No fit	0.01[b]	NA	NA	17.50 18.924
lnT3[a]	0.01	No fit	No fit	0.01[b]	NA	NA	NA
T4[a]	0.14	6e-6	Lower limit includes 0	0.01[b]	6e-4	NA	0.475 0.576
ln (T4)[a]	0.17	1.10e-5	0.00	0.01[b]	1.1e-3	∞	−0.1053

[a]Unrestricted quadratic: fit nonmonotonic, not significant. Restricted polynomial (linear): fit not significant.
[b]LOAEL; otherwise, value is NOAEL.

TABLE 7B-9. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 90-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY
(Benchmark response based on 10, 20, and 40% changes from control value.)

	Model	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	Power	0.14	0.000006 —	0.01 0.000001	15.09 0.52[a]	4.75	0.01[b]
ln(T4)	Power	0.165	0.00	0.004	4.87		0.01[b]
T3	Power	0.01		No significant fit		174.96	0.01[b]
ln(T3)	Power	0.01		No significant fit			0.01[b]
TSH	Power	0.43	0.272 0.019	8.808 2.404	285.52 73.80	16.33	0.05
ln(TSH)	Power	0.40	0.082	7.94	405.14		0.05

[a]BMDL calculation failed at a number of values. This means BMDL value may not be accurate.
[b]LOAEL not NOAEL.

TABLE 7B-10. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR HORMONE AND THYROID MORPHOMETRY DATA OF F1-GENERATION PUPS AT PND5 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY
(Argus Research Laboratories, Inc., 1998a, and Channel, 1998c)[a]
(Benchmark response based on 10% change from control value.)

Endpoint	Model	p of Fit	BMD	BMDL	NOAEL or LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR: 10% Control SD
TSH	Linear	0.50	4.64	3.77	3.0	1.55	1.26	0.45 0.465
	Power	0.31	4.48	1.43	3.0	1.49	0.48	
ln TSH	Linear	0.48	5.51	4.43	3.0	1.84	0.54	−0.1054
	Power	0.30	5.03	2.11	3.0	1.68	0.70	
T3	Neither linear, quadratic, or power FCNS fit data	<0.00001 for all	No fit	No fit	0.1	NA	NA	
lnT3	Neither linear, quadratic, or power FCNS fit data	<0.00001 for all	No fit	No fit	0.1	NA	NA	
T4	Nonmonotonic quadratic significant fit	0.50 min = 7.45 mg/kg	*1.26*	*0.98*	*1.0*	*1.26*	*0.98*	0.341 0.370
ln (T4)	Nonmonotonic quadratic significant fit	0.50 min = 7.14 mg/kg	*1.18*	*0.92*	*1.0*	*1.18*	*0.92*	
Morphometry	Control-10% Control (=31.78); SD = 0.37 Nonmonotonic quadratic significant fit Power FCN BMDL interval includes 0.00	0.19 global min = 6.81 mg/kg	*1.053*	*0.644*	*1.00*	*1.053*	*0.644*	
ln (morph)	Control-10% Control (= 0.341); SD = 0.37 Nonmonotonic quadratic significant fit Power FCN BMDL computational failures	0.19 global min = 7.01 mg/kg	*0.822*	*0.538*	*1.00*	*0.822*	*0.538*	

[a]Italics denote estimates derived from nonmonotonic fits to data. FCN = function and SD = standard deviation.

TABLE 7B-11. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR HORMONE DATA OF F1-GENERATION PUPS AT PND5 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY (Argus Research Laboratories, Inc., 1998a, and Channel, 1998c) (Benchmark response based on 10, 20, and 40% changes from control value.)

	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	0.50[a]	1.26[a] 0.973[a]	2.89[a] 2.16[a]	BMD set to[a] 1,000[a]	3.41	1.0
ln(T4)	0.50[a]	0.92[a]	NC[a]	NC[a]		1.0
T3	<0.00001	NC	NC	NC	87.97	0.1
ln(T3)	<0.00001		NC	NC		0.1
TSH	0.50	4.64 3.77	9.30 7.55	18.61 15.10	4.51	3.0
ln(TSH)	0.48	NC	NC	NC		3.0

[a]Underlined values from nonmonotonic fits to data. (NC = not computed.) The BMDL calculation failed at a number of values. This means BMDL value may not be accurate.

TABLE 7B-12. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING THE LINEAR MODEL FIT TO THE MOTOR ACTIVITY DATA OF F1-GENERATION PUPS AT PND14 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY (Argus Research Laboratories, Inc., 1998a) (Benchmark response based on 10% change from control value.)

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR: 10% control SD
Movement[a]	0.72	1.94	1.04	None	NA	NA	24.45 162.75
Time[b]	0.69	1.33	0.66	None	NA	NA	18.60 184.78

[a]Number of movements.
[b]Time spent in activity.

TABLE 7B-13. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING THE POWER MODEL FIT TO THE HORMONE DATA OF FEMALE RABBITS ON GESTATION DAY 29 IN THE DEVELOPMENTAL STUDY (Argus Research Laboratories, Inc., 1998c) (Benchmark response based on 10% change from control value.)

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR
TSH, ln TSH						NA	No effect of dose
T3, ln T3						NA	No effect of dose
T4	0.06	0.54	Lower limit includes 0	0.1	5.4	NA	0.187
ln (T4)	0.0503	1.69	0.002	0.1	16.9	0.02	0.1053

TABLE 7B-14. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING THE POWER MODEL FIT TO THE HORMONE DATA OF FEMALE RABBITS ON GESTATION DAY 29 IN THE DEVELOPMENTAL STUDY (Argus Research Laboratories, Inc., 1998c) (Benchmark response based on 10, 20, and 40% changes from control value.)

	p of Fit	(10%)	(20%)	(40%)	Mean	NOAEL
T4	0.06	0.54 —	7.05 —	91.76 0.63	1.874	0.1
ln(T4)	0.05	1.69 0.0018	10.97 0.033	86.19 7.278		0.1
T3						No effect
ln(T3)						No effect
TSH						No effect
ln(TSH)						No effect

8. SCREENING ECOLOGICAL RISK ASSESSMENT FOR PERCHLORATE

8.1 INTRODUCTION

As discussed in Section 1.1, perchlorate salts including ammonium, potassium, sodium, and magnesium perchlorate, are manufactured as oxidizer components for propellants and explosives. The manufacture or use of perchlorate salts has been reported in most of the states of the continental United States (Figure 1-3). In some areas involved with the manufacture, use, or disposal of perchlorate salts, perchlorate, as the anion dissociated from these salts, has contaminated soils or ground or surface waters (Figure 1-4). These releases of perchlorate into the environment have been confirmed to have occurred in 20 states, clustered primarily in the southwestern United States where most sampling has occurred (Figures 1-3 and 1-4). Currently, there is a research need to determine whether perchlorate ion is causing any potential effects on ecosystems or ecosystem components. This chapter presents a screening-level ecological risk assessment of environmental contamination by perchlorate. In organization, it follows the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

8.1.1 Management Goals and Decisions

The discovery that perchlorate release in some sites has contaminated ground and surface waters in certain locations has raised public and regulatory agency concerns. Much concern has focused on potential public exposures through drinking water and on the possible needs to improve analytical and treatment methods and to develop drinking water regulations (Section 1.4). Consequently, an extensive scientific assessment effort is underway to address those concerns (Section 1.5). A balanced approach requires assessing ecological effects as well. The goal of this screening-level ecological risk assessment is, therefore, to indicate the likelihood that adverse ecological effects (i.e., toxicity to specific organisms or effects on aquatic or terrestrial ecosystems) will result from observed levels of environmental contamination by perchlorate. The results of this assessment may be used to address the following questions:

- Are ecological risks best characterized as *de minimis* (exposures clearly are below levels of concern), *de manifestis* (risks are clearly significant and require management action to reduce exposures); or somewhere in between and requiring further characterization?
- Are analytical detection methods for determining levels of perchlorate in the environment sufficient, or is it likely that adverse ecological effects occur at levels below current detection limits?
- Is the available ecotoxicological information on perchlorate sufficient, or are additional studies needed?

8.1.2 Scope, Complexity, and Focus

In the previous ERD version of this document (U.S. EPA, 1998d), the available information for this ecological risk assessment was characterized as "very limited" and the assessment was characterized as "screening-level." Information about the environmental levels of perchlorate to which organisms were exposed and about its effects on diverse taxonomic groups was practically nonexistent. Since then, additional information has become available that improves the database in some respects. Most significantly, additional data are available on effect levels in aquatic animals, an aquatic plant, a terrestrial plant, and a soil invertebrate; some of these data are for chronic exposures. Effect levels in rodents have been reevaluated as part of the human health risk assessment for perchlorate, and the ecological implications of those changes are reflected herein. In addition, surveys have been conducted at several sites of known or suspected perchlorate contamination, and environmental and biological materials have been analyzed for perchlorate. Nonetheless, the level of knowledge on this issue must still be characterized as limited because the number of species tested is still quite minimal, and the site surveys focused only on the range of exposures at those sites. This ecological risk assessment is therefore still a screening-level, rather than definitive, assessment. The materials used in the 1998 ERD and those that are new to this present draft, are described in this section.

Interagency Perchlorate Steering Committee Report. Perchlorate Ecological Risk Studies is a report of the IPSC's Ecological Risk/Transport and Transformation Subcommittee, dated November 13, 1998 (Interagency Perchlorate Steering Committee, 1998). This report presents a literature review on perchlorate toxicity to nonmammalian organisms, recognizing that few published studies exist, and a rationale for the selection of a battery of ecotoxicology tests

conducted for the USAF Armstrong Laboratory by EA Engineering, Science and Technology, Inc. It then summarizes those test results, discusses the findings in the context of observed exposures, discusses uncertainties, and makes recommendations for further study. The present report constitutes a reevaluation of much of the same information from EPA's perspective, except that EPA did not examine the open literature studies reviewed by the IPSC subcommittee.

Test Battery Reports. The EA Engineering, Science and Technology, Inc. (1998) final report, *Results of Acute and Chronic Toxicity Testing with Sodium Perchlorate*, dated November 1998, details the test methods and results of the ecotoxicology battery. A follow-up report (EA Engineering, Science and Technology, Inc., 2000) details the test methods and results from additional chronic toxicity testing with the freshwater amphipod *Hyalella azteca* and the fathead minnow *Pimephales promelas*.

Block Environmental Services, Inc., Report. The report, *LC_{50} Aquatic Toxicity Test Results for Ammonium Perchlorate—A Two-Species Chronic Definitive Bioassay* (Block Environmental Services, Inc., 1998) presents additional bioassay results that were not included in the IPSC report.

Algal Toxicity Testing. The EA Engineering, Science and Technology, Inc. (1999) final report, *Results of Algal Toxicity Testing with Sodium Perchlorate*, dated September 1999, details the test methods and results of the ecotoxicological testing with the algae, *Selenastrum capricornutum*.

Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) Study. The report, FETAX Analysis of Ammonium Perchlorate (Dumont and Bantle, 1998), prepared by the Department of Zoology, Oklahoma State University, and dated May 22, 1998, presents results of the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) conducted with ammonium perchlorate. Recent data received by the EPA that the Agency has not yet fully reviewed indicate effects on thyroid function, metamorphosis and sex ratio in developing *Xenopus laevis* (Goleman et al., 2002). These data are made available with this document to the external peers for their review.

Phytotransformation Study. Two sets of studies report on the accumulation and potential degradation of perchlorate by plants. The study, *Laboratory Characterization of Phyto-transformation Products of Perchloroethylene (PCE), Trichloroethylene (TCE) and Perchlorate* (Nzengung, n.d.; Nzengung et al., 1999), examined perchlorate distribution and degradation in experimental systems containing sand, aqueous perchlorate solution, and rooted

cuttings of woody plant species. This study also examined systems containing chopped leaves or microbial mats and aqueous perchlorate solution. A second study, *Potential Species for Phytoremediation of Perchlorate* (Susarla et al., 1999a; Susarla et al., 2000a), reported perchlorate depletion from test media over a ten day period by 13 vascular plant species and their potential for phytoremediation of perchlorate contaminated sites.

Biotransport Investigation Studies. These studies assess the potential for bioaccumulation of perchlorate in food webs by answering the question of whether perchlorate is present in biological receptors. The report *Scientific and Technical Report for Perchlorate Biotransport Investigation: A Study of Perchlorate Occurrence in Selected Ecosystems* (Parsons, 2001) examined perchlorate concentrations in site media and in various ecological receptors at six sites with known or suspected perchlorate contamination: (1) sites associated with withdraw of irrigation water from the Colorado River in the vicinity of Yuma, Arizona; (2) Las Vegas Wash and Lake Mead near Las Vegas, Nevada; (3) Allegany Ballistics Laboratory, Rocket Center, West Virginia; (4) Holloman Air Force Base in Otero County, New Mexico; (5) Naval Surface Warfare Center, Indian Head, Maryland; and (6) Longhorn Army Ammunition Plant, Karnack, Texas. Additional data are available for one of these sites, Longhorn Army Ammunition Plant (LHAAP), Texas, in a paper published by Smith et al. (2001). In both studies, ion chromatography with an AS-16 analytical column was used to measure for perchlorate concentrations. Analyses with this analytical column have been shown to be superior than other columns for detecting and quantifying perchlorate (Ellington and Evans, 2000; Susarla et al., 2000b).

All these sites, except for those in the vicinity of Yuma, are associated with localized contamination related to the manufacture, handling, or use of perchlorate in solid propellants. The Yuma sites are approximately 250 miles downstream along the Colorado River from the Las Vegas Wash and Lake Mead sites; the report suggests that there is no localized source of the perchlorate; therefore, the most likely potential source of any perchlorate contamination in these soils is believed to be Colorado River irrigation water. However, portions of the Yuma Proving Grounds are drained by washes that pass near some of the agricultural locations sampled, and the information provided in the report was not sufficient for ruling out the possibility of contamination from the Yuma Proving Grounds.

8.2 PROBLEM FORMULATION

The characteristics of perchlorate and its sources are described earlier in this document (Chapters 1 and 2). Because this assessment is site independent, this problem formulation focuses on the selection of assessment endpoints, derivation of the conceptual model, and the analysis plan.

8.2.1 Assessment Endpoints

In ecological risk assessment, assessment endpoints are operational definitions of the environmental values to be protected. They are chosen based on policy goals and societal values, their ecological relevance, and their susceptibility to the stressor and are defined in terms of an entity and a property of that entity. The assessment endpoints for this ecological risk assessment are described in the following five subsections.

8.2.1.1 Fish Community Richness and Productivity

Fish communities are valued societally and are ecologically important. The productivity of these communities is important in terms of the support of fisheries. Species richness is important in terms of maintaining biodiversity. This importance is reflected by the use of species sensitivity distributions in the derivation of national ambient water quality criteria and the use of fish species richness as an important component of bioassessment procedures for enforcement of the Clean Water Act.

8.2.1.2 Aquatic Invertebrate Community Richness and Productivity

Aquatic invertebrate communities have little direct societal value but are important to energy and nutrient dynamics in aquatic ecosystems. The productivity of these communities is important in terms of trophic support of fisheries, of other groups of aquatic species, and of some terrestrial insectivores. Species richness is important in terms of maintaining biodiversity. This importance is reflected by the use of species sensitivity distributions in the derivation of national ambient water quality criteria and the use of invertebrate species richness as an important component of bioassessment procedures for enforcement of the Clean Water Act.

8.2.1.3 Aquatic Plant Richness and Productivity

Algae and other aquatic plants have little direct societal value but are important to energy and nutrient dynamics in aquatic ecosystems. Species richness is important in terms of maintaining biodiversity. Because of their importance to the trophic support of fisheries and other aquatic consumers, productivity is an important endpoint for this assemblage.

8.2.1.4 Soil Invertebrate Community Richness and Productivity

Soil invertebrate communities have little direct societal value, but, in nearly all terrestrial ecosystems, they are important to energy and nutrient dynamics and to maintenance of soil structure. The productivity of these communities is also important in terms of trophic support of some terrestrial insectivores. Species richness is important in terms of the policy of maintaining biodiversity.

8.2.1.5 Terrestrial Plant Richness and Productivity

Terrestrial plants are valued highly by society for production of food, fiber, and timber, as well as their aesthetic value. The primary valued property of terrestrial plants is their productivity. As autotrophs, plants are the basis of energy and nutrient dynamics in most terrestrial or aquatic food webs. Moreover, species richness is important in terms of the policy of maintaining biodiversity.

8.2.1.6 Population Productivity of Herbivorous Wildlife

Herbivorous wildlife are included as an endpoint entity because of the apparent bioconcentration of perchlorate in plant foliage. The meadow vole (*Microtus pennsylvanicus*) is used as a representative species for this group. Population productivity is used as the endpoint property because growth and reproduction are generally sensitive properties and because herbivores are valued for their production of food for human and nonhuman carnivores.

8.2.2 Conceptual Models

The conceptual model describes the relationships between sources of perchlorate and the endpoint receptors (Figure 8-1). Sources include spills during the flushing of rockets; the combustion of rocket fuel; the improper disposal of rocket fuel, open burn or open detonation

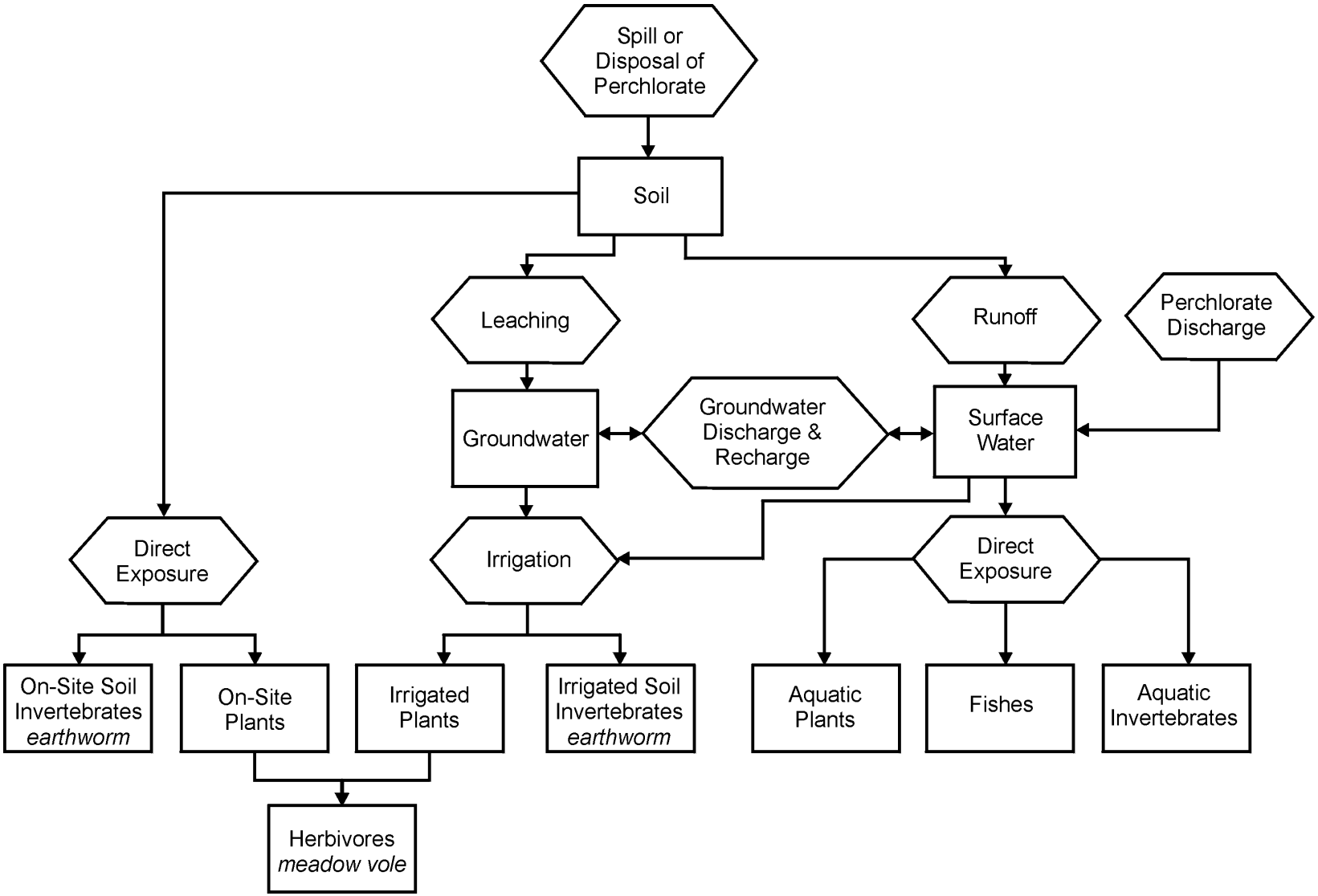

Figure 8-1. A conceptual model of exposure of ecological endpoint receptors to perchlorate. Specific endpoint taxa are identified in italics; all other endpoints are defined at the community level. Processes are designated by hexagonal boxes, compartments by rectangular boxes.

operations, explosives, or manufacturing wastes; and the aqueous discharge of waste water from manufacturing of perchlorate. The most recent information on perchlorate content in fertilizers demonstrates that fertilizer use is unlikely to constitute an environmentally significant source of perchlorate contamination, and ecological risks from this source are not considered further (see Chapter 9). Spills contaminate the soil at the site and, through leaching and run-off, contaminate the surface water and groundwater. The discharge of groundwater to surface water may result in locally high levels of perchlorate in surface waters. Aquatic communities are exposed directly to contaminated surface water; soil invertebrate and plant communities are exposed to perchlorate in soil at the spill site and through irrigation with either surface or groundwater; and herbivorous terrestrial wildlife are exposed through their consumption of plants that have bioconcentrated perchlorate. However, the potential for transfer of perchlorate further up the terrestrial food web is currently unknown.

This conceptual model is relatively simple because it excludes some potential routes and receptors. Dietary exposures are excluded from aquatic systems because, as of this writing, available data have not shown perchlorate to bioconcentrate to any significant extent. Information newly received form the U.S. Army Corps of Engineers (Condike, 2001) report on the analysis of environmental samples from perchlorate-contaminated water bodies near McGregor Naval Weapons Industrial Reserve Plant (NWIRP), TX, and purports to show fish tissue concentrations that exceed comparable water concentrations. These data suggest that perchlorate not only accumulates but is bioconcentrated. This information, which has not yet been fully reviewed by the U.S. EPA, is herewith made available with this document to external peers for their review.

Wildlife are assumed to have negligible exposure from air or from direct exposure to soil. Exposures of wetlands to groundwater or surface water are not included explicitly because their exposures and effects are assumed to be equivalent to irrigation exposures. That is, plants and invertebrates are assumed to be exposed to pore-water concentrations equal to surface or groundwater concentrations. Exposures to contaminated sediments also are not included explicitly because they are believed to be equivalent to surface water exposures. Perchlorate salts are highly water soluble and the anion is unlikely to adsorb to anionic particles, such as soils or humic substances, to a significant extent. Therefore, sediment exposures are expected to be dominated by exposure to pore water, which is assumed to be equal to surface water.

8.2.3 Analysis Plan

This screening assessment uses existing information to determine whether the existing environmental contamination by perchlorate poses a clearly significant risk, insignificant risk, or an ambiguous risk. The analysis of effects will consist of the derivation of screening benchmarks through the application of conservative extrapolation models. The analysis of exposure for ecological endpoints consists of measured concentrations reported in Chapter 1 or derived from Parsons (2001) or Smith et al. (2001). Soil exposure estimates are based on exposure to perchlorate in irrigation water.

8.3 ANALYSIS

8.3.1 Characterization of Exposure

8.3.1.1 Water Concentrations

As previously described, fishes, aquatic invertebrates, and aquatic plants may be exposed directly to concentrations of perchlorate in surface waters. These concentrations may result from surface run-off from perchlorate-contaminated soil, from leaching of perchlorate from contaminated soil via shallow groundwater, or from direct discharge of aqueous wastes. Surface or groundwater may be used for irrigation, resulting in direct exposure of soil invertebrates or plants (Figure 8-1).

Perchlorate salts are dissolved readily given the conditions under which the contamination has occurred, releasing the perchlorate anion and the associated cation. Sorption is not expected to attenuate perchlorate because it absorbs weakly to most soil minerals, and natural chemical reduction in the environment is not expected to be significant. Consequently, perchlorate is both very mobile in aqueous systems and persistent for many decades under typical ground and surface water conditions (Section 1.1).

Limited information is available on perchlorate concentrations in surface waters. Perchlorate from an ammonium perchlorate manufacturing area has been detected at 4 to 16 μg/L downstream in Lake Mead and the Colorado River (Section 1.2). Information on the frequency or central tendency (mean or median) of perchlorate detection in those water bodies was not available for this review, but it is assumed that some aquatic organisms are exposed chronically

to concentrations as high as 16 μg/L. On the other hand, perchlorate concentrations have been measured as high as 0.37% (37×10^6 μg/L) in groundwater-monitoring wells at facilities that manufacture or test rocket motors and at 280 μg/L in public water supply wells (Section 1.2) Smaller surface water bodies, including some that are supplied primarily by groundwater, are likely to exist near sites of soil contamination and to have perchlorate concentrations much higher than those reported for Lake Mead and the Colorado River. A spring associated with the Las Vegas Wash site had concentrations of 1.0 to 1.3×10^5 μg/L in surface water (Parsons, 2001). Perchlorate concentrations in a pond (INF Pond) that receives water from the pump and treat system at the Longhorn Army Ammunition Plant near Karnack, TX ranged from 30,776 to 31,438 μg/L in November 1999 (Smith et al., 2001) and ranged from 3500 to 3800 μg/L in September 2000 (Parsons, 2001). It is also possible that, within large water bodies, there are locally elevated concentrations at sites of groundwater discharge. In the vicinity of a sediment delta created by the Las Vegas Wash in Las Vegas Bay of Lake Mead, Parsons (2001) documents a maximum perchlorate concentration of 68 μg/L in surface water. At the Allegany Ballistics Laboratory in Rocket Center, WV, discharge water from a Comprehensive Environmental Response Compensation, and Liability Act (CERCLA) groundwater pump and treat facility to the North Branch Potomac River contained 250 to 280 μg/L perchlorate (Parsons 2001). Surface water concentrations in Town Gut Marsh adjacent to the Naval Surface Warfare Center at Indian Head, MD ranged from not detected (reporting limit = 4.0 μg/L) to 25 μg/L. It should be noted that the groundwater pump and treat facilities either at Longhorn Army Ammunition Plant or Allegany Ballistics Laboratory were not equipped with facilities to treat perchlorate in water.

Surface water concentrations in Harrison Bayou below the discharge point for the INF pond at LHAAP also ranged from undetectable (reporting limit = 4.0 μg/L) to 4.0 μg/L (Parsons, 2001; Smith et al., 2001). However, Smith et al. (2001) point out that water from the pond is discharged to Harrison Bayou only during periods when Harrison Bayou is flowing, and neither study apparently sampled Harrison Bayou when water was being discharged from the pond. Therefore, higher concentrations of perchlorate in surface water of Harrison Bayou are likely to be measured at other times.

It is assumed that irrigation waters pumped from Lake Mead or the Colorado River are in the range of downstream concentrations given above (i.e., 4 - 16 μg/L). Groundwater irrigation

may be contaminated at levels similar to those observed in public water supplies (≤280 μg/L), unless the well is appreciably nearer a perchlorate-contaminated site.

8.3.1.2 Aquatic Bioaccumulation

As discussed above, little information has been previously available on the potential for the perchlorate ion to accumulate in animal tissues. The studies outlined in the Parsons (2001) report sought to answer the question whether perchlorate is present in ecological receptors. In these studies, concentrations of perchlorate in aquatic vegetation, fish, amphibians, aquatic invertebrates, and birds were compared to surface-water, pore-water, and sediment concentrations from the same water body. This information is supplemented by the additional studies conducted at LHAAP by Smith et al. (2001).

When perchlorate concentrations in physical media (i.e., surface water or sediment) were greater than the reporting limits for biological media (≥300 ppb [μg/L or μg/kg] in Parsons [2001]), concentrations in aquatic vegetation were similar to or greater than the concentrations in surface water or pore water; but concentrations in fish, amphibians, or invertebrates were less. In Smith et al. (2001) reported the detection of high concentrations of perchlorate in the INF Pond and lower concentrations in aquatic vegetation and in animals than in surface water or sediments.

In Parsons (2001), when perchlorate concentrations in the physical media were lower, concentrations in aquatic vegetation or amphibians were in a few cases greater than the concentrations in surface water or sediment; but in most cases, perchlorate was not detected in aquatic receptors. However, our understanding of bioaccumulation of perchlorate in this lower concentration range is limited because the reporting limits in the Parsons (2001) studies for perchlorate in animal tissues (i.e., 300-400 μg/kg) were greater then the reporting limits for surface water or pore water (i.e., ≈ 4 μg/L) or for sediments (i.e., ≈ 80 μg/L).

Although Smith et al. (2001) do not identify their reporting limits, their reporting limits for biological tissues appear to be less (i.e., ≈70 μg/kg based on their lowest detected concentration) than those of Parsons (2001). In the Smith et al. (2001) study of LHAAP, detected perchlorate concentrations were similar in surface water (44-85 μg/L), sediments (78 μg/kg), and fish tissues (83-131 μg/kg) at Goose Prairie Creek. In Harrison Bayou, the single detected concentration in surface water (4 μg/L) was less than detected concentrations in animal tissues (86-356 μg/kg).

However, as the authors discuss, the measured concentration in surface water in Harrison Bayou is likely less than when water is being discharged from the INF Pond (Smith et al., 2001). In addition, the study did not collect sufficient samples from any one site and medium or species for any significant statistical comparisons to be made.

Information newly received form the U.S. Army Corps of Engineers (Condike, 2001) report on the analysis of environmental samples from perchlorate-contaminated water bodies near McGregor Naval Weapons Industrial Reserve plant (NWIRP), TX, and purports to show fish tissue concentrations that exceed comparable water concentrations. These data suggest that perchlorate not only accumulates but is bioconcentrated. This information, which has not yet been fully reviewed by the U.S. EPA, is herewith made available with this document to external peers for their review.

The above information indicates that perchlorate may bioaccumulate in aquatic organisms living in contaminated waters, but it does not resolve the question of whether perchlorate may bioconcentrate in the tissues of aquatic organisms to levels exceeding the surface water concentrations. The existing data are also insufficient to determine whether there is further trophic transfer of perchlorate within aquatic food webs.

8.3.1.3 Soil Levels

On-site soils may be contaminated by direct spills of perchlorate solutions from flushing rockets, combustion of rocket fuel, improper disposal of rocket fuel, open burn/open detonation operations, explosives, or manufacturing wastes. Perchlorate concentration measurements at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001). Off-site soils may be contaminated via irrigation (Figure 8-1). Because of the high water solubility of perchlorate salts, perchlorate is unlikely to accumulate via adsorption to irrigated soils, and aqueous perchlorate was not found to adsorb to sand in laboratory reactors (Nzengung, n.d.). By gross approximation, then, soil concentrations (expressed as milligrams per kilogram) would be unlikely to exceed the concentrations (expressed as milligrams per liter) in irrigation water. Similarly, concentrations of perchlorate in soil pore water may be assumed to be equal to the concentration in irrigation water, both in the field and in soil toxicity tests. However, the concentration of perchlorate salts in irrigated soils with high evaporation rates cannot be ruled out. At the Yuma site, soils are irrigated with water from the Colorado River, and concentrations

of perchlorate in surface-water samples collected near the irrigation intake locations ranged from 0.003 to 0.006 mg/L. In surface soil, the single detection (0.090 mg/kg) was well above the concurrently-measured water concentrations, as were the perchlorate detection limits in soil (0.079 to 0.080 mg/kg). The relatively higher detection limits in soil, the limited nature of the sampling in soil and water, and the lack of information about potential sources other than irrigation water (see Section 8.1.2) complicate the interpretation of the presence and fate of perchlorate in irrigated soils.

8.3.1.4 Uptake by Vegetation

Several laboratory experiments have examined plant uptake of perchlorate from solution culture. Experiments with candidate plants for use in the phytoremediation of perchlorate-contaminated sites showed that perchlorate may concentrate in vegetation (Nzengung, n.d.; Susarla et al., 2000a). Nzengung (n.d.) used rooted cuttings of woody plants, willow (*Salix* spp.), Eastern Cottonwood (further identified only as "poplar"), and eucalyptus (*Eucalyptus cineria*) planted in sand with nutrient solution containing perchlorate at 20 or 100 mg/L for 24 to 42 days. In each case, perchlorate was taken up and concentrated in aerial plant parts, especially leaves. Concentration factors, expressed as the ratio of leaf concentration (mg/kg wet weight) to initial solution concentration (mg/L), ranged from 7.5 to 25.

Susarla et al. (2000a) used seedlings or rooted cuttings of 13 vascular plant species, planted in sand with nutrients, and exposed for ten days to 0.2, 2.0 or 20 mg/L perchlorate. These researchers also reported depletion of perchlorate from test media. Qualitative analyses suggested accumulation of perchlorate in the aerial tissues of most of the species analyzed. Using their data and the approach reported by Nzengung above, we calculated concentration factors ranging from 0 to 330.

Nzengung (n.d.) and Susarla et al. (2000a) reported that perchlorate accumulated primarily in the leaves, followed by stems, then roots. Predicted perchlorate breakdown products, chlorate, chlorite, and chloride were detected in plant tissues in both studies, but quantitative evidence was not presented. In addition to this lack of quantitative data, there are other concerns related to the potential for plants to degrade perchlorate. First, information concerning accumulation and potential transformation is limited to a few studies by these two laboratories. Second, the method used for perchlorate analysis yielded estimates of perchlorate in fertilizer that were

subsequently found to be overestimated by 30 to 150% (Susarla et al., 2000b). Third, no physiological explanation has been suggested for why plants should accumulate this salt far in excess of concentrations in water or groundwater, though it appears this may be simply a function of water uptake rates to meet transpirational losses. Fourth, these two studies were short-term, material depletion studies, a type of study we believe will overestimate long-term accumulation rates because some of the "response" is likely the result of factors not related to the chemical in question. There is ample evidence from salt accumulation studies of plants to suggest that the initial increases in perchlorate accumulation by plants may be due to a salt effect; that is, nutrient salts are initially indistinguishable from perchlorate salts in that they all represent an ionic imbalance across the cell wall. One of the confounding issues that can only be determined with longer-term studies is the effect of increased cell sap salinity on additional perchlorate uptake. As sap salinity increases, there should be an increase in H_2O uptake, further increasing the perchlorate concentrations. This will continue only until a certain concentration of salts, including perchlorate, is reached, at which time the plant will close its stomata and shunt sap salts to vacuoles.

In addition to the above stated concerns, there is no reason to expect that these are steady-state concentration factors. These experiments were designed to quantify phytotransformation of an initially introduced perchlorate quantity, rather than bioconcentration, with respect to an ambient perchlorate concentration. As the perchlorate-amended nutrient solution was transpired, and some perchlorate was taken up, it was replenished by solution, without added perchlorate; thus, perchlorate in the test chamber diminished throughout the experiment. Concentration factors that would be observed at steady state, such as may result from continual irrigation with perchlorate-contaminated water, cannot be estimated from this study. Because of the uncertainties associated with both perchlorate accumulation and degradation by plants, a simple, conservative, screening-level assumption that concentrations in leaves can exceed water concentrations by a factor of 100 was made.

If irrigation is from surface water sources similar to the Colorado River or Lake Mead, with concentrations as high as 16 μg/L, then plant concentrations are assumed to be as high as 1.6 mg/kg. If irrigation is from groundwater sources similar to potable water supplies, with concentrations as high as 280 μg/L, then plant concentrations will be assumed to be as high as 28 mg/kg.

Concentrations in plant tissues and soils also have been measured in the field. Ellington et al. (2001) measured perchlorate concentrations in leaves of tobacco, *Nicotiana tabacum* var. K326, field-grown in soil amended with Chilean saltpeter, which is naturally high in perchlorate. Perchlorate concentrations (± SD) in leaf lamina from the 1999 crop were 96.0 ± 0.6 mg/kg dry weight and 14.6 ± 0.1 mg/kg wet weight; concentration in a composite soil sample collected in December 1999 was 0.34 ± <0.01 mg/kg dry weight. The concentration factor in this study was approximately 43, on the basis of wet weight in leaf lamina and dry weight in soil.

The field studies by Parsons (2001) found that, for various sites, wet-weight perchlorate concentrations in terrestrial vegetation samples were 1.5 to 80 times the wet-weight concentrations in soil samples. The data from one site (i.e., Building 25C) at LHAAP (Smith et al., 2001) seem to indicate greater concentration factors, but the soil and plant samples were taken at different times of the year (i.e., January and October, respectively) and only one sample each of three plant species was analyzed.

Soil-to-vegetation concentration factors derived from the above field studies were similar in magnitude, but when using them for risk assessment care should be taken to note the different bases; exposure concentration was variously reported as mg/kg wet weight in soil or mg/kg dry weight in soil. The maximum measured concentration in vegetation at irrigated sites in the vicinity of Yuma, Arizona was 1.0 mg/kg wet weight. At sites with soil contamination related to the manufacture, handling, or use of perchlorate in solid propellants, maximum plant concentrations were 428 mg/kg wet weight at a spring; 99.2 mg/kg wet weight at a site upstream from Lake Las Vegas in the Las Vegas Wash area of the Lake Mead Recreational Area, Nevada; and 300 mg/kg wet weight at the Burn Area of Allegany Ballistics Laboratory, West Virginia. In most cases, detection limits were ~0.4 mg/kg wet weight.

8.3.1.5 Herbivore Exposure

The representative herbivore selected for this assessment, *M. pennsylvanicus*, has a diet consisting mainly of monocot and dicot shoots, has an estimated food consumption rate of 0.005 kg/day wet weight, and a body weight of 0.044 kg (Sample and Suter, 1994). Using the assumptions stated above, daily exposures resulting from surface water and groundwater irrigation may be as high as 0.18 mg/kg-day and 3.2 mg/kg-day, respectively. Daily exposures resulting from maximum measured concentrations in plants range from 0.11 mg/kg-day at the

irrigated sites in the vicinity of Yuma to 49 mg/kg-day for the sites with direct soil contamination.

In the Parsons (2001) studies, except when concentrations in surface soils were high (i.e., ≥9000 μg/kg), perchlorate was not detected in terrestrial birds, mammals, or insects with reporting limits of 300 to 400 μg/kg. The vertebrates collected varied substantially between sites, but the birds collected include the mourning dove (*Zenaida macroura*), tree swallow (*Tachycineta bicolor*), roughwing swallow (*Stelgidopteryx serripennis*), lesser nighthawk (*Chordeiles acutipennis*), nighthawk (*C. minor*); Gambel's quail (Callipepla gambelii), starling (*Sturnus vulgaris*); American crow (*Corvus brachyrhynchus*), eastern bluebird (*Sialia sialis*), eastern phoebe (*Sayornis phoebe*), and blue grosbeak (*Guiraca caerulea*). The mammals collected include the cactus mouse (*Peromyscus eremicus*), rock pocket mouse (*Chaetodipus intermeius*), Audubon's cottontail (*Sylvilagus audubonii*), deer mouse (*P. maniculatus*), long-tailed pocket mouse (*Perognathus formosus*), western pipestrelle (*Pipistrellus hesperus*), house mouse (*Mus musculus*), white-footed mouse (*Peromyscus leucopus*) meadow vole (*Microtus pennsylvanicus*), Merriam's kangaroo rat (*Dipodomys merriami*), desert pocket mouse (*C. penicillatus*), hispid cotton rat (*Sigmodon hispidus*), western harvest mouse (*Reithrodontomys megalotis*), marsh rice rat (*Oryzomys palustris*); northern short-tailed shrew (*Blarina brevicauda*), racoon (*Procyon lotor*), eastern harvest mouse (*R. fulvescens*), little brown bat (*Myotis lucifugus*), eastern cottontail (*S. floridanus*). At those sites where perchlorate concentrations in surface soils were high, perchlorate concentrations in potential herbivore tissues were generally an order of magnitude or more less than that in vegetation. At one site, Yuma, with lower perchlorate concentrations in soil (i.e., mean of all results = 81 μg/kg), perchlorate was detected in a single terrestrial reptile sample (brush lizard, *Urosaurus graciosus*), but this detection was lower than the mean perchlorate concentration in vegetation. Although detected soil concentrations were lower (i.e., 50 to 322 μg/kg) in Smith et al. (2001), the concentrations of perchlorate in two composite samples of livers from harvest mice (*Reithrodontomys fulvescens*) were several orders of magnitude less than the detected concentrations in their potential food, plant leaves or seeds.

8.3.2 Characterization of Effects

8.3.2.1 Aquatic Organisms

Effects on the richness and productivity of fish, aquatic invertebrate, and aquatic plant communities are jointly characterized using the procedures for deriving Tier II water quality values (U.S. Environmental Protection Agency, 1993; Suter and Tsao, 1996). Tier II values are derived where data are not sufficient for deriving ambient water quality criteria (AWQC). The Tier II value derivation procedures account for missing information with approximately 80% confidence.

Test results potentially useful for deriving Tier II values were available for five aquatic species (Table 8-1). In acute tests (48 and 96 h) with sodium perchlorate, using the water flea *Daphnia magna*, the amphipod *Hyalella azteca*, and the fathead minnow *Pimephales promelas*, the endpoints lethality and inhibition were studied. In seven-day tests with a different water flea (*Ceriodaphnia dubia*) and with *P. promelas*, acute lethality was studied in addition to more sensitive endpoints, including the number of offspring per female (*C. dubia*) and growth rate (i.e., body weight; *P. promelas*). A seven-day test with *C. dubia* generally is here used in place of a chronic (i.e., twenty-one day) test because test organisms produce three broods during the test; a seven-day test with *P. promelas* is arguably subchronic because of the test's short duration relative to the organism's lifespan (Suter, 1990; Norberg-King, 1990). A 35-day, early-life-stage (ELS) test with *Pimephales*, here used in place of a chronic test, showed no significant effects on any standard endpoint (survival, growth or biomass) at the highest concentration tested. However, all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling that was not observed in the larvae exposed to the control water. This finding suggests the presence of subtle effects that could be ecologically significant and raises doubt about whether a chronic No-Observed-Effect-Concentration (NOEC) has been adequately determined for this species.

Steps followed in the derivation of the Tier II value for sodium perchlorate are presented in Table 8-2. The secondary acute value (SAV), 5 mg/L (as ClO_4^-), is derived to be protective of 95% of species during short-term exposures with 80% confidence. The secondary chronic value (SCV), 0.6 mg/L (as ClO_4^-), likewise is derived to be protective of 95% of species during long-term exposures with 80% confidence. A sodium chloride control test showed that some of the toxicity to *P. promelas* was potentially attributable to the sodium cation. These tests suggest

TABLE 8-1. RESULTS OF PERCHLORATE TOXICITY TESTS IN AQUATIC AND TERRESTRIAL SPECIES

	Test Description		Endpoints (as mg/L perchlorate in water or mg/kg in soil or sand)[a]				
Test Species	**Age**	**Duration**	**Acute LC_{50} (95% CL)**	**NOEC**	**LOEC**	**ChV**	**IC_{25} (95% CL)**
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science and Technology, Inc., 1998)							
Daphnia magna	<24 hr	Acute (48-hr)	490 (406 - 591)	—	—	—	—
Pimephales promelas	12 - 13 days	Acute (96-hr)	1,655 (1,507 - 1,817)	—	—	—	—
Ceriodaphnia dubia	<24 hr	Chronic (7-day)	66 (40-144) [48-h]	10	33	18.2	17 (8.1 - 20.5)
Pimephales promelas	<24 hr	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280[c]	208[c]	212[c] (175 - 231)[c]
Lactuca sativa	<24 hr	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280[c]	208[c]	212[c] (175 - 231)[c]
Lactuca sativa		Chronic definitive (28-d, sand)		<80	80	<80	41
Lactuca sativa		Chronic definitive (28-d, soil)		40	40	56.6	30
Lactuca sativa		Chronic definitive (28-d, sand)		20	40	28.3	34.3
Eisenia foetida		Acute definitive (7 day/14 day, soil)	4,450/4,450	—	—	—	—
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science and Technology, Inc., 2000)							
Pimephales promelas	Embryo	Chronic (35-day, early life stage)	> 490 [96-hr]	> 490[d] <28[e]	> 490[d] 28[e]	> 490[d]	> 490[d] <28[e]
Hyalella azteca	7 - 14 days	Chronic definitive (28-day)		> 1000	> 1000	> 1000	> 1000

TABLE 8-1 (cont'd). RESULTS OF PERCHLORATE TOXICITY TESTS IN AQUATIC AND TERRESTRIAL SPECIES

	Test Description		Endpoints (as mg/L perchlorate in water or mg/kg in soil or sand)[a]				
Test Species	**Age**	**Duration**	**Acute LC_{50} (95% CL)**	**NOEC**	**LOEC**	**ChV**	**IC_{25} (95% CL)**
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science, and Technology, 1999)							
Selenastrum capriconutum	7 days	Acute (96-hr)	—	500	1,200	775	615 (149-1,126)
Ammonium perchlorate (NH_4ClO_4)[f] tests (Block Environmental Services, Inc., 1998)							
Ceriodaphnia dubia	<24 hr [g]	Chronic (6-day)	77.8 [6-days]	9.6	24	15	24
Pimephales promelas	<24 hr [g]	Subchronic (7-day)	270 [7-days]	9.6	96	30	114
Ammonium perchlorate (NH_4ClO_4)[f] tests (Dumont and Bantle, 1998)							
Xenopus	Embryo	96-hr	420[h]	—	—	—	—
Xenopus	Embryo	96-hr	336[h]	—	—	—	—

[a]Notation: LC_{50} = Concentration lethal to 50% of individuals; NOEC = No-observed-effect concentration; LOEC = Lowest-observed-effect concentration; ChV = Chronic value; IC_{25} = Concentration inhibiting a process (e.g., growth, reproduction) by 25%; CL = confidence limits.
[b]Sodium chloride control showed no adverse effects of sodium ion except as indicated. Reported values are based on nominal concentrations.
[c]Sodium chloride control showed significant adverse effects attributable to sodium cation at highest test concentration. Effects observed at this perchlorate concentration may have been caused in part by sodium ion toxicity.
[d]Standard endpoints: survival, growth, biomass
[e]Although there were no effects on standard endpoints at any tested concentration, the investigators reported that all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling, which was not observed in the larvae exposed to the control water.
[f]Ammonium control was not used; adverse effects of ammonium ion cannot be ruled out at all effect concentrations. *C. dubia* and *P. promelas* results are based on measured concentrations. *Xenopus* results are based on nominal concentrations. Confidence limits are not reported.
[g]Not reported; assumed based on standard protocols.
[h]IC_{50} for malformations.

TABLE 8-2. PROCEDURE FOR DERIVING TIER II WATER QUALITY VALUES FOR SODIUM PERCHLORATE

Step	Value ($mg/L\ ClO_4^-$)	Rationale
Identify the lowest genus mean acute value (GMAV)	66	Lowest GMAV is for genus *Ceriodaphnia* (based on *C. dubia*)
Determine the final acute value factor (FAVF), a factor that compensates for lack of data on a sufficient number of taxonomic groups	13.2 (unitless)	The FAVF varies according to the number of specified taxonomic groups for which GMAVs were available. In this case, two specified values were available (a nonsalmonid fish and a planktonic crustacean), of which one is a daphnid; the value selected from the FAVF table (U.S. Environmental Protection Agency, 1993; Suter and Tsao, 1996) is 13.2.
Calculate the secondary acute value (SAV)	5.0	SAV=GMAV ÷ FAVF = 66 ÷ 13.2
Identify three or more acute-chronic ratios (ACRs), which are ratios of acute value (AV) to chronic value (CV) for a given species (but ratios must be geometrically averaged within any single genus)	3.6, 8.0 (range, <3.4 - >59), 17.9	ACRs can be derived for two species in different genera. For *C. dubia*: ACR=AV ÷ CV = 66 ÷ 18.2 = 3.6 For *P. promelas*, two AVs are available. The lower (614) is thrown out because the larval stage is not standard for acute tests; the higher (1,655) is used. Three CVs are available: >490 for standard endpoints, and <28 for redness and swelling, in the 35-d ELS test; and 208 for survival in the 7-d test. There is uncertainty as to the interpretation of the ELS test results; the 7-d result is used and the two results from the ELS are used to determine a range, shown in parentheses: ACR = 1,655 ÷ 208 (range, >490 - <28) = 8.0 (range, <3.4 - >59) No ratio is possible for *H. azteca* because we are unable to calculate CV due to no acute toxicity. Because a third value is not available, a default value of 17.9 (which provides 80% confidence based on other toxicants) is substituted, according to the Tier II method.
Derive the secondary acute-chronic ratio (SACR)	8.0 (range, <6.0 - >15.6)	The SACR is the geometric mean of the ACRs. (The uncertainty range associated with the *P. promelas* value is carried through and shown in parentheses.)
Derive the secondary chronic value (SCV)	0.60 (range, <0.32 - >0.83)	SCV=SAV ÷ SACR, 5.0 ÷ 8.0 (The uncertainty range associated with the *P. promelas* value is carried through and shown in parentheses.)

the possibility that if perchlorate were associated with a less toxic cation, the SCV may be lower than is necessary to protect against perchlorate ion toxicity. Further tests with perchlorate may be needed to assess potentially less toxic cations.

Similar chronic (or subchronic) tests were conducted with ammonium perchlorate (Table 8-1). Results, expressed as ClO_4^-, were very similar for *C. dubia*, but *P. promelas* was more sensitive to ammonium perchlorate than to sodium perchlorate. However, Tier II values for ammonium perchlorate are not presented for several reasons, including the lack of ammonium controls which makes it difficult to determine whether the observed effects were caused by the perchlorate anion; the lack of acute values for *C. dubia* and *P. Pimephales*; and the fact that the FETAX (*Xenopus*) test is designed to detect teratogenic potential, and the embryo is not a particularly sensitive life stage for toxicity. When perchlorate is administered as the ammonium salt, the ammonium ion concentration expressed on an ammonia-nitrogen (in milligrams of NH_3-N/L) basis is 14% of the respective perchlorate ion concentration. A Lowest-Observed-Effect-Concentration (LOEC) for *C. dubia* of 24 mg/L perchlorate (Table 8-1) thus corresponds to 3.4 mg NH_3-N/L. Based on a species mean chronic value (SMCV) of 13 mg NH_3-N/L for *C. dubia* exposed to ammonia alone (U.S. Environmental Protection Agency, 1998h), the former value is probably too low to be responsible for the observed effects[1]. On the other hand, the LOEC observed with *P. promelas* at 96 mg/L (Table 8-1) corresponds to 14 mg NH_3-N/L, which exceeds a SMCV of 3.09 mg NH_3-N/L (U.S. Environmental Protection Agency, 1998h). Therefore, ammonium exposure alone could have been responsible for the effects of ammonium perchlorate observed in *P. promelas*.

The SAV and SCV derived above based on sodium perchlorate are probably protective even if ammonium perchlorate is the form released, however. Calculated NH_3-N concentrations corresponding to those values are below the acute and chronic ambient water quality criteria for ammonia, regardless of pH (U.S. Environmental Protection Agency, 1998h). While SAV and SCV are not calculated for plants, it appears that there is little perchlorate or ammonium toxicity to the alga *Selenastrum* in toxicity studies (Table 8-1).

[1]Ammonia/ammonium toxicity increases as test-water pH increases (U.S. Environmental Protection Agency, 1998e). The value of 13 mg NH_3-N/L corresponds to a pH of 8.0; however, unless the test water pH had exceeded 8.8, it is doubtful that 3.4 mg NH_3-N/L was responsible for the observed effects.

8.3.2.2 Terrestrial Organisms

Plants. The only available phytotoxicity information comes from 28-day seedling growth tests of lettuce (*Lactuca sativa*) performed in soil and sand cultures with sodium perchlorate (EA Engineering, Science and Technology, Inc., 1998). Although the exposure was to sodium perchlorate solution added to the solid media, the results may be expressed as milligrams per kilogram soil or sand, wet weight, or as milligrams per liter of irrigation solution. Growth was a more sensitive response than germination or survival. The quartile inhibitory wet-weight concentrations (IC_{25}s) for growth in soil and sand were 78 mg/kg (293 mg/L) and 41mg/kg (160 mg/L), respectively. Survival was reduced 26% at 420 mg/kg (2,520 mg/L) in soil and 39% at 180 mg/kg (840 mg/L) in sand. To account for interspecies variance, a factor of 10 is applied to the lowest IC_{25} to obtain a screening benchmark of 4 mg/kg as a wet-weight concentration in soil (or 16 mg/L as a concentration in irrigation solution).

Soil Invertebrates. The only available toxicity data for soil invertebrates is a 14-day acute lethality test of the earthworm (*Eisenia foetida*) performed in artificial soil irrigated with sodium perchlorate. The LC_{50} at both 7 and 14 days was 4,450 mg/kg as a wet-weight concentration in soil. No factors or other models are available to extrapolate from that LC_{50} to chronic effects on survival, growth, or fecundity or to extrapolate from this species to the soil invertebrate community as a whole. Therefore, the factors applied for aquatic communities in cases where there is only one LC_{50} (see Section 8.3.2.1) to obtain a conservative estimate of a soil screening benchmark for soil community effects, are as follows:

$$\begin{aligned} \text{Threshold} &= LC_{50} \div (\text{factor for interspecies variance} \times \text{acute-chronic ratio}) \\ &= 4{,}450 \text{ mg/kg} \div (242 \times 18) \\ &= 1 \text{ mg/kg as a wet-weight concentration in soil.} \end{aligned}$$

The equivalent aqueous phase benchmark is 2.8 mg/L. This approach requires the assumptions that the variance among soil species is approximately the same as among aquatic species, and that the distribution of acute-chronic ratios across chemicals is approximately the same for both communities. The interspecies variance factor is the one for a test species that has not been demonstrated to be highly sensitive.

Herbivores. The human health risk assessment for perchlorate uses 0.01 mg/kg-day as the LOAEL from which the RfD is derived (Chapter 7). That value is based on perturbations in thyroid and pituitary hormones, thyroid histopathology, and changes in brain morphometry in P0

dams on GD21 and F1-generation rat pups on PND5, PND10, and PND22. Because the representative species for the herbivore endpoint (meadow vole) is a rodent, that value is used here as well. The population-level implications of this effect are unknown; however, it seems likely that such effects on the thyroid, pituitary, and brain could diminish survivorship and fecundity and diminish population production. To account for interspecies variance and LOAEL to NOAEL extrapolation, an uncertainty factor of 10 is applied to obtain a dietary screening benchmark for herbivores of 0.001 mg/kg body weight-day, or ~0.01 mg/kg as a wet-weight concentration in plant tissue (see exposure assumptions in Section 8.3.1.5).

CHAPTER 9. EVALUATION OF EVIDENCE FOR INDIRECT EXPOSURES

The primary purpose of this document is to derive human and ecological risk estimates for perchlorate. As indicated in Chapter 1, pollution of drinking water supplies is the major concern. Most perchlorate salts are used as solid oxidants or energy boosters in rockets or ordnance; therefore, much of the perchlorate-tainted waterways in the U.S. can be traced to military operations, defense contracting, or associated manufacturing facilities. Figure 1-5 shows that the perchlorate anion could potentially be found in many natural waterways that are used for irrigation or consumed by livestock or wildlife. Thus, it is logical to question whether there are means through which humans might consume perchlorate other than drinking water. This question is compounded by the chemical nature of perchlorate, which grants it long life under typical environmental conditions (Urbansky, 1998; Urbansky and Schock, 1999; Espenson, 2000).

As discussed in Section 7.1.5, once a reference dose for perchlorate is established, any burden posed by exposure routes other than potable water necessarily requires that the contaminant's concentration in a water supply be lowered by an equivalent amount if it is determined to calculate a maximum contaminant level goal (MCLG). A relative source contribution (RSC) of between 20% to 80% is used to adjust the RfD according to the decision framework presented in the EPA's methodology for deriving ambient water quality criteria (U.S. Environmental Protection Agency, 2000).

Because polluted waters are used for irrigation, there are also questions concerning absorption, elimination, and retention in food plants. However, this issue becomes considerably less important if it can be demonstrated that the irrigation water is perchlorate-free. Likewise, there are concerns that animals raised for food would consume plants that had received perchlorate-tainted water. As described in Chapter 8, studies are being conducted to assess the occurrence of perchlorate in biological fluids and tissues of animals and plants in affected regions in recognition of the inter-connectedness of the food chain/food web continuum.

While much of the perchlorate problem can be traced to specific sites, a few reports have suggested that fertilizers could represent another source of perchlorate in the environment (TRC Environmental Corporation, 1998). These will be addressed in further detail in Section 9.1.1. Sporadic detection of perchlorate in fertilizers was initially alarming because of the widespread use of fertilizers in production farming. In addition to the ecological impact, this raised the issue of assigning responsibility for clean-up costs. Because of the dependence of U.S. agriculture on chemical commodity fertilizers, it was clear that assessment of any possible role of fertilizers would require investigation.

This chapter summarizes the available data on the potential for exposure through runoff, erosion, fertilizer, and groundwater movement. Evidence concerning the potential of perchlorate to contaminate soil, sediment, vegetation, livestock and wildlife is also evaluated.

9.1 FERTILIZERS AS SOURCES OF PERCHLORATE SALTS

9.1.1 The Potential Role of Fertilizers

Recently, attention has been drawn to the possible roles of fertilizers as a source of perchlorate contamination for two reasons. First, perchlorate-tainted agricultural runoff could lead to pollution of natural waterways used as drinking water sources. Second, there is a potential for food plants to take up and retain any soluble compounds—including perchlorate salts—and thus provide an alternate route of exposure. It has long been known that Chile possesses caliche ores rich in sodium nitrate ($NaNO_3$) that coincidentally are also a natural source of perchlorate (Schilt, 1979; Ericksen, 1983). The origin of the sodium perchlorate ($NaClO_4$) in the caliche deposits remains an area of debate, but perchlorate is present and can be incorporated into any products made from the caliche.

An examination of two manufacturing lots found perchlorate concentrations below 2 mg/g, (i.e., < 0.2% w/w) with some lot-to-lot variability (Urbansky et al., 2001). Presently, the caliche-derived products are sold in the U.S. only by Sociedad Quimíca y Minera (SQM), but other companies have mineral rights to some Chilean deposits and mines (U.S. Environmental Protection Agency 2001b) and are potential sources of caliche-derived products. SQM has now modified its refining process to produce a fertilizer that contains less than 0.1 mg/g (<100 µg/g)

of perchlorate, further reducing any environmental release (Lauterbach, 2001). Because nitrate salts (saltpeters) find use as fertilizers, these natural resources have been mined and refined to produce commercial fertilizers for domestic use or for export. Chilean nitrates make up about 0.1% of the U.S. market. Most U.S. fertilizers are derived from other raw materials other than sodium nitrate and ammonium nitrate (NH_4NO_3), which is often used for purposes similar to $NaNO_3$, is manufactured from methane, nitrogen, and oxygen. There is no evidence that any ammonium nitrate is derived from Chilean caliche. On account of its low usage, perchlorate from Chilean nitrates cannot represent a continuing, significant anthropogenic source of perchlorate nationwide, especially with its lowered perchlorate content.

9.1.2 Raw Material Use

As with many commodity chemicals, large scale purchases are dictated by cost of raw materials, which are in turn influenced by transportation costs. Consequently, proximate (rather than distant) sources of agricultural chemicals are likely to play the greatest roles in production farming. Additionally, processing aids (e.g., clays) are likely to be derived from the nearest sources.

Commodity chemicals used as agricultural fertilizers contain fairly high concentrations of one, or sometimes two, of the primary plant nutrients, expressed as nitrogen (N), phosphorous (as the oxide P_2O_5), or potassium (as the oxide K_2O). Trace metals (e.g., copper) can be applied separately or along with these primary nutrients on a farm site. The primary phosphorus sources are ammonium phosphates and triplesuperphosphate (a hydrous calcium phosphate). The primary potassium source is potassium chloride. A mixture of synthetic and natural components are used in fertilizer manufacture, described in detail elsewhere (U.S. Environmental Protection Agency, 2001b).

Fertilizer application in production farming is highly dependent on the crop and the native soil. Agriculture is influenced by climate, weather, topography, soil type, and other factors that are generally similar within a geographical region; therefore, crops and fertilizer use are also similar within such a region. For example, the Corn Belt relies heavily on urea and anhydrous ammonia as nitrogen sources. Ammonium nitrate finds greater use in tobacco farming, and potassium magnesium sulfate finds more use in milk-producing states. Because all plants require the same primary nutrients, there is some fertilizer usage to provide these regardless of crop.

Local soil conditions also dictate what nutrients should be augmented, causing there to be large regional variations.

Consumer fertilizer (specialty) products can be distributed over large geographical regions because of the nature of the market. For example, major manufacturers have a limited number of sites dedicated to blending multiple-nutrient formulations. These products are often sold as bagged fertilizers through home-improvement centers, nurseries, florists, horticulturists, and department (or other retail) stores. Unlike agricultural fertilizers, consumer products are usually multi-nutrient formulations. In addition, trace metals are sometimes incorporated directly into them. Because the average user will apply only a very small amount of trace metals (or even primary nutrients) relative to a production farm, it is more economical, more practical, and more convenient to use multiple-nutrient formulations. Moreover, the average consumer does not have the wherewithal to disperse careful doses of several single-component fertilizers at the appropriate times of the growing season.

Because fertilizer application on production farms is geographically delimited, there is considerable interest in knowing which commodity chemicals might contain perchlorate—at least in terms of dosing. Such information might suggest regions which should be investigated for perchlorate contamination. Moreover, it will be important to know what crops might be affected—if any.

9.1.3 Fertilizer Analysis Studies

Aside from the analyses of Chilean caliche, there were no studies to suggest that any other processed fertilizer or raw material might contain perchlorate prior to 1998. That year, the Ecosystems Research Division of the EPA's National Exposure Research Laboratory (NERL-ERD) found perchlorate in several samples that were not derived from Chile saltpeter (Susarla, 1999a). This finding was later duplicated by other investigators from the North Carolina State University College of Agriculture. However, the presence of perchlorate could only be confirmed in consumer products, not in agricultural fertilizers. Moreover, subsequent analyses of bags of the same materials acquired at a later date (likely from different manufacturing lots) did not show perchlorate (Susarla et al., 2000). The choice of fertilizers did not account for the possibility that the same raw materials must have been used in a variety of products at a point in time. Additionally, a few major companies are responsible for making a large number of

products under several brand names. Furthermore, some companies rely on toll manufacturing so that the products are actually made by another company to meet a specific formulation. Accordingly, an error or contamination associated with one raw material could affect a variety of products without regard to company or application.

Perchlorate was found in six of eight lawn and garden fertilizers tested, according to a report provided to the EPA by the U.S. Air Force Materiel Command (TRC, 1998). However, the report's authors were careful to point out that the results were obtained from a single sampling event and that raw material usage was variable; therefore, no general conclusions could be drawn. These qualifiers are consistent with the limitations enumerated above, but they do point towards a temporal contamination of some products.

This study helped bring to light a number of important issues for trace analysis of fertilizers. First, most of the research on determining perchlorate to that time had been focused on either finished potable water or raw source water (Urbansky, 2000). Second, fertilizers are considerably more complicated matrixes than dilute water solutions. Third, a solid fertilizer is not a homogeneous substance. In particular, multi-component formulations used as lawn and garden fertilizers are macroscopically heterogeneous and it is possible to sort out the particles visually. Thus, representative subsampling becomes a key issue. Fourth, the effectiveness of leaching out any perchlorate ion into an aqueous phase was unknown. Fifth, the products chosen did not reflect the chemical fertilizers used for production farming, but rather the ingredients used for lawn and garden fertilizers during a specific time period.

Around the same time, the U.S. Air Force Research Laboratories (AFRL) performed a study to assess interlaboratory corroboration; that is, the ability of different labs to analyze the same sample and get the same result (AFRL, 1999; Eldridge, 2000). A variety of techniques performed by multiple laboratories showed acceptable agreement on the concentrations of perchlorate in solutions prepared from the purchased products. Several limitations (such as product choice and sampling difficulties with heterogeneous solid products) made it impossible to gain an understanding of agricultural fertilizer use, and the AFRL intentionally restricted its use of the data to evaluating interlaboratory agreement. However, data from the AFRL study was sufficient to confirm independently that some lawn and garden fertilizer products did contain perchlorate during a certain period of time.

Subsequently, the Water Supply and Water Resources Division of the EPA's National Risk Management Research Laboratory (NRMRL) conducted its own survey of fertilizers in a collaboration with the Oak Ridge National Laboratory (Urbansky et al., 2000a; Urbansky et al., 2000b). In addition to a variety of products purchased from retailers, products were purchased from farming supply stores (e.g., 50-lb bags of urea or ammonium nitrate) in Indiana, Ohio, Kentucky, Pennsylvania, and Tennessee. In addition, commodity chemical samples were collected from local distributors in Ohio and Indiana. These included urea, potassium chloride, ammonium monohydrogen phosphate, and granular triplesuperphosphate, among others. Samples were leached or dissolved and subjected to complexation electrospray ionization mass spectrometry (cESI-MS) or ion chromatography (IC). Of 45 tested products, the only ones that were found to contain any perchlorate were those based on Chile saltpeter. While this study was the first to include the same products used on agricultural production farms, it did not address the issues of sampling, product inhomogeneity, or geographical source variation.

In an effort to better address sampling, raw material usage, and other issues, the EPA undertook an additional study of fertilizers. The project was divided into two phases, the first part of which evaluated the testing laboratories for their ability to identify and quantitate perchlorate in a fertilizer matrix. In the second phase, samples gathered under the supervision of state agricultural agents were homogenized and sent to the laboratories for analysis using a method established by the EPA (U.S. Environmental Protection Agency, 2001a). This investigation was the most thorough in terms of including agriculturally relevant materials used to manufacture a wide variety of specialty products or sold directly to farmers. It also spanned all major national suppliers of these products. Although it reflected only a temporal snapshot, as had all of the other studies, the survey of fertilizers incoporated the greatest number of unique samples, quality control tests, and standardized practices, as well as other design improvements. Four laboratories analyzed all of the materials, and some samples were analyzed by additional laboratories. No other materials were found to contain perchlorate at measurable concentrations, and the EPA concluded that the only clearly identifiable fertilizer source of perchlorate was caliche. The data collected in this endeavor were additionally used to evaluate laboratory performance and further validate the method (Urbansky and Collette, 2001). A set of archived samples of all the Phase 2 materials was analyzed while evaluating an alternate ion

chromatographic column and independently verified all of the results reported in U.S. Environmental Protection Agency (2001a) (DeBorba and Urbansky, 2001).

The findings reported in U.S. Environmental Protection Agency (2001a) are the most comprehensive in terms of the types of materials tested, the manufacturers, the number of laboratories analyzing each field sample of material, and the quality control checks. In these regards, it represents our best understanding of fertilizers in terms of perchlorate content. While the presence of perchlorate in the materials gathered in late 1998 through early 1999 remains enigmatic, there is no evidence to support the concern that there is ongoing or routine perchlorate contamination in the U.S. fertilizer supply. Reports in 1999 may have reflected the temporal contamination of one or more raw materials or merely an error in manufacture. Based on the studies reported to date (Collette and Williams, 2000; Gu et al., 2000; Urbansky et al., 2000a; Urbansky et al., 2000b; Robarge et al., 2000; EPA, 2001b; Williams et al., 2001; DeBorba and Urbansky, 2001), there is a consensus among researchers from the EPA, the fertilizer industry, and other federal and state laboratories that currently used fertilizers are negligible contributors to environmental perchlorate contamination. Even imported Chile saltpeter or products derived from it contribute minimally due to their low use and low perchlorate content. Consequently, the EPA has concluded that further investigation is unwarranted (U.S. Environmental Protection Agency, 2001b).

IMC-Agrico, a major North American fertilizer manufacturer, has instituted its own monitoring program for its raw materials and products as a result of continuing interest among the scientific, industrial, and regulatory communities. These products include various potassium ores (langbeinite, sylvinite), potash-based products (potassium chloride, potassium sulfate and potassium magnesium sulfate), and phosphate products (ammonium monohydrogen phosphate, ammonium dihydrogen phosphate and granular triplesuperphosphate). After more than 100 analyses using the latest method (EPA, 2001a), IMC reported to the EPA that no perchlorate was detected in any of the materials it tested during a period spanning nearly three years. In addition, IMC states that it has analyzed Magruder check samples for perchlorate. The Magruder check sample program is jointly administered by the Association of American Plant Food Control Officials and The Fertilizer Institute; it bears the name of a chemist from the F. S. Royster Guano Company named E. W. Magruder, who initiated the program in 1922. The program selects, prepares, and distributes samples of various materials and finished products to subscribing

laboratories and then collects and analyzes the data. Magruder samples reflect monthly snapshots taken from the entire fertilizer industry. Perchlorate has not been detected in any IMC product or any of 16 Magruder samples, according to IMC (personal communication from William L. Hall).

9.1.4 Complicating Factors

It is worth pointing out at the U.S. Geological Survey (USGS) and Air Force Research Laboratories have found perchlorate in isolated samples of sylvite taken from New Mexico (Harvey et al., 1999). The USGS is engaged in additional sampling of North American mining sites in order to assess whether there are natural mineral deposits of potassium perchlorate in sylvite or sylvinite. Because little is known about the mechanisms of perchlorate formation in the natural environment (which are assumed to be meteorological in nature), it is not clear whether these findings represent a low-level background to be expected in evaporite mineral deposits or not. Nonetheless, perchlorate has not been detected in any samples of agricultural grade potassium chloride (0-0-62 or 0-0-60) taken under the direction of the EPA or by IMC-Agrico. Accordingly, it appears that this mineral commodity does not suffer from inclusions of perchlorate salts to any environmentally relevant extent.

Decades ago, ammonium nitrate was prepared from Chilean sodium nitrate by ion exchange rather than by gaseous reactants. It appears that cost began to prohibit this practice for fertilizer-grade ammonium nitrate. Nonetheless, some facilities appear to have continued the practice for explosives-grade ammonium nitrate that was used for blasting in mining operations throughout the American Southwest. It is unlikely that reliable data can be obtained from more than the past 10 years or so. Prior to the establishment of nitric acid and ammonia factories, natural saltpeters played significant roles in American agriculture. Thus, there may be contamination of groundwater in regions where these materials were used historically. The lack of information concerning natural attenuation, as well as a limited knowledge of hydrogeology, makes it difficult to determine where and how such problem sites might be found. For this reason, monitoring for perchlorate under the EPA's Unregulated Contaminant Monitoring Rule can be expected to provide some of the most useful information.

Even though perchlorate was identified in some fertilizer products and was presumably introduced through a contaminated raw material, this incident appears to have been entirely

isolated. Furthermore, awareness within the fertilizer industry and the environmental community is now heightened to the point that it appears unlikely to happen again.

9.2 MONITORING FATE AND TRANSPORT IN LIVING PLANTS

Due to the reported occurrence of perchlorate in certain water resources and in certain fertilizer products, several groups have begun to address the extent and significance of perchlorate uptake by plants. For example, if produce is grown using irrigation water tainted with perchlorate, or if agricultural soil is amended with perchlorate-tainted fertilizer, this might constitute a route of human exposure if perchlorate is taken up and retained in the edible parts of produce plants. The possibility of a relevant exposure route would be increased if perchlorate was found to bioaccumulate and if it was shown to survive the various processes that edible plants undergo before being consumed. Unfortunately, experimental results that definitively gauge the extent of risk from this route of exposure have not yet been published. However, some progress toward this goal has been made.

9.2.1 Difficulties in Analyzing Plant Tissues and Other Environmental Samples for Perchlorate

One problem that has delayed accurate and definitive studies of perchlorate uptake by edible plants is the difficulty of analyzing for perchlorate in plant materials. Ion chromatography is currently the recommended method for routine analysis of inorganic ions such as perchlorate. It is a sensitive, reliable, and easily-implemented technique when perchlorate occurs in a matrix that has a relatively low level of total dissolved solids (TDS). Unfortunately, extracts of plant materials contain high concentrations of TDS, inorganic ions, amino acids, sugars, fatty acids, and nucleotides—all of which contribute to the ionic strength of the sample (Ellington and Evans, 2000). In such matrices with high TDS/ionic strength, other ions can overwhelm the conductivity detector and effectively mask the signal from perchlorate. Ion chromatography is not unique in this regard. Other techniques and methods suitable for reasonably dilute drinking water matrices (Urbansky et al., 2000c; Magnuson et al., 2000a, b; Urbansky et al., 1999; Urbansky and Magunson, 2000) cannot be readily applied to fertilizers or botanical and physiological fluids. The problems of trace ionic analysis have led to the development of other

methods that rely on expensive instrumentation, but are not generally available, such as asymmetric waveform ion mobility mass spectrometry (Handy et al., 2000; Ells et al., 2000) or tandem mass-spec (MS-MS) systems (Koester et al., 2000).

Recently Ellington and Evans (2000) have reported an IC-based method using an enhanced clean-up procedure for the quantitation of perchlorate in plant materials that greatly reduces interferences from dissolved matter. The minimum reporting level (MRL) of perchlorate in lettuce and tomato was found to be approximately 250 μg/g on a wet mass basis. Lettuce and tomato were chosen as representative plants because they are considered high priority candidates for screening foodstuffs (Ellington and Evans, 2000). Perchlorate was spiked into the extraction water for one half of the duplicate freeze-dried samples, while one half were extracted with pure water. In the absence of other ions, some perchlorate is lost to the alumina used for the clean-up; however, this should not impact application of the method to plant material because most extracts have sufficient ionic strength. Note that perchlorate was not detected in any produce, nor was the method applied to any edible plants that were grown with intentional exposure to perchlorate.

9.2.2 Ecological Transport

In the laboratory setting, some plant species will absorb perchlorate when exposed to contaminated irrigation water. Uptake by plants has been explored for possible use in phytoremediation (Nzengung, 1999; 2000). Some investigators have speculated that bacteria are responsible for this phenomenon in plants. Perchlorate-reducing monera have been identified by several laboratories, and cultured from a variety of sources (including Las Vegas Wash sediments, food processing sludge, soils, and sewage sludge); (Logan, 1998; Coates et al., 1999; Coates et al., 2000; Kim and Logan, 2001; Wu et al., 2001; Logan, 2001). Recent work showing perchlorate reduction in saline solution suggests that attenuation may be possible even in briny locations (e.g., the Las Vegas Wash) or in fertilizer-laden farm runoff (Logan et al., 2001; Okeke et al., 2001). This suggests that perchlorate-reducing bacteria are present at significant levels in the environment. On the other hand, the bacteria isolated thus far prefer oxygen over nitrate over perchlorate. In order to for perchlorate reduction to occur, the water must be anoxic and all of the nitrate must have been consumed. Moreover, these bacterial cultures require a suitably moist environment; arid soils or regions with low rainfall may not sustain their growth. Natural

attenuation probably varies around the nation, depending on local factors. Accordingly, it is not possible to draw any meaningful conclusions about the ecological impact of fertilizers that contain perchlorate, for they may or may not be applied in areas where this type of bacterial degradation can occur.

Another factor that has prevented the early materialization of definitive data on risk from perchlorate in edible plants is that many researchers who have addressed plant uptake of perchlorate are primarily interested in other aspects of the problem. For example, Ellington et al. (2001) have applied the optimized IC-based method described above first to the analysis of perchlorate in tobacco plants and tobacco products. Tobacco was chosen because it is grown in some locations in soils amended with Chile saltpeter.

Ellington and Evans (2000) obtained green (uncured) tobacco leaves from the Coastal Plain Experiment Station (CPES) in Tifton, GA in late July 1999. The plants grew in soil that had been amended with two fertilizer products, one of which was Chile saltpeter. The perchlorate level in the Chile saltpeter was 1.5 mg/g, consistent with contemporaneous reports (Urbansky et al., 2001; personal communication from W.P. Robarge). Perchlorate was also found in a 6-6-18 plant food that had been applied to the same soil. While 3% of the nitrogen was from nitrate, the perchlorate concentration was only 36 μg/g; whereas, based on the typical perchlorate content in Chile saltpeter, it should have been about eight times larger if all of the nitrate were from Chile saltpeter. This suggests that synthetic nitrates were also part of the fertilizer's constitution. Perchlorate concentrations in the dried tissue varied from 12.5 to 165 μg/g, depending on the portion of the leaf examined and the curing process employed. Soil samples leached with deionized water contained 0.3 μg/g on a dry weight basis. EPA researchers also analyzed several off-the-shelf cigarettes (2 brands), cigars (1 brand), and chewing tobacco (7 brands) and found perchlorate concentrations ranging from 0.4 to 21.5 μg/g (undried), and only one product that contained none (Wolfe et al., 1999; Ellington et al., 2001). They confirmed the IC results by chlorine NMR spectrometry and capillary electrophoresis. Collectively, these observations argue that tobacco plants can take up perchlorate from perchlorate-contaminated fertilizers via the soil. Furthermore, they indicate the importance of investigating whether crop plants can accumulate perchlorate in their edible portions and whether any contamination can persist through the processing that precedes consumption.

Several groups have looked at the accumulation of perchlorate in various inedible plants as a potential means of fate and remediation. Perchlorate-tainted water from the Las Vegas Wash enters Lake Mead and the Colorado River and therefore has the potential to affect the potable water of many people as well as the irrigation water used for much of the lettuce produced in the U.S. Salt cedar (*Tamarix ramosissima*) is an invasive woody plant that grows prolifically in and around the Las Vegas Wash. Salt cedar consumes and transpires an enormous amount of water when it is actively growing. Furthermore, it accumulates and secretes salt. For these reasons, Urbansky et al., (2000d) have analyzed samples of salt cedar that were taken from the Las Vegas Wash. They found perchlorate at 5-6 μg/g in dry twigs extending above the water and 300 μg/g in stalks immersed in the water from a plant growing in a contaminated stream, suggesting that salt cedar plays a role in the ecological distribution of perchlorate.

Still others have investigated plant uptake with the specific goal of identifying remediation strategies for perchlorate. The biodegradation of perchlorate in woody plants has been investigated as a means of phytoremediation (Nzengung et al., 1999; Nzengung and Wang, 2000). Nzengung et al. (1999) and Nzengung and Wang (2000) found that willow trees (genus *Salix*) were able to decontaminate aqueous solutions containing 10–100 mg/L of perchlorate to below the method detection limit of 2 μg/L and suggest that two distinct phytoprocesses were at work in their studies. Specifically, they observe evidence for rhizodegradation from the exudates released from the plant, and—more importantly from the standpoint of relevance for food safety issues—they see accumulation in branches and leaves. Only about 11% of the perchlorate spiked into the water in which the trees were grown was found to phytodegrade in 26 days. The majority of perchlorate that was removed from solution after 26 days was found in the leafs. Longer term experiments suggest that the perchlorate did not accumulate in the leaves, but was very slowly transformed there as well. Generally, the perchlorate level in the leaves increased to a maximum before decreasing to undetectable levels after perchlorate was completely removed from solution. Nzengung et al. assumed that the phytodegradation pathway of perchlorate leads to chloride. Moreover, Nzengung et al. explored the role of other anions in the removal of perchlorate in solution. They found that the perchlorate removal rate was decreased as the NO_3^- level was increased. This was attributed to competing reactions in which both anions were utilized as electron acceptors. Clearly this has relevance for the food safety issue and should be investigated further. For example, the type of fertilizer used in food crop production may have

an effect on the degree to which perchlorate is taken up, depending on the major components of the fertilizer.

Susarla and coworkers have published results of their investigations on transformation of perchlorate by a wide range of plant types. For example, Susarla et al. (1999b,c) have performed screening studies to determine what species might show potential for further investigations of perchlorate phytoremediation. Thirteen vascular plant species were selected for evaluation in these preliminary experiments. This included four tree species, four herbaceous wetland species, four aquatic species, and one herbaceous upland species. Laboratory-scale experiments were conducted in order to, among other things, evaluate the ability of these plants to remove perchlorate from solution, evaluate the role of nutrients on perchlorate removal, and determine the fate of perchlorate removed form solution (e.g., plant tissue distribution, accumulation versus breakdown). Each of these topics is indirectly relevant to the issue of uptake by edible plants.

For all of these experiments, perchlorate concentrations of 0.2, 2.0 and 20 mg/L were tested in aqueous and sand treatments for ten-day periods. Perchlorate was found to be depleted from solution in the presence of all but two species. Susarla et al. (1999a,c) used a system of five categories to classify the performance of the species based on the degree to which they depleted the solution. None of the trees tested were included in the highest category of performance, but some of the wetland and aquatic plants were. Plant tissue (e.g., roots, stems, leaves) were analyzed from samples that demonstrated the maximum drop in perchlorate concentration. Susarla et al. (1999a,c) report perchlorate, or some transformation metabolite (chlorate, chlorite, chloride), in all tissues analyzed. Results of these studies suggested significant influences on depletion of perchlorate from, among other things, growth substrate (sand versus aqueous treatment), the level of nutrients, stage of plant maturity, and the presence of other ions. All of these influences should prove to be valuable insights when considering the uptake of perchlorate by edible plants. Based on screening studies, additional studies focused on the phytotransformation of perchlorate by the aquatic plant parrot-feather (*Myriophyllum aquaticum*); (Susarla et al., 1999b; Susarla et al., 1999c).

Tobacco is one crop for which the use of Chilean nitrate salts can be documented in some locations. In northern Kentucky, these products are primarily used for seedling beddings rather than fertilizing fields; for various reasons, ammonium nitrate is preferred by many farmers in Kentucky. Such preferences vary throughout tobacco-producing states and regions, however.

Data on application of Chile saltpeter is sparse, and it is not possible to estimate the ecological impact in any meaningful way. There can be no question that at least some vascular plants absorb perchlorate from their local environments. Furthermore, perchlorate has been found in a number of plants and animals living in contaminated environs (Smith et al., 2001). An obvious concern raised by finding measurable perchlorate concentrations in plant tissues is whether this ion can affect food crops and what factors might influence its uptake and accumulations. These issues shall be considered next.

9.2.3 Extrapolating to Food Plants

Because so much U.S. produce is fertilized with perchlorate-free chemical commodities, the risk from exposures via fertilizers is small. Some crops (e.g., corn, wheat, and rice) are fertilized with materials that are unquestionably perchlorate-free. Additionally, there is no reason to suspect any perchlorate associated with growing grains. However, the risk of exposure resulting from irrigation with perchlorate-tainted water in the American Southwest is unknown. At present, there are no efforts to test fruits and vegetables for perchlorate. Many of the studies on uptake by plants have been based on concentrations higher than those encountered in irrigation water. Furthermore, some products derived from Chile saltpeter are known to be among those used on California citrus crops.

One of the few studies of perchlorate uptake by edible plants is the ongoing work of Hutchinson and coworkers with lettuce grown in a greenhouse with perchlorate-tainted irrigation water. Lettuce is of particular importance for assessing the risk of perchlorate to the food supply since much of the lettuce produced in the U.S. is irrigated by water that is fed by the Las Vegas Wash, which is contaminated with perchlorate. Also, lettuce has a high water content and virtually the entire above-ground plant is consumed without cooking or processing. These characteristics would present a potential risk if lettuce efficiently accumulates perchlorate. Hutchinson and coworkers are irrigating lettuce plants with five different concentrations of perchlorate (0.1, 0.5, 1.0, 5.0, and 10.0 μg/L) for a period of 90 days following planting. At various intervals of time they divide the plants into green tissue and root samples and analyze each sample for perchlorate using an analytical method adapted from Ellington and Evans (2000). Their results show an accumulation of perchlorate into the green tissue. The level of perchlorate built up steadily over the first 50–60 days of the experiments, then generally leveled

off. At about 50 days into the experiment, the lettuce irrigated with 10.0 ppm perchlorate exhibits a perchlorate content of about 3 mg/g on a lettuce dry matter basis. Since lettuce is about 90% water, this would amount to about 0.300 mg/g on a wet weight basis. The amount of perchlorate detected in the leaves is generally linear with dosing level for a given day. Experiments are underway to determine whether lettuce has the capability to degrade perchlorate if the supply of the contaminant is stopped. However, this determination is frustrated by the fact that lettuce continues to grow. Therefore, a decline in concentration (e.g., expressed in mg/g) does not adequately reflect the situation. The preliminary results from these studies (Hutchinson et al., 2000) suggest that, when complete, they will constitute considerable progress on the issue of exposure to perchlorate from edible plants.

Even if many food plants can be shown to absorb and retain perchlorate, the primary source of the contaminant is irrigation water polluted from defense-related activities. Because these activities are reasonably localized geographically, most of the country's agricultural products should be perchlorate-free, e.g., corn, wheat, rice, milk. On the other hand, some types of produce are supplied almost entirely by regions dependent on contaminated irrigation water. Therefore, these sites represent possible exposure routes for most of the nation via foods such as lettuce.

Historically, much of the emphasis on fertilizer pollution from agricultural runoff has been on fertilizers applied to the soil. However, potassium nitrate is usually applied to the leaves of citrus trees when a potassium deficiency is found by analyzing leaf tissue. Such foliar application would not necessarily contribute significantly to runoff type pollution of waterways, but could lead to the absorption of contaminants through the leaves and wood. There are no reliable data on the sources of potassium nitrate used for citrus crops. While it is known that absorption of anions similar to perchlorate (e.g., pertechnetate) are affected by the ionic strength and compostion of the surrounding solution, little is known about the factors that influence perchlorate influx via roots or leaves. In addition, the fate of absorbed perchlorate in the plants is also unknown. It may be that xylem-supplied tissues, such as leaves, are the final repository rather than phloem-supplied tissues, such as fruits.

These issues and more have begun to be examined by the EPA, but there are many unknowns (U.S. Environmental Protection Agency, 2001b). Until such time as quantitative studies are performed on various species to determine what factors influence the absorption,

accumulation, and distribution of perchlorate in plants, it is not possible to estimate whether foods can serve as meaningful contribution to the body burden or to the risk posed to humans from perchlorate contamination. Even if they do, there is considerable peace of mind in knowing that fertilizers and water supplies are generally not providing any perchlorate to the plants in the first place. Consequently, only a small number of foods are worth considering for further study. On the other hand, it is not known to what extent other countries rely on natural saltpeters to fertilize food crops. Moreover, it is not known whether fruits and vegetables absorb and retain the perchlorate ion. Therefore, it is not possible to say whether fruits and vegetables grown outside the U.S. serve as a possible exposure routes at this time. Depending on the season, imported oranges, apples, and grapes and their juices are consumed throughout the U.S.

Because there are no data on perchlorate in imported produce, no data on perchlorate in U.S. produce, and no data from controlled laboratory experiments on uptake in fruit crops, it is impossible to assess whether these foods can contribute to perchlorate consumption in humans or whether drinking water constitutes the entire body burden. However, the available information on fertilizers and irrigation water suggests that foods do not contribute to the body burden. At the present time, the available data point towards drinking water as the principal exposure pathway for humans.

9.3 SUMMARY

Despite some initial findings implicating fertilizers as a source of perchlorate, more thorough and better designed studies that were conducted subsequently have not found this to be the case. Current fertilizer manufacturing practices and raw material sources make it unlikely that perchlorate contamination could occur widely and without discovery. While some plants may absorb or even accumulate perchlorate in specific tissues, there are many unknowns with regard to the edible portions of nutritionally and agriculturally important crops. Many factors influence transport of ions, and current understandings of plant physiology and botany suggest perchlorate uptake would be reduced as a result of such factors. Even if perchlorate uptake occurred in some food crops, perchlorate contamination is localized geographically outside of major agricultural regions, minimizing possibility of uptake in edible produce. While perchlorate-tainted irrigation water may be a source available for uptake of perchlorate by plants,

this is again localized, and has not been proven to occur at the concentrations of perchlorate that are observed environmentally. Difficulties in analyzing many plant or animal tissues originally were obstacles to executing appropriate studies, but these problems have generally been solved. Ideally, more data would be available on food plants, but current evidence suggests that drinking water is the primary exposure pathway to perchlorate for humans.

10. MAJOR RISK CHARACTERIZATION CONCLUSIONS

10.1 HUMAN HEALTH

This section summarizes major findings regarding human health presented in Chapters 1, 2, 3, 4, 5, and 7.

10.1.1 Hazard Potential

Perchlorate is an anion that originates as a contaminant in ground and surface waters from the dissolution of ammonium, potassium, magnesium, or sodium salts. Ammonium perchlorate is the oxidizer and primary ingredient in solid propellant for rocket motors. Perchlorate salts also are used on a large scale as a component of air bag inflators and in the manufacture of pyrotechnics and explosives. Solid rocket inventories are growing at a significant rate as systems reach the end of their service life: the solid rocket disposal inventory is expected to be over 164 million lb by the year 2005. Because the accepted method for removal and recovery of solid rocket propellant is high-pressure water washout, a large amount of aqueous solution containing ammonium perchlorate is generated. A number of locations where perchlorate has been detected in groundwater or surface waters are in areas associated with the development, testing, or manufacturing of aerospace materials. Perchlorate contamination also occurs when explosives are used extensively, e.g., open burn/open detonation operations and some mining activities.

Perchlorate is rapidly absorbed from the gastrointestinal tract, whereas dermal and inhalation exposures are not expected to be significant exposure routes for the general public. The known mode of action for perchlorate is that it acts as a competitive inhibitor of active iodide uptake by the *sodium (Na^+)-iodide (I^-) symporter (NIS)* in most mammals, including humans, laboratory test species, and wildlife. This decrease in intrathyroidal iodide results in a decreased production of T3 and T4 thyroid hormones. Decrements in thyroid hormones can cause permanent neurodevelopmental deficits and impair adult organisms as well. A decrease in thyroid hormones can also potentially perturb the hypothalamic-pituitary-thyroid axis to increase the pituitary's production of TSH and, consequently, stimulate the thyroid to increase production

of thyroid hormone in an attempt to compensate. Prolonged stimulation of the thyroid by TSH may result in thyroid neoplasia, particularly in rodents known to be sensitive. Tumors have occurred in rats dosed with high levels of perchlorate for long periods and at much lower doses in relatively young adult animals (19 weeks) dosed *in utero* and during development. These findings have raised concerns about the *in utero* imprinting of the regulatory system responsible for controlling thyroid hormone economy.

The target tissue for systemic effects of perchlorate has been identified as the thyroid. The key event of its mode of action is iodide uptake inhibition at the NIS. Changes in the thyroid hormone homeostasis result in histopathological changes in the thyroid, including: colloid depletion, follicular hypertrophy, follicular hyperplasia, and decrease in follicular lumen size. If perchlorate exposure is stopped, the thyroid histopathological effects have been shown to be reversible after exposures as long as 90-days in rats, but incomplete recovery of thyroid hormones occurs in this same time period. There are also some case studies in humans treated therapeutically with perchlorate that indicate reversibility of thyroid hormone changes after years of exposure.

Other potentially adverse and permanent effects from decreased thyroid hormone include effects during development *in utero* and early growth, particularly effects on the nervous system if the pregnant mother was hypothyroxinemic or hypothyroid. Laboratory animal assays performed in response to recommendations made at the peer review in 1999 and as part of the perchlorate testing strategy confirmed neurodevelopmental effects observed in previous studies. Changes in brain morphometry and motor activity were observed. The potential for major disturbances in thyroid hormone homeostasis to disturb reproductive capacity or to induce immune effects also exists. The ability of perchlorate to cause contact hypersensitivity is suggested but remains not well characterized. Finally, a remarkable conservation of the thyroid hormone regulatory system has been demonstrated across species. Inhibition of iodide uptake by the NIS has been shown in pharmacokinetic studies to be very similar across species, including humans.

10.1.2 Dose Response

The revised RfD is based on an assessment that reviewed a set of studies that were developed to explicitly evaluate these potential toxicities. The quantitative estimate of risk is

based on laboratory animal data because there are no good observational epidemiological data concerning human subjects representative of the critical sensitive populations (hypothyroxinemic pregnant women or children) or that have evaluated neurodevelopmental outcomes; nor have adequate clinical studies been performed. A harmonized approach was proposed based on the key event of iodide inhibition and its relationship to disturbances in the hypothalamic-pituitary-thyroid axis as evidenced by effects on thyroid and pituitary hormones, thyroid histopathology, and brain morphometry. Using these precursor lesions as the basis for the point-of-departure is considered to be protective for cancer development as well as for neurodevelopmental sequelae.

The database supported a point-of-departure for the RfD deviation at 0.01 mg/kg-day based on changes in maternal thyroid and pituitary hormones and on changes in the brain morphometry and thyroid and pituitary hormones of fetal and neonatal pups. A composite uncertainty factor of 300 was applied in the derivation. An adjustment also was made for administration of perchlorate as ammonium perchlorate. The RfD is for perchlorate as the anion because that is what is sampled and analyzed in environmental media and because the salts of perchlorate readily dissolve. Uncertainty factors were applied for intrahuman extrapolation, the use of a LOAEL, concern regarding the lack of studies of longer duration and database deficiencies. Confidence in the study, the database, and the RfD is rated as medium. A major uncertainty is the sensitivity that the screening neurodevelopmental studies provide to protect against neuropsychological deficits of exposures that might occur within critical developmental windows or in susceptible human populations.

The daily perchlorate exposure to the human population that is likely to be without appreciable risk of either cancer or noncancer toxicity during a lifetime is 0.00003 mg perchlorate/kg-day. It again is noted that this RfD is specific for the anion because that is what is detected in most environmental samples and because most salts of perchlorate readily dissolve. Because of the application of uncertainty factors, this dose is approximately 1/300 of the dose that resulted in brain morphometry and thyroid changes in pups and hypothyroid status (decreased T4 and increased TSH) in rat mothers (Argus Research Laboratories Inc, 2001) and in their pups both during gestation (GD21) and in the post-natal period (PND4 through PND21).

10.1.3 Risk Characterization

Comprehensive risk characterization for the perchlorate contamination issue, as discussed in Chapter 1 (see Figure 1-5), requires accurate information on exposure levels determined by a validated analytical method. Dose-response estimates such as the value derived herein can then be used to gauge the potential toxicity of those exposures. Exposure can be either direct, most likely by ingestion, or indirect, such as by consumption of contaminated food. When using the dose-response assessment derived herein to compare with exposure estimates, one should remain keenly aware that many of these exposure aspects have not yet been characterized accurately for perchlorate. Fate and transport information do not exist to track the spatial and temporal distribution of perchlorate; the potential for evaporative concentration in soils has not been characterized, nor has its uptake in plants or herbivores. In addition, there are uncertainties remaining in the dose-response estimate itself. These concerns also should be considered whenever attempting to characterize the risk to a specific human population exposed to a particular scenario.

10.1.3.1 Direct Exposures

Typically the RfD is used as a comparison for oral ingestion, such as by drinking water. The RfD is compared with an exposure estimate of the drinking water concentration to characterize potential toxicity. When making this comparison, the assumptions underlying derivation of the RfD must be kept in mind. The RfD is intended to be protective of susceptible populations exposed daily. The frequency and magnitude of exposure is a key attribute of accurate dose-response characterization (Jarabek, 1995c) and an equally important component of risk characterization. Transient decreases in T4 can cause permanent neurodevelopmental deficits. Thus, the degree to which the particular suspected population at risk fits with the underlying assumptions of the RfD derivation should be kept in mind. Finally, the degree of imprecision in the derivation of an RfD should be taken into account. The RfD estimates are not intended to serve as “bright line” estimates. By definition, there is an order of magnitude uncertainty around the estimate. This generally translates into a range of approximately three-fold below to three-fold above the RfD, but also depends on the nature of the effects used as the basis.

10.1.3.2 Indirect Exposures

Where crops are irrigated with perchlorate-contaminated water, indirect human exposures may result. A number of factors need to be considered in estimating human exposure through crops.

Concentration in plant parts as a result of root uptake normally is calculated using a soil-to-plant transfer factor that is expressed as the ratio of plant to soil concentration. If perchlorate is subject to evaporative concentration in irrigated soils, then soil concentration, and therefore uptake, may be higher than that expected simply based on concentration in irrigation water. If a leaf crop such as lettuce is spray-irrigated, perchlorate could be concentrated evaporatively on external leaf surfaces. Because perchlorate salts have high water solubility, this contamination probably would be removed largely by washing. On the other hand, if perchlorate is phytodegraded, as one study has suggested (Nzengung, n.d.), soil or plant concentrations may be lower than otherwise expected. Studies are needed to determine the behavior and fate of perchlorate in plant-soil-water systems, including studies that simulate leaf crop irrigation and that account for full life cycles of crops.

Besides estimates of perchlorate concentrations in crops, the calculation of human daily intake depends on the number of crop types that are contaminated, the extent to which a particular individual obtains the crops from a contaminated source, and the individual's daily consumption of the crops. These factors may vary widely in the exposed population, and methods for accounting for the combined variability should be used in characterizing these exposures.

Methods for estimating human exposures resulting from crop uptake of soil-deposited contaminants are presented in Chapters 6 (Determining Exposure Through the Terrestrial Food Chain) and 10 (Risk Assessment) of the EPA document, "Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions (EPA 600/ R-98/137)." That document currently is undergoing revision and is scheduled for final release in January 2002. If the needed information can be obtained on perchlorate behavior and fate, the methods described therein can be used to develop estimates of human exposure and risk.

10.1.4 Major Uncertainties and Research Needs

Reliable exposure estimates are required to accurately and comprehensively characterize the risk of perchlorate contamination. This section will briefly summarize research needs associated with aspects of uncertainty about the human health risk dose-response estimate that were highlighted in Chapter 7.

The greatest need for continued improvement in the dose-response assessment is a more accurate characterization of the linkage between the key event of the mode of action (i.e., inhibition of iodide uptake in the thyroid gland), subsequent changes in thyroid hormones, and the correlation to outcome measures in hypothyroxinemic pregnant animals and their pups. Because this need must be addressed in the fetal compartment as well, accurate characterization of toxicokinetics during pregnancy and lactation also are required. More definitive studies of the degree of change in perturbation of the hypothalamic-pituitary-thyroid axis (i.e., change in hormone levels) that is associated with thyroid histology, and with neurobehavioral deficits especially, would improve the confidence in the accuracy of the exposure-dose-response continuum. The current studies may need to be repeated with larger sample sizes and lower doses, and new studies may be needed to evaluate effects on fetal hormone levels and neurodevelopmental measures both in the laboratory and in a survey of the human population. Research on potential factors influencing sensitivity is also critically requisite. Animal models of thyroid impairment such as iodide deficiency and "womb to tomb" exposure designs should be explored. Finally, mechanistic determinants of these toxicokinetic and toxicodynamic parameters and processes should be further characterized.

10.2 ECOTOXICOLOGY

10.2.1 Aquatic Life

Procedures for deriving Tier II water quality values were used in Section 8.3.2.1 to jointly characterize the potential effects of the perchlorate ion on the richness and productivity of fish, aquatic invertebrate, and plant communities. Tier II values are derived when data are not sufficient for deriving ambient water quality criteria. The Tier II value derivation procedures account for missing information with approximately 80% confidence. In this case, the Tier II

values derived, termed secondary acute and chronic values, were 5 and 0.6 mg/L (i.e., 5,000 and 600 μg/L), respectively; difficulties associated with the interpretation of one test result in an uncertainty range for the secondary chronic value of < 0.32 to > 0.83 mg/L (< 320 to > 830 μg/L). Perchlorate levels reported for large surface waters (as high as 16 μg/L) and ground waters (as high as 280 μg/L in public supply wells) are well below the secondary acute and chronic values. Thus, at these exposure levels, the likelihood of effects on the richness and productivity of fish, aquatic invertebrate, and plant communities appears to be low. However, because much higher perchlorate concentrations have been reported in monitoring wells at rocket motor manufacturing or testing sites (37×10^6 μg/L) and in groundwater-dominated surface water systems close to sites of contamination (3500 to 1.3×10^5 μg/L), sites clearly exist that have perchlorate concentrations high enough to cause toxicity to aquatic life. These sites include springs, such as that sampled along Las Vegas Wash in Nevada (Parsons, 2001) and the INF Pond at Longhorn Army Ammunition Plant in Texas (Parsons, 2001; Smith et al., 2001). On the other hand, concentrations below the Tier II values were detected in larger water bodies immediately adjacent to sites of contaminations, such as in Lake Mead immediately adjacent to the mouth of the Las Vegas Wash (less than 4 to 68 μg/L). Water discharged from a CERCLA groundwater pump-and-treat facility that was not equipped to treat perchlorate at Allegany Ballistics Laboratory to the North Branch Potomac River contained 250 to 280 μg/L perchlorate (Parsons, 2001).

Where high levels of contamination exist, sensitive aquatic organisms such as daphnids may be the most likely to experience effects; in the reported tests, effects were seen on both survival and reproduction (neonates per organism). A teratogenicity assay, FETAX, showed malformations in frog embryos occurring at only slightly lower concentrations than lethality, indicating that perchlorate is probably not a potent developmental toxicant. Tier II values are not estimated for plants, but results from algal toxicity tests suggest that even at the higher perchlorate concentrations associated with rocket motor manufacturing, risk of toxicity to aquatic plants is low.

The perchlorate anion can be associated with various cations including sodium, ammonium, and potassium. When sodium perchlorate was tested, the sodium cation was not toxic to daphnids in sodium chloride control tests but did show toxicity to minnows. Ammonium controls were not used in tests with ammonium perchlorate, but ammonium ion is a

known toxicant with toxicity that varies according to water temperature and pH. In any aquatic system where perchlorate is present, attention should be given to determining the concentrations of potentially toxic cations that may contribute to ecological effects.

Based on a secondary chronic value of 600 μg/L (uncertainty range, < 320 to > 830 μg/L) for perchlorate, the analytical detection methods for perchlorate in water are sufficient. The detection limit achieved for perchlorate in water was 4 μg/L (Parsons, 2001; Smith et al., 2001), which is much less than the secondary chronic value. Thus, the likelihood that adverse ecological effects will occur below detection limits is low.

10.2.2 Risks to Consumers of Aquatic Life

Information from Parsons (2001) and Smith et al. (2001) indicate that perchlorate may bioaccumulate in aquatic invertebrates and fish in contaminated waters, but perchlorate is not expected to bioconcentrate in these organisms to levels exceeding the surface water concentrations. Therefore, there currently is no indication that consumers of aquatic invertebrates or fish are at increased risk of effects from bioconcentration in areas where perchlorate concentrations in surface water occur. However, there is some uncertainty about the potential for bioaccumulation of perchlorate at low concentrations (i.e., 4 to 300 μg/L in water) because of the higher detection limits for perchlorate in animal tissues, which were 300 to 400 μg/kg in Parsons (2001) and about 70 μg/kg in Smith et al. (2001). Furthermore, perchlorate may bioconcentrate (i.e., to levels exceeding those in water) in aquatic plants; therefore, consumers of aquatic plants may be at greater risk than consumers of aquatic invertebrates or fish, but information is not available concerning effect levels in aquatic herbivores.

10.2.3 Terrestrial Life

10.2.3.1 Plants

Terrestrial plants may be exposed to perchlorate in soil at disposal sites and at sites irrigated with contaminated surface water or groundwater. Perchlorate concentrations in soil at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001) and can be higher than the screening benchmark of 4 mg/kg and even higher than the lethal concentrations ($\geq$180 mg/kg; EA Engineering, Science and Technology, Inc., 1998).

In the absence of reliable information concerning the accumulation of perchlorate in irrigated soils, it may be assumed that soil concentrations equal irrigation-water concentrations (Section 8.3.1.3). Reported surface-water concentrations in the Colorado River, 4 to 16 μg/L, would translate to 0.004 to 0.016 mg/kg. At the Yuma site, there was a single detection in surface soil of 0.090 mg/kg; all other measurements were below the detection limits of 0.079 to 0.080 mg/kg (Parsons, 2001). Even the single detected concentration is a factor of 44 lower than the benchmark value. The reported groundwater concentration in public wells of 280 μg/L would translate to 0.28 mg/kg, which is a factor of 14 lower than the benchmark value. Hence, perchlorate does not appear to constitute a hazard to plants irrigated with surface water. However, given the large uncertainties concerning exposure, a hazard from groundwater irrigation cannot be precluded.

Based on this screening benchmark of 4 mg/kg for perchlorate, the analytical detection methods for perchlorate in soil are sufficient for determining whether soils will cause toxicity to plants, and there is little likelihood of adverse ecological effects occurring at levels below detection limits. The detection limit achieved for perchlorate in soils was generally 75-80 μg/kg (Parsons, 2001), but there was at least one soil sample where the reporting limit was 803 μg/kg. However, all of these limits are less than the screening benchmark.

10.2.3.2 Soil Invertebrates

Soil invertebrates may be exposed to perchlorate in soil at disposal sites and at sites irrigated with contaminated surface water or groundwater. Perchlorate concentration measurements at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001) and, therefore, can exceed the soil screening benchmark of 1 mg/kg. In the absence of reliable information concerning the accumulation of perchlorate in irrigated soils, it may be assumed that soil concentrations equal irrigation water concentrations (Section 8.3.1.3). Reported surface water concentrations in the Colorado River, 4 to 16 μg/L, would translate to 0.004 to 0.016 mg/kg in soils. At the Yuma site, the single detection in surface soil was 0.090 mg/kg with detection limits of 0.079 to 0.080 mg/kg. This detected concentration is a factor of 11 lower than the soil screening benchmark value (1 mg/kg). The reported groundwater concentration in public wells of 280 μg/L would translate to 0.28 mg/kg, which is a factor of 4 lower than the benchmark value. Hence, perchlorate does not appear to constitute a hazard to soil invertebrates

in soil irrigated with surface water. However, given the large uncertainties concerning exposure, a hazard from groundwater irrigation cannot be precluded.

Based on this screening benchmark of 1 mg/kg for perchlorate, the analytical detection methods for perchlorate in soil are sufficient, and there is little likelihood of adverse ecological effects occurring at levels below detection limits. The detection limit achieved for perchlorate in soils was generally 75-80 μg/kg (Parsons, 2001), but there was at least one soil sample where the reporting limit was 803 μg/kg. However, all of these limits are less than this screening benchmark.

10.2.3.3 Herbivores

Exposures of voles to perchlorate based on measured plant concentrations at rocket motor manufacturing or testing sites (0.11 mg/kg day to a maximum of 49 mg/kg day) exceed both the LOAEL of 0.01 mg/kg/day and the screening benchmark of 0.001 mg/kg day**.** Estimated exposures of voles consuming plants on sites irrigated with surface water (0.18 mg/kg day) and groundwater (3.2 mg/kg day) also exceed the LOAEL and the screening benchmark. Hence, there is a potential hazard to all herbivorous wildlife living in areas that may be irrigated with contaminated water. At disposal sites, wildlife would be at risk from the effects of loss of food and habitat from toxic effects on plants, as well as the potential for direct toxic effects via consumption of perchlorate-tainted food or water.

Assuming a water ingestion rate of 0.21 g/g-day (U.S. EPA, 1993a,b), the screening benchmark for herbivores is equivalent to a water concentration of 4.8 μg/L. Perchlorate levels reported for large surface waters (as high as 16 μg/L) are greater than this concentration. Much higher perchlorate concentrations have been reported in monitoring wells at rocket motor manufacturing or testing sites (37×10^6 μg/L) and in groundwater-dominated surface water systems close to sites of contamination (3500 to 1.3×10^5 μg/L), and rodent exposures via drinking water at these sites would exceed the rodent NOAEL.

Based on screening level benchmarks for herbivores, the analytical detection methods for perchlorate in plant tissues may not be sufficient for the detection of concentrations potentially toxic to herbivores even though the analytical detection methods for perchlorate in water are sufficient. The detection limits achieved for perchlorate in water and in plant tissues were 4 μg/L and 0.4 mg/kg, respectively (Parsons, 2001; Smith et al., 2001).

10.2.3.4 Carnivores

Available evidence indicates that concentrations in terrestrial invertebrates are less than the concentrations in plants and similar to that in soils. As a result, there currently is no indication that terrestrial carnivores are at additional risk from perchlorate. Risks of direct toxic effects are therefore lower for carnivores than herbivores. In locations where perchlorate levels are sufficient to significantly affect herbivores, carnivores are more likely to be affected by loss of prey than by perclorate toxicity. Therefore toxic effects are not quantified.

10.2.4 Uncertainties

This discussion of uncertainties is limited to qualitative uncertainties associated with major gaps in the data available for ecological risk assessment of perchlorate. This is because, as with other screening assessments, quantitative uncertainties are treated through the use of conservative assumptions. It is also because the data gaps are the major sources of uncertainty, not imprecision or inaccuracy of the available data.

10.2.4.1 Uncertainties Concerning Aquatic Risks

Aquatic Exposures. The primary uncertainty associated with this assessment of aquatic risks is the paucity of data on perchlorate occurrence in surface waters. For lack of systematic sampling and analysis, the spatial and temporal distribution of perchlorate in water is unknown. It is not certain whether the reported concentrations in water represent the highest existing levels. This is not a large source of uncertainty for this screening assessment if it is assumed that sampling has been biased to areas of highest likely contamination. However, it would be a major source of uncertainty in any subsequent definitive assessment.

Aquatic Effects. While the effects of perchlorate on some species of algae are known, the effects on aquatic macrophytes are unknown. As a result, risks to aquatic primary producers are estimated using only the chronic toxicity test results for the alga *Selenastrum*. Because of physiological differences between algae and vascular plants, effects on aquatic primary producers are not adequately assessed. In addition, it us unknown how or if physiological variations among various species of algae or plants may affect their susceptibility to perchlorate.

Algae, aquatic macrophytes, and terrestrial leaf litter are the bases of food chains in many aquatic ecosystems. Because perchlorate has been shown to concentrate in leaves of terrestrial

plants and aquatic plants, the potential for direct impacts to primary consumers (i.e., planktonic and benthic invertebrate communities) is a concern that could not be addressed in this assessment.

A 35-day, early-life stage (ELS) test with *Pimephales*, generally regarded as a chronic test but short of a full-life-cycle test, showed no significant effects on any standard endpoint (survival, growth or biomass) at the highest concentration tested (490 mg/L). However, all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling, which was not observed in the larvae exposed to the control water. This finding suggests the presence of subtle effects that could be ecologically significant and raises doubt about whether a chronic NOEC has been adequately determined for this species. This uncertainty is displayed as a range surrounding the secondary chronic value (i.e., < 0.32 to > 0.83 mg/L). Because of the inequality signs, even the width of the range is uncertain. For this reason, and because of the potential for chronic effects caused by thyroid dysfunction, chronic effects should be investigated in a full life cycle test.

The uncertainty factors in the secondary chronic value are high because of the lack of test results for aquatic organisms other than fathead minnows, amphipods, and daphnids.

10.2.4.2 Uncertainties Concerning Terrestrial Risks

Terrestrial Exposure. The available data concerning aqueous perchlorate levels is sparse and has not been collected systematically. As a result, the spatial and temporal distribution of perchlorate in irrigation water is unknown. It is not clear that the reported concentrations in water represent the highest existing levels. This is not a major source of uncertainty for this screening assessment if it is assumed that sampling has been biased to areas of highest likely contamination. However, it would be a major source of uncertainty in any subsequent definitive assessment.

The fate of perchlorate in soil, including its tendency for evaporative concentration, is not well characterized. As a result, soil concentrations were assumed to be equal to irrigation water concentrations. This assumption could be low by multiple orders of magnitude if evaporative concentration occurs with perchlorate, as it does with metals. The limited data for irrigated soils near Yuma (Parsons, 2001) do not support the occurrence of such a high degree of evaporative

concentration, but neither are they sufficient to rule out concentration by up to a factor of 10 or so. More information on the fate of perchlorate in irrigated soils is needed.

The bioconcentration of perchlorate by plants suggests that perchlorate may be elevated in leaves and leaf litter to levels that may affect invertebrate herbivores and soil invertebrate communities. For lack of data concerning dietary toxicity, risks to invertebrates by this route were not assessed.

Available toxicity data for rodents suggest that vertebrate herbivores may be sensitive to low levels of perchlorate in plant tissues; concentrations potentially causing toxicity are calculated to be lower than those currently detectable by chemical analyses of plants. In Parsons (2001), detection limits for plants were generally about 0.4 mg/kg wet weight; similar detection limits were achieved by Ellington and Ellis (2000) and Ellington et al. (2001), as compared to an exposure benchmark of 0.01 mg/kg in plant tissue for a representative herbivore (see Section 8.3.2.2). Therefore, lower detection limits for perchlorate in plant tissues may be needed to completely assess the risks to vertebrate herbivores.

Terrestrial Effects. The toxicity of perchlorate to nonmammalian vertebrate wildlife is unknown. As a result, risks to birds, reptiles, and amphibians could not be assessed.

The toxicity of perchlorate to terrestrial invertebrates, other than acute lethality to earthworms, is unknown. As a result, risks to other terrestrial invertebrates were inadequately assessed.

10.2.5 Research Needs

Three questions were asked of the screening ecological risk assessment for perchlorate:

- Are ecological risks best characterized as *de minimis* (exposures clearly are below levels of concern), *de manifestis* (risks are clearly significant and require management action to reduce exposures); or somewhere in between and requiring further characterization?
- Are analytical detection methods for determining levels of perchlorate in the environment sufficient, or is there a likelihood of adverse ecological effects occurring at levels below current detection limits?
- Is the available ecotoxicological information on perchlorate sufficient, or are additional studies needed?

In the immediate vicinity of facilities that were involved in the manufacture, use, or disposal of perchlorate salts, particularly facilities involved in handling of solid rocket propellents, ecological exposure can exceed levels of concern and management actions may be needed to reduce these exposures. Site-specific risk assessments would be needed to guide remediation of such locally contaminated sites. Farther from such facilities, ecological exposures appear to be below levels of concern.

The analytical detection methods for perchlorate are generally sufficient, and there appears to be no indication of adverse ecological effects occurring at levels below detection limits, except that detection limits in plant tissues are not low enough to ensure that risks to herbivores are detected. Additionally, there is some uncertainty about the potential for bioaccumulation at low concentrations of perchlorate in surface water, because of differences in the analytical detection limits between water and animal tissues.

The available ecotoxicological information on perchlorate is sufficient for this screening-level ecological risk assessment. However, additional ecotoxicological studies could reduce the uncertainties about the toxicity of perchlorate to other potential ecological receptors.

While the available information may yield an adequate screening level ecological risk assessment, the following research needs for exposure and effects analysis deserve mention.

10.2.5.1 Exposure

Concerning exposure, at least three important issues remain unresolved:

- Because the available data on accumulation in terrestrial and aquatic vascular plants are from studies that were not designed to quantify accumulation factors, the accumulation of perchlorate in terrestrial and aquatic plants should be further investigated.
- Because of the potential for evaporative concentration, the fate of perchlorate in irrigated soils should be investigated.
- Because the concentrations that have potential for dietary toxicity to vertebrate herbivores are less than the limits of detection currently achievable by chemical analysis of plants, analytical methods for plant tissues that could lower the limits of detection should be investigated.

10.2.5.2 Effects

Also requiring further attention are issues related to the effects of potential perchlorate exposure:

- The effects of exposure of aquatic plants should be determined.
- The effects of exposure of noncrustacean invertebrates should be determined.
- The effects of dietary exposure to perchlorate should be determined in birds and in herbivorous or litter-feeding invertebrates.
- The effects of dietary and cutaneous exposure to perchlorate should be determined for adult amphibians and aquatic reptiles.
- If perchlorate occurs at significant levels in estuarine systems, its toxicity in saline waters should be determined.

10.2.5.3 Site-Specific Investigations

Some of the research needs that were listed in the previous ERD of this document have been met by the research conducted by the US Air Force IERA (Parsons, 2001) in which perchlorate concentrations in environmental media (i.e., surface soils, surface water, sediments, and pore water) and biological tissues (i.e., terrestrial plants, invertebrates, reptiles, birds, and mammals and aquatic vegetation, invertebrates, fish, amphibians, reptiles, and birds) were surveyed at six sites with known perchlorate contamination. These data are supplemented by additional sampling at one of the sites, Longhorn Army Ammunition Plant in Texas, by Smith et al. (2001). These studies do address some questions about exposure that were expressed in the previous ERD of this document (U.S. EPA, 1998d), i.e:

- Because concentrations of perchlorate in water are poorly known, and concentrations in soil and biota are unknown, a survey of perchlorate contamination should be conducted.
- Because, contrary to expectations, perchlorate accumulates to high concentrations in terrestrial vascular plants, the accumulation of perchlorate in aquatic plants and in animals should be investigated.

However, these studies were screening-level surveys that took small numbers of samples during limited periods of time. In addition, the studies were not designed to address questions about the effects of exposure. In some locations, concentrations in environmental media were high enough

that toxicity to ecological receptors was highly likely (i.e., the risks were *de manifestis*), and in other locations toxicity could not be ruled out (i.e., the risks could not be termed *de minimus*). Therefore, systematic sampling is needed in these locations to more definitively quantify exposures and effects, so that the likelihood, nature and extent of ecological risks may be quantified, appropriate remedial alternatives may be designed, and effectiveness of site cleanup may be judged. In addition, site surveys may be required in other locations where perchlorate contamination is suspected.

10.3 CHARACTERIZATION PROGRESS SUMMARY

Despite the fact that the appreciation of widespread perchlorate contamination emerged only five years ago, considerable progress has been made in hazard identification and quantitative dose-response characterization for both the human health and ecotoxicological risks of potential perchlorate exposures. The thyroid has been confirmed as the target tissue in humans, laboratory animals, and wildlife. The key event of the mode of action for perchlorate is iodide uptake inhibition at the NIS with the potential for both subsequent neurodevelopmental and neoplastic sequelae. A harmonized human health reference dose has been proposed to be protective for both sequelae based on a mode of action model. Data insufficiencies for various ecotoxicological receptors and for accurate exposure estimates precludes other than a screening-level assessment at this time. Additional research is needed to determine the contribution of exposure sources other than drinking water. This requires more progress in the area of analytical methods to extend current approaches to other media.

As with any risk assessment, additional insights and new research will continue to change our understanding as the knowledge base is informed with new data and as the scientific and technical areas relevant to the particular risk characterization mature and evolve. Work dedicated to the areas defined in this chapter should allow continued improvement of the risk characterizations for perchlorate in the future.

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APPENDIX A

Schematics of Study Designs for Neurodevelopmental, Two-Generation Reproductive and Developmental Studies

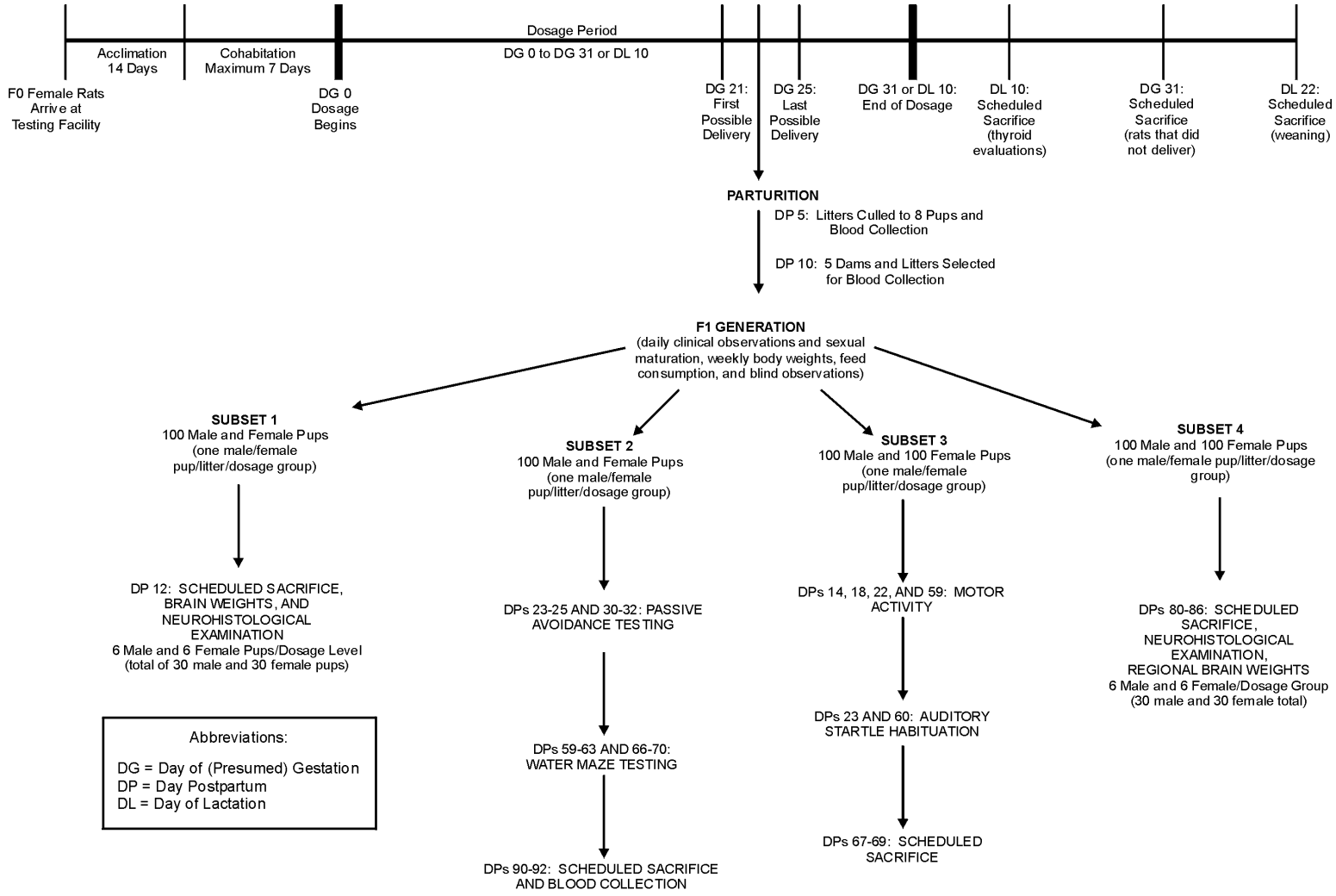


Figure A-1. Schematic of the neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to SD rats (Argus Research Laboratories, Inc., 1998a).

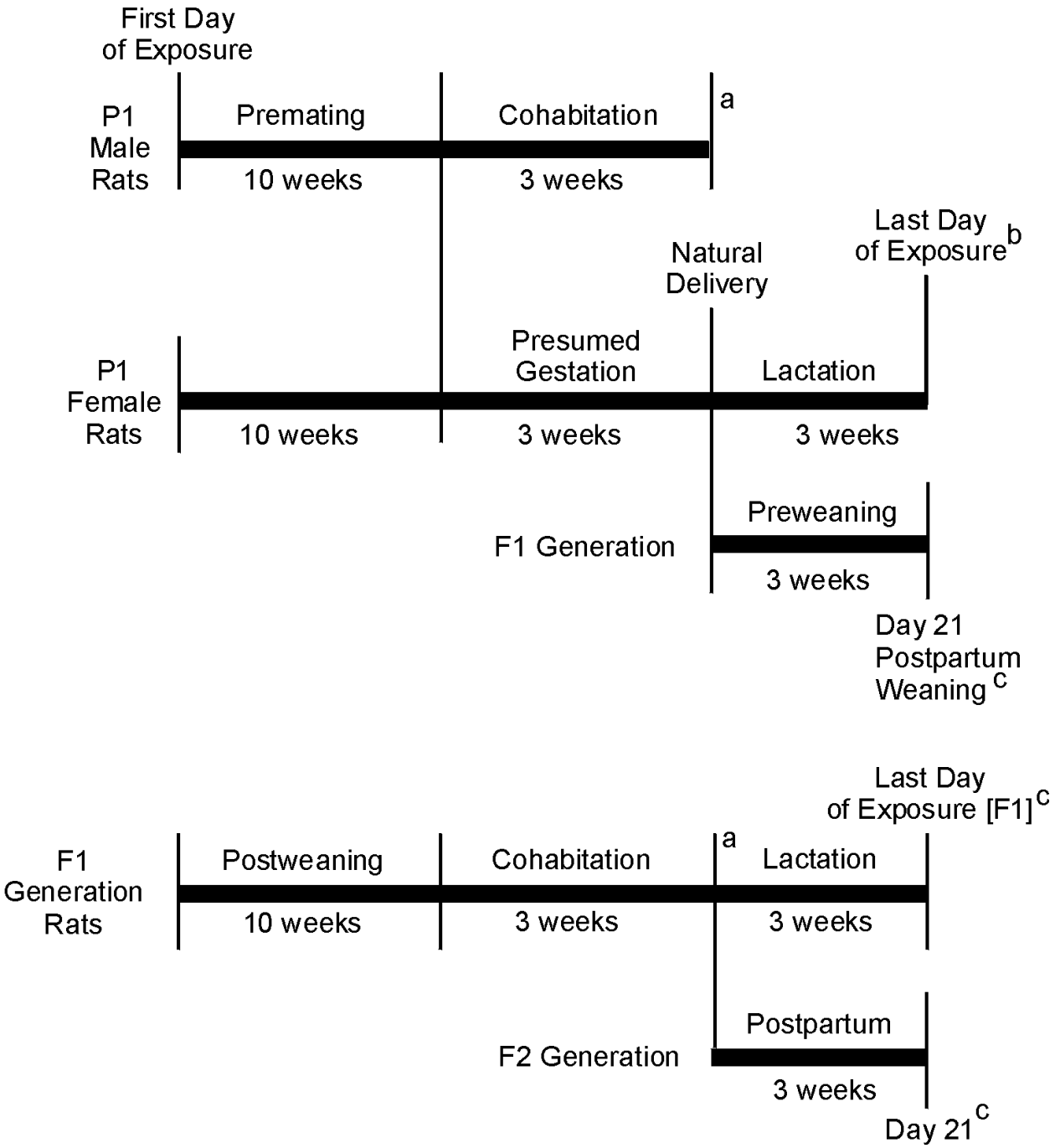
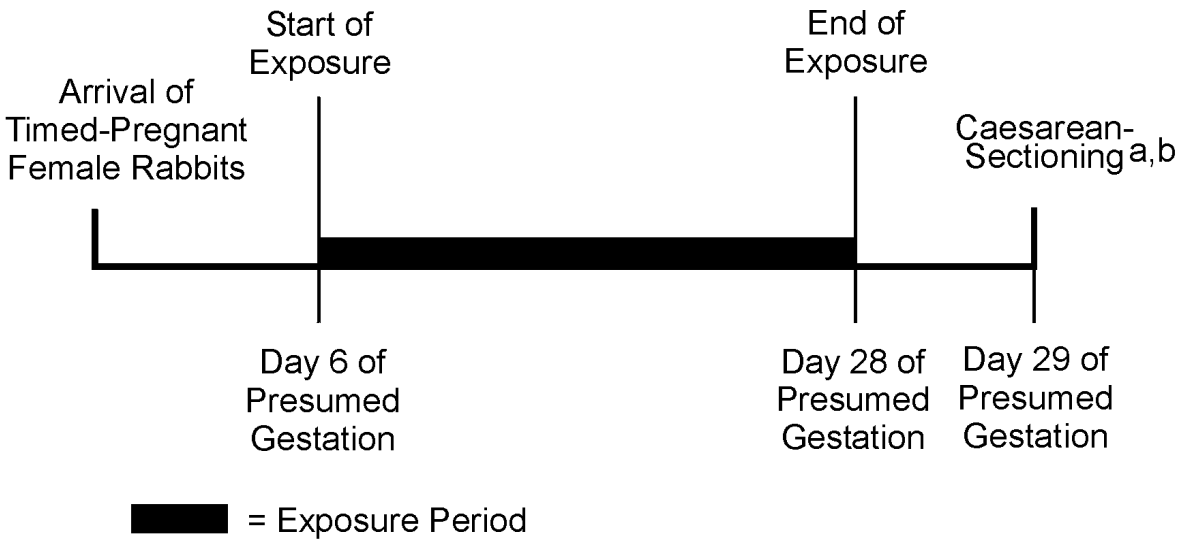


Figure A-2. Schematic of the oral (drinking water), two-generation (one litter per generation) reproduction study of ammonium perchlorate in SD rats (Argus Research Laboratories, Inc., 1998b).



a = Blood samples taken from does for thyroid and pituitary hormone (T3, T4, TSH) analyses.
b = Fetal evaluations (external examinations and soft tissue and skeletal examinations).

Figure A-3. Schematic of the oral (drinking water) developmental toxicity study of ammonium perchlorate in New Zealand rabbits (Argus Research Laboratories, Inc., 1998c).

APPENDIX B

List of Acronyms and Abbreviations

Acronym	Definition
$\Delta A°_{rxn}$	Helmholtz free energy of reaction
$\Delta G°_{f}$	Gibbs free energy of formation
$\Delta G°_{rxn}$	Gibbs free energy of reaction
$\Delta S°_{univ}$	net entropy of the universe
a-p	anterior-posterior
Ab	antibody
ACSL	advanced continuous simulation language
ADHD	attention deficit hyperactivity disorder
ADME	absorption, distribution, metabolism, and elimination
AFB	air force base
AFRL	U.S. Air Force Research Laboratories
AFRL/HEST	Air Force Research Laboratory/Human Effectiveness Directorate
AIDS	acquired immunodeficiency syndrome
AITD	autoimmune thyroid disease
ANCOVA	analysis of covariance
AP	ammonium perchlorate
ATP	adenosine triphosphate
AUC	area-under-the-curve
AV	acute value
AWQC	ambient water quality criteria
BF_4^-	tetrafluoroborate
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMR	benchmark response

Acronym	Definition
BW	body weight
C’	complement
CA DHS	California Department of Health Services
cAMP	cyclic adenosine monophosphate
CBC	complete blood count
CCL	Contaminant Candidate List
CD4/CD8	cluster of differentiation — cellular markers 4 and 8
CDC	Centers for Disease Control and Prevention
CERCLA	Comprehensive Environmental Response Compensation Liability Act
cESI-MS	complexation electrospray ionization mass spectrometry
CFU	colony-forming units
CHS	contact hypersensitivity
ChV	chronic value
Cl_2	chlorine
CI	confidence interval
ClO^-	hypochlorite
ClO_4^-	perchlorate
ClUC-p	perchlorate urinary clearance
CNS	central nervous system
CP	cyclophosphamide
CPES	Coastal Plain Experiment Station
CPM	counts per minute
Cs^+	cesium
CsCl	cesium chloride
CTL	cytotoxic T-lymphocyte
CV	coefficient of variation
DAF	dosimetric adjustment factor

Acronym	Definition
DEQ	Department of Environmental Quality
DIT	diiodotyrosine
DNA	deoxyribonucleic acid
DNCB	dinitrochlorobenzene
DoD	Department of Defense
DoE	Department of Energy
DTH	delayed-type hypersensitivity
DWEL	drinking water equivalent level
E:T	effector to target cell
EAR	estimated average requirement
EGF	epidermal growth factor
ELISA	enzyme linked immunosorbant assay
ELS	early-life stage
EPA	U.S. Environmental Protection Agency
EPL	Experimental Pathology Laboratories, Inc.
ER	endoplasmic reticulum
$E°$	standard electric potential
F	Faraday constant
F1	first generation
F2	second generation
FAVF	Final acute value factor
FCN	function
FETAX	Frog Embryo Teratogenesis Assay: *Xenopus*
FGF	fibroblast growth factor
FH	follicular epithelial cell hypertrophy or hyperplasia
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fT4	free thyroxine

Acronym	Definition
GA	Golgi apparatus
GD	gestation day
GGTP	g-glutamyl transpeptidase
GI	gastrointestinal
GMAV	Genus mean acute value
gsp	GTP-binding protein mutation
Gy	Gray (equal to 100 rads)
H^+	hydrogen
H_2O_2	hydrogen peroxide
hCG	human chorionic gonadotropin
$HClO_4$	perchloric acid
HEE	human equivalent exposure
HOCl	hypochlorous
I^-	iodide
IC	ion chromatography
IC_{25}	quartile inhibitory concentration
ICD-9	International Classification of Diseases, 9th Revision
ID	iodine deficiency
IFN	interferon
IGF-1	insulin-like growth factor
IgG	immunoglobulin G
IgM	immunoglobulin M
ip	intraperitoneally
IPSC	Interagency Perchlorate Steering Committee
IRIS	Integrated Risk Information System
IU	international unit
IUDR	uridine

Acronym	Definition
iv	intravenously
K^+	potassium
K_2O	potassium oxide
Km	Michaelis-Menten affinity constant
KNO_3	potassium nitrate
LC_{50}	concentration lethal to 50% of population
LD	lactation day
LHAAP	Longhorn Army Ammunition Plant
Li^+	lithium
LLNA	local lymph node assay
ln	natural log
LOAEL	lowest-observed-adverse-effect level
LOEC	lowest-observed-effect concentration
LOEL	lowest-observed effect level
LP	lymphoproliferation
LS	Lumen size
LY	lysosomes
M-W RST	Mann-Whitney Rank Sum Test
MCA	3-methyl cholanthrene
MCL	maximum contaminant level
MDL	minimum detection limit
MF	modifying factor
$Mg(ClO_4)_2$	magnesium perchlorate
MIT	monoiodotyrosine
MMIA	1-methyl-2-mercaptoimidazole
MANOVA	multiple analysis of variance
MCLG	maximum contaminant level goal

Acronym	Definition
MRL	minimum reporting limit
mRNA	messenger ribonucleic acid
MS-MS	mass spec — mass spec
MTD	maximum tolerated dose
n	number of electrons or number of moles
n.d.	no date
N-P-K ratio	nitrogen-phosphorous-potassium ratio
Na^+	sodium
$NaClO_4$	sodium perchlorate
$NaNO_3$	sodium nitrate
NAS	National Academy of Sciences
NASA	National Aeronautics and Space Administration
NCE	Normochromatic erythrocyte
NCEA	National Center for Environmental Assessment
NDEP	Nevada Division of Environmental Protection
NERL-ERD	Natural Exposure Research Laboratory's Ecosystems Research Division
NH_4^+	ammonium
NH_4ClO_4	ammonium perchlorate
NH_4NO_3	ammonium nitrate
NHEERL	National Health and Environmental Effects Research Laboratory
NIEHS	National Institute for Environmental Health Sciences
NIS	sodium iodide symporter
NK	natural killer
NMR	nuclear magnetic resinance
NO_3^-	nitrate
NOAEL	No-Observed-Adverse-Effect Level
NOEC	No-Observed-Effect Concentration

Acronym	Definition
NPDWR	National Primary Drinking Water Regulations
NRMRL	National Risk Management Research Laboratory
NTP	National Toxicology Program
O_2	oxygen
OEHHA	Office of Environmental Health Hazard Assessment
OEPP	Office of Emergency Response and Remediation
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response
OW	Office of Water
p	probability
P	pressure
P1	parental generation
P_2O_5	disphosphorus pentoxide
p53	*p53* tumor suppressor gene
PA	prealbumin
PAS	periodic acid shift
PBI	protein-bound iodide
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocyte
PCB	polychlorinated biphenyl
PFC	plaque-forming cell
PHG	public health goal
PII	plasma inorganic iodide
PND	post-natal day
PP	post partum
PP-TH	plasma protein-thyroid hormone

Acronym	Definition
ppb	parts per billion
ppm	parts per million
PQL	practical quantitation limit
PSG	Perchlorate Study Group — consortium of defense contractors
PT-p	thyroid follicle:stroma partition coefficient
PTU	propylthiouracil
PWG	Pathology Work Group
QA/QC	quality assurance/quality control
R	ideal gas constant
RAIU	radioactive iodine uptake
ras	*ras* protooncogene
Rb^+	rubidium
RDA	recommended dietary allowance
RfC	inhalation reference concentration
RfD	oral reference dose
RIA	radioimmunoassay
RL	reproducibility limits
RO	reverse osmosis
r_s	Spearman's rank order
RSC	relative source contribution
rT_3	reverse triiodothyronine
SACR	secondary acute-chronic ratio
SAV	secondary acute value
sc	subcutaneously
SCN	thiocyanate
SCV	secondary chronic value
SD	standard deviation

Acronym	Definition
SD rats	Spraque-Dawley strain
SDWA	Safe Drinking Water Act
SE	standard error of the mean
SGOT	serum glutamyl oxacetic transsaminase
SGPT	serum glutamyl pyruvic transaminase
SLA	soluble *Listeria* antigen
SMCV	species mean chronic value
SNK	Student Newman Keuls
SRBC	sheep red blood cell
SRLB	Sanitation and Radiation Laboratory Branch
T	temperature
T2	diiodothyronine
T3	triiodothyronine
T4	thyroxine or tetraiodothyronine
T4 GLUC	T4-glucuronide conjugate
TBG	thyroid-binding globulin
TCE	trichloroethylene
TDS	total dissolved solids
Tg	thyroglobulin
TH	thyroid hormone
TPO	thyroid peroxidase
TRH	thyrotropin-releasing hormone
TSCA	Toxic Substances Control Act
TSH	thyroid-stimulating hormone
tT4	total thyroxine
UCMR	Unregulated Contaminant Monitoring Rule
UDPGTs	uridine diphosphyl glucuronosyl transferases

Acronym	Definition
UF	uncertainty factor
USAF	United States Air Force
USGS	United States Geological Survey
USN	United States Navy
V	volume
Vmaxc	Michaelis-Menten maximum velocity capacity
W_{exp}	expansion work
WHO	World Health Organization
WPAFB	Wright Patterson Air Force Base
WSWRD	Water Supply and Water Resources Division

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Perchlorate: Not Only Rocket Science

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It's Not Only Rocket Science

The perchlorate story follows the arc of other major controversial chemical management challenges, such as dioxin, where the initial focus on end-of-pipe controls missed key sources in the environment

By Andrew Rak

Andrew Rak is a Manager within Noblis's Center for Sustainability. Noblis is a nonprofit science, technology, and strategy organization based in Falls Church, Virginia.

The ongoing debate over the potential health effects from exposure to low levels of perchlorate is overshadowing an equally important dialogue on the sources of contamination. Perchlorate first became a headline concern when it was discovered that it entered the environment from rocket fuel and munitions at Department of Defense sites — seemingly just another legacy of the Cold War — but it can also be found in fertilizer, bleach, fireworks, road flares, and blasting compounds. It can be present as an ingredient or impurity in lubricating oils, matches, and automotive airbag deployment initiators. It is associated with aluminum, rubber, dye, and paint manufacturing, leather tanning, and pulp processing. Solutions used in water and wastewater treatment plants for disinfection have also been identified as a potential problem.

While some research has addressed the relative importance of multiple sources, the perception that military activities are the overwhelming contributor to the environment may be hindering appropriate and effective regulation. While perfect knowledge about the sources of perchlorate is unobtainable, continuing to focus solely on a few point sources will not adequately spur effective public health regulation.

Extensive toxicological studies have been undertaken related to the potential health risks from exposure to perchlorate, including a National Research Council review of the Environmental Protection Agency's toxicity assessment. The chemical may pose a health risk if taken up by the thyroid, where it disrupts the production of hormones and affects metabolism and neurodevelopment. But toxicity is only part of a risk-based approach to public health. As the NRC famously declared in 1983 in the Red Book — more properly "Risk Assessment in the Federal Government: Managing the Process" — only by combining toxicity data with information about the sources of exposure to a contaminant can a thorough risk-based approach be constructed. In other words, the same scientific rigor and expertise applied to the investigation of a chemical's toxicity should be applied to the identification of sources that lead to significant exposures.

A thorough examination of the sources of exposure to perchlorate has not been completed, and this lack of knowledge hinders attempts by regulatory agencies to provide an effective risk-based approach for protecting public health. Through an

understanding of sources in the environment and releases to the environment, regulatory agencies can take appropriate steps to directly reduce exposure and control public health risk.

While defense activities have often been assumed to be the dominant fraction of perchlorate's environmental presence, military contributions alone cannot be the explanation for the geographically dispersed detection of the compound. Nor can military sources be responsible for the perchlorate detected in a wide variety of food stuffs, including dairy items and produce. Sampling of dairy products from Maryland and organic lettuce from Wisconsin have found trace amounts of perchlorate; however, any direct connection to military point sources at these locations is lacking, thereby suggesting the importance of other contributors.

To holistically address the risks a more comprehensive assessment of the sources contributing to exposures is necessary

Early impressions about perchlorate sources helped federal and state regulators develop preliminary plans for addressing contamination. However, more complete evaluations may increase the focus on newly identified sources. In a 2005 letter to the federal Environmental Protection Agency, state regulatory officials wrote that "none of the nine water supplies that have tested positive for perchlorate in Massachusetts appear to have any connection to military bases or activities." The use of perchlorate-containing explosives in construction activities, and even in bleach, were among the new sources identified by the state. Earlier this year, Maine Drilling and Blasting agreed to contribute $1 million to the city of Westford, Massachusetts, for remediating perchlorate associated with construction explosives. Grappling with the risk from other sources is just a matter of time.

The advent of new scientific fingerprinting methods that distinguish natural from man-made sources will allow regulatory managers to tackle this multi-faceted source challenge with targeted risk reduction measures. As new sources are identified and quantified, lingering misperceptions about the role of point and non-point sources of perchlorate should be displaced.

The perchlorate story follows the arc of other major controversial chemical management challenges, such as dioxin, where the initial focus on end-of-pipe controls from incinerators and chlorine, and pesticide production proved to miss key sources in the environment. During the multi-decade interagency and public debate over the appropriate health risk estimates for dioxin, EPA issued a pivotal emissions report. In 2000, the emissions inventory analysis for dioxin found that backyard burning of plastics and other waste in rural areas was a major remaining non-point source of deposition to soil. This surprising finding shifted the regulatory debate and highlighted areas where the traditional focus on point sources and end-of-pipe enforcement approaches could not be applied. Any new reduction strategy for dioxin emissions would likely have to include multiple non-point sources in order to be effective. The same dynamic appears to be developing for perchlorate.

While early data helped regulatory agencies focus on perchlorate as an emerging problem, to holistically address its risks a more comprehensive assessment of the sources contributing to environmental exposures is necessary. With improved sampling technology and the additional data that are being generated relating to dietary and other exposure routes, food safety regulators, environmental agencies, and local governments will be able to more effectively regulate the use, disposal, and cleanup of perchlorate, and reduce public exposure. Information on unusual suspects will increase the government's grasp of the problem and enhance risk mitigation as our understanding advances.

At the federal level, EPA has elected to address

exposure to perchlorate in groundwater and drinking water using authorities under Superfund and the Safe Drinking Water Act. Although the agency has yet to make a final regulatory determination for perchlorate in drinking water, some state regulatory agencies have already promulgated regulations, with California and Massachusetts setting enforceable limits of 6 parts per billion and 2 parts per billion, respectively. However, it is not clear if these regulations will result in a meaningful public health benefit because we do not know what role drinking water plays in overall exposure to perchlorate. Addressing the need for appropriate regulation requires a more complete understanding of the sources (and relative amounts) of synthetic and natural perchlorate in the environment.

While EPA has elected to address perchlorate in groundwater and drinking water, other regulatory agencies and researchers have paid attention to potentially more widespread exposures in the diet. Ongoing food monitoring surveys have found perchlorate in mothers' milk and cows' milk, as well as in infant-formula. Perchlorate has also been found in lettuce, spinach, cantaloupe, cabbages, and beets. The Food and Drug Administration has completed studies of the occurrence of perchlorate in food items, but has yet to issue any health advisories. Likewise the Department of Agriculture conducts monitoring through its National Residue Program. But an analysis of the relative sources of perchlorate and how these sources may influence entry into the food chain has not been conducted. In the future, the NRP may generate key opportunities for risk reductions should trends related to perchlorate in food warrant mitigation.

A continued focus on military sources will not provide a complete analysis of the sources of perchlorate. The defense establishment uses perchlorate in numerous items as an oxidizer. While the military continues to purchase large amounts of perchlorate for solid rocket motors and other items (6.2–8.1 million pounds per year from 2004–2006), solid perchlorate inside a rocket motor casing is unavailable for release to the environment. Industrial recycling of rocket motors, which previously released wash water into the environment, is now a closed loop system. Annual military training uses of perchlorate in detonators, initiators, and ground burst simulators, which are confined to certain ranges, is approximately 1.6 million pounds, and substitutes are now being employed for some of these uses. As a result of remediation activities, substitution efforts, best management practices, and compliance with Clean Water Act permits, defense-related releases of perchlorate should be diminishing.

In California, one of the states thought to be most contaminated with perchlorate, the military collaborated with state officials to investigate reports of widespread contamination. In 2003, the Defense Department and state regulators established a partnership to investigate the presence of perchlorate throughout California to locate previously unidentified threats to public water supplies. More than 900 military sites were screened using a consensus-based protocol; 870 of these sites were deemed to be not of concern. State regulators agreed that military installations and formerly used defense sites did not appear to be significantly impacting California public drinking water wells. As a result of the collaboration, California also adopted many of the department's best management practices for perchlorate.

The department began an extensive perchlorate monitoring program and, by 2008, had collected more than 47,500 samples at 309 locations nationwide to define the scope of its perchlorate contamination problem. The results of this monitoring program — posted and annually updated on the Defense Environmental Network Information Exchange website — show that the vast majority of perchlorate samples from military locations were below 4 parts per billion, and large groundwater plumes of perchlorate exist at only a limited number or military sites.

While the military appears to be defining and controlling many of its potential sources of perchlorate, it is unclear what controls are being put in place elsewhere. Recent research announced in the Fourth National Report on Human Exposure to Environmental Chemicals by the Centers for Disease Control and Prevention together with a number of academic findings suggest that exposure to perchlorate is widespread, indicating there may be many undocumented sources. These sources should be thoroughly evaluated in order to better understand total human exposure.

The contribution of perchlorate from the use of sodium hypochlorite for various household, drinking water, and commercial disinfection purposes may be an important non-point source contributor to total perchlorate exposure. Sodium hypochlorite, a bleach, generates perchlorate in storage and when it is exposed to sunlight. The compound is used in a variety of household applications (household bleach is a 3–6 percent solution) and industrial applications (water and waste water disinfection solutions are 12–15 percent solutions) related to disin-

fection. Nearly $3 billion of sodium hypochlorite bleach is sold globally each year. In a 2008 study, perchlorate contamination was found to occur in more than 90 percent of sodium hypochlorite samples.

The most recent part of the perchlorate source picture to come to light is the volume of the compound entering the United States in fireworks. Recent data from the Department of Commerce indicate a large increase in firework imports over a five-year span, from 174.7 million pounds in 2002 to 271.2 million pounds in 2006. Potassium perchlorate constitutes up to 70 percent of the chemical fraction in fireworks. Based on these data, the American Pyrotechnics Association estimates that 14.2 million pounds of perchlorate entered the country in fireworks in 2002, and 21.8 million pounds in 2006. Imports represent about 90 percent of fireworks used in the United States. For 2006, the amount of perchlorate imported in fireworks represents nearly three times the amount purchased by the military. While importation does not necessarily equate with amounts released into the environment, these data highlight an important potential nonpoint source.

Recent field studies also support focusing on perchlorate discharges from fireworks. EPA analyzed water in an Oklahoma lake before and after fireworks displays. Testing performed 14 hours after an event showed that perchlorate levels rose 24 to 1,028 times above the predisplay baseline. The agency found that concentrations of perchlorate peak about 24 hours after a display and then decrease to the baseline within 20 to 80 days. Thus, Fourth of July activities and sporting events may be important in future efforts to define sources and routes of exposure. Efforts to restrict and manage releases at the local level may be able to control exposure.

The millions of emergency road

Perchlorate Will Accumulate

In 2008 NASA's Phoenix Lander found perchlorate in three different Martian soil samples. That discovery prompted a re-analysis of the soil samples we had brought back from an expedition a year earlier to Antarctica's Dry Valleys, a site used as a Mars analogue for training because of the subzero temperatures and extreme aridity. For both Earth and Mars, the evidence implies that given the right conditions and a source of chlorine, perchlorate will accumulate.

Free of anthropogenic influences, the Dry Valleys provide an ideal location for such studies. The Antarctic soil samples had been collected from pits dug in three types of Dry Valley microclimate zones: coastal (wet), inland (arid), and highland (hyperarid). Samples were collected from every identifiable soil horizon (boundaries between different kinds of soil) down to the ice-cemented soil. All the samples were reanalyzed for perchlorate.


Samuel Kounaves

To our amazement, perchlorate was found in all the highland soils, in all the horizons from the surface to the ice-cemented soil. Its concentration ranged up to 630 parts per billion and in a continuous vertical profile. In contrast, for the inland valley soil horizons, it was found to vary with a more heterogeneous distribution, while in the coastal valleys it appeared very randomly distributed, approaching 1,100 ppb in one isolated soil horizon, and totally absent in others, with no regular or discernible pattern.

How the perchlorate is deposited in these valley soils is clearly evident from its correlation to nitrate and chloride, both of which have been shown to be atmospherically formed and deposited. Thus, the best explanation for its changing distribution in the soil when moving from the upper to lower valleys is the difference in the amount of liquid water present and its effect, leading to depletion or concentration of the perchlorate. In the highlands the atmospheric deposition of perchlorate is left undisturbed, while in the lower wet valleys its distribution becomes chaotic.

Combining our findings with those for the Arctic, North America, and other regions provides an emerging picture for the global presence of natural perchlorate. In addition, our results from Antarctica clearly point to the conclusion that even though natural perchlorate is atmospherically and homogeneously deposited, over time it will accumulate at high levels at some locations while it will be nonexistent at others.

Our results support the hypothesis that perchlorate must also have a variety of long-term, widely, and irregularly distributed sinks. Since aqueous perchlorate is chemically stable in the natural environment, its lack of accumulation in the ocean or aquifers may also be attributable to microbial utilization in anaerobic or low nitrate media.

To help us understand the full impact of perchlorate, we need to more accurately determine its global distribution and accumulation patterns, its interactions with terrestrial ecology, and its atmospheric formation mechanisms.

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For more information see: S. P. Kounaves, et al., Environmental Science and Technology, 2010, 44, 236-2364, doi:10.1021/es9033606.

flares sold annually contain significant concentrations of perchlorate, which is often released into the environment. A study in Santa Clara, California, found that 88,184 pounds of road flares are used in the county annually with a corresponding perchlorate content of 5,732 pounds. Field testing has shown maximum concentrations of perchlorate in runoff leaving highways of 314,000 parts per billion after road flare use. Considering the volume nationwide, discarded or partially used emergency flares may be another important non-point source of exposure. Communities could take steps to consider the use of alternative safety-lighting systems such as those adopted by Seattle and other local police organizations to reduce the contribution from flares.

Natural sources of perchlorate from geological materials, such as potash ore, playa crust, and hanksite, also contribute to environmental perchlorate. Natural deposits have been found in the Texas panhandle and north-central New Mexico. Other research suggests that perchlorate may be produced via atmospheric processes, then enters the food chain through rainfall. Atmospheric formation may explain the discovery of perchlorate in soil and ice from the Antarctic [See Another View, page 41] where concentrations reach up to 1100 micrograms per kilogram. Finally, there is a possible connection between tropospheric ozone and the formation of perchlorate in plants, a factor that could in part explain the presence of perchlorate in agricultural products.

One source of perchlorate in the environment that is receiving some additional attention is the over 100 million pounds of Chilean nitrate fertilizer — which contains perchlorate at levels of 100,000 parts per billion —that have been applied in the United States. There are over 400,000 pounds still being applied annually to commercial agricultural land and homeowner gardens. In 2006, Texas Tech University scientists reported in the journal *Environmental Science & Technology* that perchlorate-containing Chilean nitrate fertilizer likely accounts for more low-level contamination in the United States than all military and industrial sources put together.

Perhaps the most important development driving the understanding of sources of perchlorate in the environment is new forensic fingerprinting technology that can help differentiate man-made and natural types of perchlorate. Isotopic methods are powerful tools when applied to the intractable problems of source attribution for groundwater contaminants. Elements in compounds can have widely different isotopic ratios based on their mode of formation. Stable ratios have a fingerprint, allowing scientists to distinguish sources from one another. The isotopic method for perchlorate is available for use through some university and Department of Energy laboratories. Scientific validation of the new method is underway.

The new clarity made available through forensic inquiries into sources may provide a much better understanding about the proportion of contributions of perchlorate in the environment from commercial, consumer, agricultural, and military sources. Forensic methods distinguished synthetic from natural sources of perchlorate at contaminated sites in southern California and elsewhere, and proved helpful in disentangling possible sources. A 2009 study by the U.S. Geological Survey used forensic isotopic methods to identify historic use of fertilizer as the most likely cause of groundwater contamination in areas of Long Island and concludes that these findings may have national implications.

The growing concern over the possible health effects of exposure to low levels of perchlorate should not overshadow the investigation of perchlorate sources. There appears to be a growing body of evidence that perchlorate sources should be reappraised, particularly in light of findings that perchlorate in fireworks, bleach, safety flares, and Chilean fertilizer may outweigh military-industrial uses. The new data on sources in the environment and releases to the environment should be used to better inform regulatory decisions on controlling exposure. More importantly, the application of fingerprinting methods that distinguish natural from man-made sources will empower regulatory managers to tackle this multi-faceted source challenge with tailored risk reduction measures. As consumer, natural, military, and agricultural sources continue to be identified and quantified, misperceptions about the role of point and non-point sources should be displaced.

While early data allowed regulatory agencies to focus initially on perchlorate as an emerging problem, to holistically address the risks from exposure a more comprehensive assessment of sources is necessary. More information about the unusual suspects among perchlorate sources will enhance risk mitigation and protect public health. With improved sampling technology, food safety regulators, environmental agencies, and local governments will be able to control the use, disposal, and cleanup of perchlorate to reduce public exposure. •

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Particulate Oxidative Burden Associated with Firework Activity

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Firework events are capable of inducing particulate matter (PM) episodes that lead to exceedances of regulatory limit values. As short-term peaks in ambient PM concentration have been associated with negative impacts on respiratory and cardiovascular health, we performed a detailed study of the consequences of firework events in London on ambient air quality and PM composition. These changes were further related to the oxidative activity of daily PM samples by assessing their capacity to drive the oxidation of physiologically important lung antioxidants including ascorbate, glutathione and urate (oxidative potential, OP). Twenty-four hour ambient PM samples were collected at the Marylebone Road sampling site in Central London over a three week period, including two major festivals celebrated with pyrotechnic events: Guy Fawkes Night and Diwali. Pyrotechnic combustion events were characterized by increased gas phase pollutants levels (NO_x and SO_2), elevated PM mass concentrations, and trace metal concentrations (specifically Sr, Mg, K, Ba, and Pb). Relationships between NO_x, benzene, and PM_{10} were used to apportion firework and traffic source fractions. A positive significant relationship was found between PM oxidative burden and individual trace metals associated with each of these apportioned source fractions. The level of exposure to each source fraction was significantly associated with the total OP. The firework contribution to PM total OP, on a unit mass basis, was greater than that associated with traffic sources: a 1 μg elevation in firework and traffic PM fraction concentration was associated with a 6.5 $\pm$ 1.5 OP^T μg^{-1} and 5.2 $\pm$ 1.4 OP^T μg^{-1} increase, respectively. In the case of glutathione depletion, firework particulate OP (3.5 $\pm$ 0.8 OP^{GSH} μg^{-1}) considerably exceeded that due to traffic particles (2.2 $\pm$ 0.8 OP^{GSH} μg^{-1}). Therefore, in light of the elevated PM concentrations caused by firework activity and the increased oxidative activity of this PM source, there is value in examining if firework derived PM is related to acute respiratory outcomes.

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Introduction

Anthropogenic emissions from firework displays are responsible for particulate matter (PM) episodes with the greatest concentrations measured in the United Kingdom (*1*). Nightly celebrations occur annually over a two to three week period to celebrate Diwali and Guy Fawkes Night during October and November. Small size pyrotechnic events (including hand-held sparklers, cherry bombs, roman candles representing British category 2 and US class 1.4G) are set off by individuals in parks and back gardens. Larger professional displays (British category 4 and US class 1.3G) also occur where pyrotechnics are projected to attain explosion heights of up to 200 m. Regardless of the type of firework ignited, the combustion products are similar: high SO_2 and NO gaseous releases (*3, 2*), dense metal-rich PM plumes with a large organic carbon component (*4*).

The firework physicochemical signature has been extensively characterized internationally: Washington State, US (*5*); California, US (*6*); Texas, US (*7*); Montreal, Canada (*8*), Hisar, Hyderabad, and Thumba, India (*10, 9, 2*); Beijing, China (*11*); Saalbach, Austria (*12*); United Kingdom (*1*); Mainz, Germany (*4*); Milan, Italy (*13*); and Valencia, Spain (*3*). These previous reports unanimously agree firework activity contributes to elevated ambient particulate metal concentrations in respirable size fraction (i.e., PM with an aerodynamic diameter of <10 μm, PM_{10}). Fireworks and related explosives account for substantial airshed emissions in the UK: 3 tonnes Cu, 10 tonnes Sr, 65 tonnes Ba, 73 tonnes Mg, and 100 tonnes K (*14*). These ambient emissions for Cu and Mg represented 6 and 8% respectively of annual atmospheric releases in the UK during 2000 (*14*). Each of these metals acts as a coloring agent and are paired with oxidizers, including nitrates, sulfates, and perchlorates. This is known as black powder and approximately 74% consists of KNO_3, 10% sulfur, and 16% carbon in the form of charcoal (*15*). Pyrotechnic displays on celebratory dates often cause PM_{10} mass concentrations to exceed 24 h limit values (50 $\mu g\ m^{-3}$) as set by the European Directive 2008/50/EC. Even though this concentration is not to be exceeded on more than 35 days annually, the UK has declared in its National Air Quality Strategy that it is inappropriate to control air quality infringements resulting from social and cultural activities, including bonfires and pyrotechnic displays (*16*).

To date only a handful of small scale studies have addressed the potential adverse health effects stemming from exposure to firework emissions. Pulmonary function monitored in nine patients (seven healthy and two with chronic respiratory disease) exposed to a six times increase in PM_{10} (110 $\mu g\ m^{-3}$ five hour experiment average which included a fifteen minute peak attaining concentrations in excess of 3.8 $mg\ m^{-3}$) from firework events was noted to cause a significant maximal midexpiratory flow rate decrease in the susceptible individuals (*17*). A potential impact on lung function was also supported by observations from a 1972 study where a 113% increase in emergency room visits by individuals with chronic respiratory disease was reported following a firework episode in Honolulu (*18*). More recently, Becker et al. (*19*) reported a near fatal and a fatal asthma exacerbation of two severely asthmatic children following exposure to elevated PM concentration from hand-held fireworks.

Despite the extensive use of fireworks to commemorate special events internationally, no literature has yet described the toxicity associated with firework PM on the respiratory system. We therefore investigated toxicologically relevant features of ambient PM collected over a three week period

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in central London during a period of intense firework activity including Diwali and Guy Fawkes Night. An oxidative potential (OP) metric was formulated for each daily PM sample based on their capacity to deplete antioxidants from a validated synthetic respiratory track lining fluid (RTLF) (*20*). The biological pertinence of this acellular model relates to the contention that inhaled PM mediates adverse health effects through the induction of oxidative stress at the air-lung interface (*21*). Generation of reactive oxygen species has been demonstrated following PM challenge in various extracellular and cellular compartments (*21*), with evidence that different components (biological material, polycyclic aromatic hydrocarbons/quinones, trace metals) can cause oxidative stress through different pathways. Thus the OP metric used in this study integrates the contribution of a range of PM components into a single biologically informative expression.

Materials and Methods

Sampling Site Description. Sampling was conducted at the Marylebone Road kerbside site in central London, UK. Details of sampling site have been previously reported (*22*). Given the proximity of the sampling site to traffic emissions, considerable PM and gas species contribution from traffic sources was found. Fireworks and bonfires were ignited across the city during the sampling period by individuals and at large-scale organized public displays. Large-scale organized events commenced after dusk and extended for approximately 30 min on November second (8 displays), third (17 displays), fourth (4 displays), fifth (6 displays), ninth (2 displays), and 10th (2 displays), 2007. The number of firework incidences reported to the London Fire Brigade was used as a surrogate measure of firework displays ignited by individuals. A total of 2427 incidences were logged in the inner London Boroughs over the first ten days of November 2007, significantly greater than the outer Boroughs (1720) (*23*). Specifically in the London Borough of Westminster, an inner city borough in which the Marylebone Road sampling site is located, 99 firework related incidences occurred with 26 of these events reported in the immediate Marylebone area (*23*). An indication of the number of bonfires lit by individuals was also estimated using London Fire Brigade statistics: 262 incidences of runaway bonfires, recorded as secondary fires (nonproperty location fires free of injury), were logged on November 5, 2007, representing the highest number of calls during the year (*23*).

Instrumentation. PM with an aerodynamic diameter between 1 and 10 μm (PM_{1-10}) was collected on polyurethane foams (PUFs) using the Airborne Sample Analysis Platform (Thermo ASAP) located on the roof of the monitoring station at the Marylebone Road site. Foams were exposed for 24 h periods at 200 L min^{-1}. PUFs were changed daily at 9:30GMT for samples collected between October 24, 2007 and November 6, 2007 and at 15:00GMT November 7, 2007 to November 13, 2007. PM collected on these foams was used for trace metal analysis and assessment of PM oxidative potential.

Ambient PM_{10} mass concentrations were measured by the UK Automatic Urban and Rural Network at the Marylebone Road sampling site using a Tapered Element Oscillating Microbalance (TEOM) with 15 min resolution. In addition, gaseous pollutant concentrations (SO_2, NO, NO_2) and meteorological parameters (temperature, wind direction, and wind speed) were measured on an equivalent time basis. Ambient benzene concentrations were measured at an hourly resolution by Defra's Automatic Hydrocarbon Monitoring Network using a Perkin-Elmer Online system. This consisted of an ATD400, sampling onto a carbon impregnated trap at −30 °C for 40 min every hour and an Autosystem XL gas chromatograph with a PLOT column and flame ionization detector.

Chemicals and Chelex Water Preparation. All water, unless otherwise stated, was deionized and ultrafiltered using an Elga-stat filtration system. All chemicals used were obtained from Sigma Chemical Company Ltd. and were of analytical grade. Ultrapure Chelex100 resin treated water was utilized to eliminate background metal contamination when assessing oxidative potential (*20*).

Sample Preparation. PM_{1-10} samples collected on PUFs were extracted in a 5% high pressure liquid chromatography (HPLC)-grade methanol prepared in Chelex resin treated ultrapure water, pH 7.0 to achieve a final PM concentration of 150 μg mL^{-1}. To ensure PM suspensions were homogeneous, resuspension was performed using a probe sonicator (MSE Soniprep150, 23 kHz, generator with a titanium probe) operated at an amplitude of 15 μm for 30 s. Further details are provided in the Supporting Information regarding the extraction efficiency of the PUFs and comparison to hourly PM_{1-10} mass concentrations measurements. Interexperimental positive (residual oil fly ash) and negative (M120, carbon black) PM control samples were also resuspended using the same procedure. Compositional details of the residual oil fly ash (*24, 25*) and M120 (*27, 26*) control particles have been published previously.

Assessment of Oxidative Potential. The oxidative potential of PM was assessed *in vitro* by measuring the depletion of antioxidants by standardized concentrations of resuspended particulate in a synthetic RTLF. This chemical model contained physiologically relevant concentrations (200 μM) of urate (UA), ascorbate (AA), and glutathione (GSH) adjusted to pH 7.0 and incubated at 37 °C with equal mass concentrations of PM samples (50 μg mL^{-1}) for four hours. Briefly, AA and UA concentrations were determined using reverse phase HPLC with electrochemical detection (Jones Chromatography, Hengoed, Wales). Total glutathione (GSx) and glutathione disulfide (GSSG) concentrations were determined with the GSSG-reductase-5,5′-dithio-bis(2-nitrobenzoic acid) (DNTB) recycling assay. Subtracting two times the measured GSSG from the GSx concentration yielded GSH. Further information regarding antioxidant measurements have been described elsewhere (*28*). The Supporting Information contains details describing the calculation method of the AA and GSH OP metrics.

Total PM Metal Analysis. An aqua regia solution was prepared in Chelex-100 resin treated water. This digestion media was added to the resuspended PM solution such that final concentrations of 2.8% HNO_3, 5.1% HCl, and 7.5 μg mL^{-1} PM were achieved. Samples were digested in a microwave system (Mars 240/50) for 30 min at 180 °C on full power (1600W). Following digestion, samples were further diluted with Chelex-100 resin treated water to attain a final acid and PM concentration of ca. 1.9% HNO_3, 3.4% HCl, and 5 μg mL^{-1} PM. Concentrations of K, Ca, Mg, Fe, Cu, Zn, Sr, V, Ba, and Pb in solution were determined using inductively coupled plasma mass spectrometry (ELAN DRC ICP-MS, MSF008). Dilutions of a certified multielement standard solution (VI CertiPUR Merck, Lot. No.OC529648) were used for calibration. Six NIST 1648a Urban PM control samples and water blanks were run in addition to samples; 3σ of the water blank elemental concentrations were used to establish minimum detection limits.

Results and Discussion

Air Mass Origin. The dominating parameters influencing the concentration of PM species measured at the Marylebone Road sampling site were local and regional emissions. Pyrotechnic and biomass burning (in the form of Guy Fawkes Night celebratory bonfires) were known to occur locally but

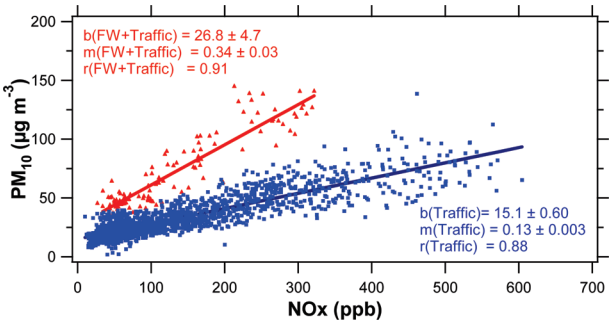

FIGURE 1. Relationship between 15 min mean NO_x and PM_{10} mass concentrations for the entire campaign period. The fraction of PM_{10} derived from vehicle sources is colored in blue squares. Red triangular markers indicate the PM attributed to firework and bonfire events as estimated by NO_x concentrations.

also took place across the United Kingdom, likely contributing to an elevated regional PM background level. The relevance of each combustion event, local or regional, to the sampling site was influenced by varying wind patterns. However, proximity of the known firework events to the sampling site and PM_{10} episode timings implicated local London combustion events with the observed PM episodes. A single large-scale firework event (the Lord Mayor Show) occurred at 18:30GMT on November 10, 2007 along the River Thames (3 km southeast of the sampling site) but did not result in a PM_{10} episode at Marylebone Road. Meteorological data obtained from Heathrow Airport indicated winds were from the northwest inhibiting the firework plume from reaching the sampling site. In contrast, firework activity on November 3 and 9, 2007 (at locations north and northwest of Marylebone Road) enhanced the magnitude of measured PM_{10} concentration given the sampled air mass on these days originated from the north and northwest, respectively. The highest PM_{10} concentrations measured during the campaign, however, occurred on the early morning of November 5, 2007 and were associated with an air mass which traveled through London from the northeast. Unlike all other discussed events, the latter period was dominated by high pressure conditions with near stagnant wind speeds (1.24 ± 0.44 m s^{-1}) which limited PM dispersion.

Nitrogen Oxides (NO_x) and PM_{10} Relationship. A relationship between NO_x and PM_{10} mass concentration was used to determine the fraction of PM_{10} attributable to differing combustion contributions (Figure 1) (*29, 30*). Complete regression source apportionment details are described in the Supporting Information. Briefly, this regression analysis segregated the data set into two groups to represent pyrotechnics/bonfires and traffic (*31*) and traffic alone (including vehicular tail pipe emissions, road dust resuspension, and tire and brake pad wear) sources such that each group was characterized by distinct PM_{10}-NO_x slope. The former mass concentration grouping corresponded to (i) periods of organized firework-bonfire displays, November 3, 2007; (ii) Guy Fawkes Night, November 4 and 5, 2007; (iii) Diwali, November 9, 2007. Parallel NO_x increases with firework activity have been identified previously (*2, 32*) and were suggested to originate from the black powder KNO_3 component (*4*). Allan et al. (*33*) evaluated the contribution of biomass combustion sources to regional NO_x concentrations during the REPARTEE 2 campaign (October 16, 2007-November 9, 2007) which overlapped with the current study: a high resolution time-of-flight Aerosol Mass Spectrometer (AMS) was operated at the Regent's Park urban background site (further site details are provided in the Supporting Information). The AMS measured $PM_{2.5}$ total organics concentrations were deconvolved using Positive Matrix Factorization into four factors profiling different source attributions: oxygenated organic aerosol, cooking organic aerosol, solid fuel organic aerosol (SFOA), and hydrocarbon-like organic aerosol (HOA). A univariate linear regression between HOA and NO_x was performed in additional to a bilinear fit including both HOA and SFOA factors with NO_x. This latter approach did not yield improved Pearson r-values compared to univariate regression results. The authors concluded that despite the NO_x emissions from solid fuel combustion, on the regional scale during REPARTEE 2, traffic dominated NO_x emissions relative to solid fuel combustion in central London as more local sources were present. Thus, the enhanced rate of PM_{10} emission relative to NO_x identified via the regression analysis in the current study is likely isolated to pyrotechnic combustion sources and not from bonfire or other solid fuel combustion emissions.

The validity of assigning the lower PM_{10}-NO_x slope to traffic-related emissions was evaluated by considering two week periods void of firework and bonfire activity before and after the campaign (October 9-24, 2007 and November 15-28, 2007). A similar traffic PM_{10}-NO_x relationship was found for these periods (slope 0.14 μg m^{-3} ppb^{-1}; y-intercept 19 μg m^{-3}; $r = 0.81$; $p < 0.001$; $N = 2695$) as during the campaign (slope 0.13 μg m^{-3} ppb^{-1}; y-intercept 15 μg m^{-3}; $r = 0.87$; $p < 0.001$; $N = 1817$).

The uncertainty of traffic and firework/bonfire apportioned PM_{10} fractions were determined as the combined uncertainties of each input variable derived in accordance with the ISO Guide to the expression of uncertainty in measurement (GUM) (*34*). Calculation details are provided in the Supporting Information. The average uncertainty for 15 min resolution firework apportioned PM_{10} mass concentrations was 3.7 μg m^{-3}. For the 15 min averaged traffic PM_{10} fraction the uncertainty on mass concentrations was estimated as 1.5 μg m^{-3}.

When interpreting temporal traffic PM_{10} source fraction fluctuations (Figure 2), it is useful to note that the Marylebone Road site is located on the south side of the carriageway in a street canyon such that a vortex is created at sufficiently high wind speeds coming perpendicular to the street (*22, 35, 36*). Consequently, strong southerly winds and those flowing parallel to the street yield increased PM concentrations. Two scenarios may result from northerly wind depending on wind speed: high velocities will cause air recirculation leading to the lowest contribution of local traffic emissions to the site, while low wind speeds enable local traffic emissions to be measured directly and thus the highest PM concentrations. During the campaign, northerly winds were associated with near-stagnant wind conditions (<1 m s^{-1}). Consequentially the maximum traffic component PM_{10} concentration measured was 16.3 ± 14.2 μg m^{-3} (33 ± 26% of the total PM_{10} mass concentration) on average during these periods. In contrast, the lowest concentrations of this source fraction (5.1 ± 7.8 μg m^{-3} or 15 ± 14% of the total PM_{10} mass concentration) were measured when higher wind speeds induced the microscale recirculation effect within this street canyon.

The firework PM component was isolated to episodes which were rapidly dispersed with only acute air quality degradations. These celebratory combustion events included (i) November 3, 2007 19:45GMT - November 4, 2007 3:45GMT (15.7 ± 5.1 μg m^{-3} PM_{10} average), (ii) November 4, 2007 18:15GMT - November 5, 2007 5:00GMT (44.3 ± 17.6 μg m^{-3}), and (iii) November 9, 2007 20:45−23:45GMT (49.9 ± 10.0 μg m^{-3}). This source fraction represented 33 ± 9%, 41 ± 13%, and 60 ± 5% of these total PM_{10} mass concentrations for each of the noted periods.

To further elucidate differences between these two combustion processes and other possible contributing anthropogenic emissions, PM_{1-10} chemical characteristics, PM number concentration, and gaseous SO_2 fluctuations were

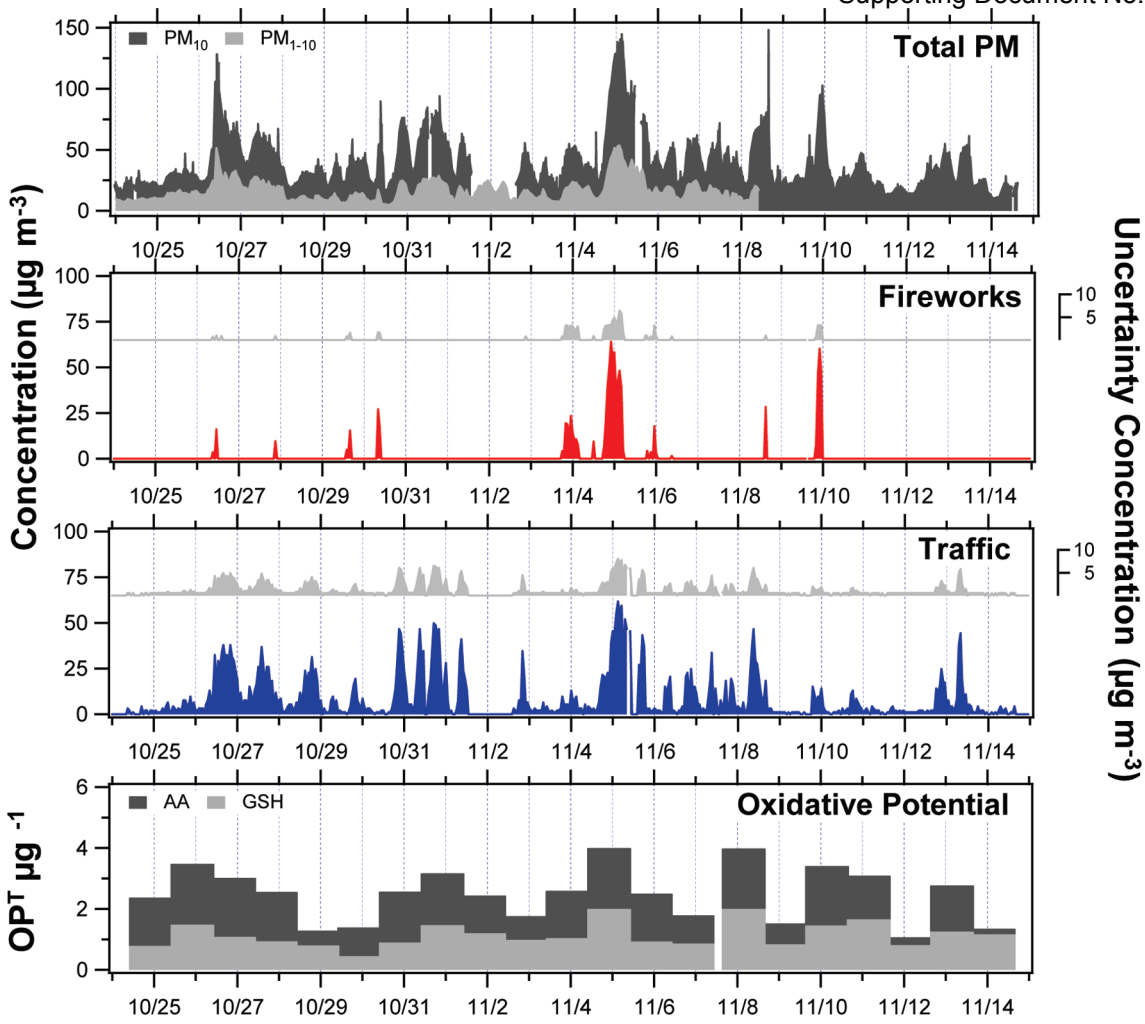

FIGURE 2. Time series of (i) PM_{10} mass concentrations measured by the TEOM (1 h average) and PM_{1-10} mass concentrations estimated by merged SMPS-APS measurements (1 h average). One hour resolution source apportioned PM_{10} firework-bonfire (ii) and traffic (iii) fractions (left axis) with the associated uncertainty for each fraction colored in light gray (right axis). Twenty-four hour averaged PM_{1-10} ascorbate (AA, light gray) and glutathione (GSH, dark gray) oxidative potential per unit mass.

assessed; the latter two are described in the Supporting Information.

Trace Metals. The temporal fluctuations of PM_{1-10} Sr, K, Mg, Ba, and Pb concentrations were found to be similar; the maximum concentration measured for all these metals was for the 24-h sample collected over November 4 to 5, 2007, representing a period of intense pyrotechnic activity (Figure 3-A). An additional rise was observed for the 24-h November 9 to 10, 2007 p.m. sample, also corresponding with a firework combustion period. No significant concentration changes during these celebratory periods were found for metals predominately associated with vehicular nontail pipe emissions (Figure 3-B). Despite the elevated Cu firework emission factors estimated by Passant et al. (*14*), no Cu concentrations increases were found during periods of known firework activity. Ba concentrations reflected firework and traffic emission fluctuations, where the latter was the result of brake pad abrasion (*37, 38*). Following PM size distribution results presented in the Supporting Information, the authors acknowledge that the sampled size fraction (PM_{1-10}) does not represent the total metal concentration associated with firework activity. As instrumentation available for daily PM filter sampling was limited to the 1 and 10 μm cut points, the reported firework PM parameters measured were therefore underestimated given that much of the PM derived from pyrotechnic combustion events was found below 1 μm.

Trace metal enrichment factors were determined for 24-h PM_{1-10} samples collected on November 4−5, 2007 and October 28−29, 2007 relative to the November 13−14, 2007 sample (Figure 4). Pyrotechnic combustion related emissions were sampled during the former as predicted from source apportionment results and the occasion of city-wide organized firework displays. In contrast, the latter two samples were primarily subject to continuous traffic emissions given the lack of apportioned firework PM component in parallel with no organized firework events. The sample collected over Guy Fawkes Night celebrations was associated with large increases of firework combustion derived trace metals. The enrichment factor calculated for the nonfirework period maintained approximately unit values for trace metals without a vehicular source. Ba, Sr, and Pb sustained the largest increases (28-, 25-, and 19-fold, respectively) on the pyrotechnic date considered. However, Ba, unlike Sr and Pb, was also associated with an increase factor of 1.4 on the nonfirework days reflective of a traffic emission contribution.

All of the measured trace metals had the potential of originating from either pyrotechnic or urban sources. To determine the elemental composition associated specifically with firework derived PM, bivariate correlations were performed with Sr and the other measured trace metals. The strongest correlation ($p < 0.0001$, $N = 21$) existed between K ($r = 0.98$), Pb ($r = 0.96$), Ba ($r = 0.92$), and Mg ($r = 0.73$)

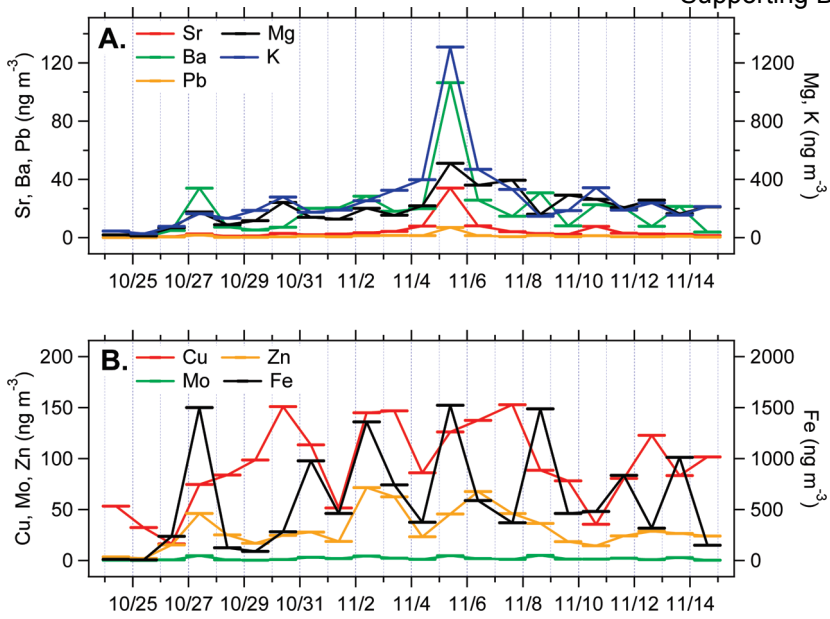

FIGURE 3. Twenty-four hour averaged PM_{1-10} concentrations for metals attributable (A; Sr, Ba, Pb, Mg, K) and not attributable (B; Cu, Mo, Zn, Fe) to firework combustion emissions.

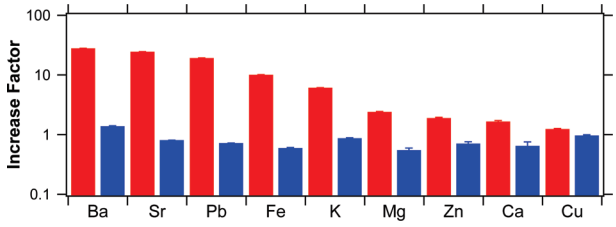

FIGURE 4. Ratio of trace metal concentrations as measured for PM samples run on (1) October 28, 2007 9:30 GMT - October 29, 2007 9:30 GMT (no fireworks events, blue bars) and (2) November 4, 2007 9:30 GMT - November 5, 2007 9:30 GMT (firework events, red bars) to PM samples collected between November 13, 2007 15:00 GMT - November 14, 2007 15:00 GMT.

confirming the elemental firework composition described by previous studies with the exception of Cu (*3, 5*). A weaker significant association was identified with Fe concentrations ($r = 0.43$, $p = 0.05$). No correlation was established between Sr with Ca, Cu, and Zn despite reported use of these species in fireworks as orange ($CaCO_3$, $CaCl_2$, $CaSO_4 \bullet xH_2O$), blue ($CuCO_3$, $CuSO_4 \bullet 5H_2O$, CuCl), and white (ZnO) coloring, respectively (*39*).

Oxidative Potential. Bivariate correlations were conducted with the bulk PM mass and trace metals for the ascorbate and glutathione dependent OP components on a unit mass basis. Individual metals, representative of the source apportioned firework and traffic PM fractions, were all found to be positively associated the OP parameters. A combination of the combustion derived metals were correlated with OP^{GSH} μg^{-1} related to both traffic (Ba, $r = 0.62$, $p = 0.003$; Mo, $r = 0.57$, $p = 0.007$; Fe $r = 0.57$, $p = 0.01$; Cu) and firework (Pb, $r = 0.54$, $p = 0.01$; Sr, $r = 0.48$, $p = 0.03$) emissions. However only traffic emissions related metals were significantly associated with OP^{AA} μg^{-1}: Mo ($r = 0.48$, $p = 0.03$), Fe ($r = 0.47$, $p = 0.03$) and Ba ($r = 0.46$, $p = 0.04$). A subset of the measured trace metals (Zn, Ca, Cu) lacked an association with antioxidant depletion. The oxidative loss of antioxidants from the synthetic RTLF model is sensitive only to intrinsic redox active PM constituents: redox active metals and quinones. With the exception of Fe and Pb, the contribution of the trace metals noted in the bivariate correlation analysis to reactive oxygen species production, and thus AA and GSH depletion, has not been reported in the literature. These species probably represent surrogates of constituents with intrinsic redox active properties which were not quantified.

A heterogeneous panel of metals, representing different source fractions, were found to be predictor/surrogate measures of OP^{AA} μg^{-1} and OP^{GSH} μg^{-1}; the former was related to traffic and the latter was associated with both traffic and firework related combustion products. Consideration of the individual trace metals in a multivariate regression method was not feasible as the colinearity of the metal concentrations would confound results. Multivariate regressions were instead performed between the ascorbate, glutathione, and total OP ($\mu g\, PM_{1-10})^{-1}$ metrics and apportioned PM_{10} source fractions such that OP dose effects were estimated for an increase of each PM source fraction in the ambient airshed. The PM_{10}(traffic), PM_{10}(firework), and PM_{10}(other) fractions were averaged to a 24-h periods to correspond with the ASAP PUF PM_{1-10} sampling intervals. As only transient firework concentrations were measured during the campaign, the validity of averaged daily firework PM_{10} concentrations was assessed based on the number of 15 min measurements included in the 24 h average. For averaging periods comprised of only a single firework concentration measurement ($N = 5$), the timing of the episode was evaluated to avoid inclusion of misclassified firework data points; this possibility is acknowledged given the limitations in the methodology employed to segregated traffic from traffic+firework measurements (Figure 1). For these firework events isolated to a 15 min averaged period, the authors dismiss the likelihood that measurements which occurred during the late morning hours (October 26, 2007 9:00 and November 6, 2007 9:30GMT) were in fact derived from a firework source and were excluded from the regression analysis. A further restraint on the regression analysis was caused by the significant negative correlation ($r = 0.63$, $p = 0.002$) between the PM_{10}(traffic) and PM_{10}(other) fractions, arising as an artifact of the definition of PM_{10}(other) (Equation S10). Consequently, the PM_{10}(other) fraction was excluded from the regression analysis, and the predicted mean contribution of this fraction to each OP metric was calculated from the traffic and firework regression coefficients.

Significance was sustained at the 95% confidence level for all OP metrics when a multivariate model was constructed to explain the measured variance (Table 1). The plot of

TABLE 1. Multivariate Regression Analysis of OP μg^{-1} (Ascorbate, Glutathione, and Total) with PM_{10} Traffic and Firework Fractions[a]

metric	PM source fraction (μg (μg PM_{10})$^{-1}$)	explanatory variable B	SE	p-value	model r	p-value
OP^{AA} ug^{-1}	traffic	3.1	1.2	0.05	0.79	0.05
	firework	3.1	1.3	0.05		
	constant	0.4	0.4	0.30		
OP^{GSH} ug^{-1}	traffic	2.2	0.8	0.03	0.89	0.008
	firework	3.5	0.8	0.005		
	constant	0.2	0.2	0.36		
OP^{T} ug^{-1}	traffic	5.2	1.4	0.01	0.90	0.006
	firework	6.5	1.5	0.005		
	constant	0.6	0.4	0.18		

[a] B-values indicate the estimated change in OP μg^{-1} for an increase in 1 μg of each apportioned source fraction. The associated standard errors (SE) for regression coefficients and each model are also presented.

regression residuals against standardized predicted values exhibited random scatter around the zero line for all three OP metric models. The fireworks PM factor was implicated with the greatest increase in OP^{GSH} μg^{-1} and OP^{T} μg^{-1} for each 1 μg dose (GSH: 3.5 ± 0.8 μg^{-1}, $p = 0.005$; Total: 6.5 ± 1.5 μg^{-1}, $p = 0.005$) compared to the traffic fraction (GSH: 2.2 ± 0.8 μg^{-1}, $p = 0.03$; Total: 5.2 ± 1.4 μg^{-1}, $p = 0.01$). Therefore, the firework PM contributed more substantially per unit mass to the PM OP in this acellular model than the traffic related PM_{10} component. Moreover, the predicted oxidative burden associated with the PM_{10}(other) fraction was 0.51 μg^{-1} and 0.96 μg^{-1} for the glutathione and total OP models, respectively. This suggested the bulk of the oxidative activity of the particles was related to the traffic and pyrotechnic sources. In contrast to the GSH and total OP metrics, the oxidative burden associated with the traffic and firework PM_{10} fractions was equivalent (3.1 ± 1.2 μg^{-1}, $p = 0.05$) in the OP^{AA} μg^{-1} regression model. These results further highlight that depletion of each antioxidant is sensitive to a heterogeneous panel of metals. The OP predicted to be derived from the PM_{10}(other) source fraction for the case of ascorbate related oxidative activity was low (0.44 μg^{-1}) compared to the traffic and firework sources, similar to the glutathione and total OP results. It is also important to note when interpreting these results that the reported oxidative potential, in particular for the firework component, was underpredicted: the lower cut point (1 μm) of the sampled size fraction prevented collection of the entire firework PM size distribution as described in the Supporting Information.

In conclusion, this study highlighted distinctions between ambient PM and gas phase species resulting from firework or traffic activity. Furthermore, ambient PM_{10} concentrations were deconvolved into source apportioned fractions which were coupled with measurements of particulate oxidative burden. The *in vitro* PM total OP per unit mass associated with pyrotechnic activity was shown to be significantly greater than that associated with traffic derived PM emissions. From the current study it was not clear if the measured pyrotechnic PM trace metals were the drivers for the measured OP or simply represent surrogates for some unmeasured components. Thus, the authors recommend further examination of a possible relationship between firework exposure and acute respiratory outcomes.

Acknowledgments

The authors thank Andrew Cakebread of King's College London for his assistance with ICP-MS analysis and Jim Mills from Air Monitors (Tewkesbury, UK) for the loan of the Thermo ASAP instrument.

Appendix A

AA	ascorbate
ASAP	airborne sample analysis platform
GSH	reduced glutathione
HPLC	high pressure liquid chromatography
ICP-MS	inductively coupled plasma mass spectrometry
NO_x	nitrogen oxides
OP	oxidative potential
$OP^{T}m^{-3}$	total particulate oxidative burden per cubic meter of sampled air
$OP^{T}\mu g^{-1}$	total particulate oxidative burden per unit mass PM
$OP^{AA}\mu g^{-1}$	particulate OP with respect to AA depletion per unit mass PM
$OP^{GSH}\mu g^{-1}$	particulate OP with respect to GSH depletion per unit mass PM
PM	particulate matter
PM_{10}	particulate matter with an aerodynamic diameter of 10 μm
PM_{1-10}	particulate matter with an aerodynamic diameter between 1 and 10 μm
PUF	polyurethane foams
RTLF	respiratory tract lining fluid
TEOM	tapered element oscillating microbalance

Supporting Information Available

The supplemental methodology details the instrumentation employed for continuous size distribution measurements, sampling location, campaign period, and source apportionment uncertainty calculations. Additional results are included discussing temporal variation of PM physical (size distributed PM number and mass concentrations) and gaseous pollutant (SO_2) characteristics. This material is available free of charge via the Internet at http://pubs.acs.org.

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Item No. 6
Supporting Document No. 5

CONCORD MONITOR
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Weighing fireworks' effects

By *Trent Spiner*
Created *07/03/2010 - 00:00*
Northwood lake
Research suggests contamination

When Jim Vaillancourt looks out over Northwood Lake from his porch tomorrow night, he won't be surprised to see two neighbors locked in their annual contest for the biggest, longest and most awesome fireworks show. After all, last year's battle went for about an hour and a half.

But as revelers plan to light fireworks across the area tomorrow, one state official said preliminary research shows fireworks can harm local water bodies because their residue can cause cancer, contaminate water supplies and poison wildlife.

State limnologist Jody Connor said he plans to continue researching the effect of fireworks from this weekend after finding that the heavy metals used by some manufacturers are already affecting the state's water bodies.

"I think a lot of people think fireworks are beautiful and have no toxins in them, but we are trying to educate people there are some toxins; even the cardboard can cause pollution," he said.

While Connor's research is preliminary, it has helped residents from at least two local municipalities ask for a fireworks ban near water bodies.

In Northfield, Vaillancourt asked selectmen last year to consider a ban while they were discussing a permitting process for lighting fireworks.

"I can't imagine that over the weekend people who are just pounding hours' worth of fireworks out over the lake can't have some kind of detrimental effect over the long term," he said.

Vaillancourt, president of the Northwood Lake Watershed Association, said he is trying to educate residents within a mile of the lake about how their actions can pollute the water. Since speaking with selectmen, banning fireworks has dropped down his list of issues after the lake required an emergency treatment last month to fight an invasive species of milfoil.

When he discusses it with neighbors, he said many argue that the safety concerns of using fireworks over land outweigh environmental concerns.

Item No. 6
Supporting Document No. 5

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NORTHWOOD LAKE

Weighing fireworks' effects - page 2

Research suggests contamination

In Franklin, resident Martin Russo told councilors that he sees more trash from bob houses than fireworks. Rep. David Palfrey said septic systems and runoff from other sources do more damage than fireworks, according to minutes of the meeting.

Councilors questioned how the ordinance would work and who would enforce it.

"I think they felt it was another level of regulation and they didn't want it," Smith said.

Connor said some companies are researching fireworks that contain fewer heavy metals.

"All these metals eventually fall to the earth, and they eventually get into the lakes," Connor said. "I'm not sure there's enough research out there, but eventually we'll have additional research that tells us how dangerous they may be to the atmosphere and to the water."

Trent Spiner can be reached at 369-3306 or tspiner@cmonitor.com.

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What does gas, oil and boat motor exhaust add to the water?
By Robert Frypp - 07/03/2010 - 8:10 am

Not to mention road salt that eventually makes it's way into the water as well when the snow melts? Because I'd worry a lot more about those effects than the minuscule, if any, effect a couple errant firework remnants might/could add to the water once a year.

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Item No. 6
Supporting Document No. 5

Michelle Mata - Fireworks

From: <LGreene98@aol.com>
To: <TAlo@waterboards.ca.gov>
Date: 12/16/2010 5:49 AM
Subject: Fireworks

To Whom It May Concern:

Due to family emergency I am, unable to come to the meeting today on the issue of fireworks over the water However, I hope that this statement can be entered as part of the citizen testimony.

Since it is the responsibility of this body to make sure our waters are not polluted, it is important to make sure that all chemicals that get put into the water are safe. From what I have read, fireworks over the water pollute the water, therefore strict regulation needs to be maintained for the protection of all of us.

Times change and new information is obtained. This organization needs to incorporate this new information as part of it's protection of our ever decreasing safe water supply. At one time fireworks were propelled over El Capitan, at Yosemite National Park. Wise minds decided, with new information about the danger, decided to discontinue these fireballs. This was not considered patriotic, as many people loved these fireworks. As a child, there were firecracker, and fireworks stands, all over the Los Angeles where I grew up. Our fathers would light them in the street. I can still remember the sparklers the kids used to play with. Then, one 4th of July, a child was burned and a house caught on fire from these pyrotechnics. Someone finally said enough, as homes were getting closer to each other and it was becoming dangerous. Again, not considered very patriotic, but times changed.

For the last many years it has been the policy to shoot fireworks over areas such as Mission Bay, and other bodies of water on the 4th of July. However, we know now that this is not safe for the fish, plants in the water, or for humans in the water. Therefore, it is once again time to make an unpopular decision. People will say it is not patriotic. However, people have also said many things about the environment that have been correct, and it is up to this body to listen, learn, and most of all educate yourselves to the harm that is being done by allowing a light show to be more important than safety. This is patriotic, not the other way around.

I am sure that this board will do the right thing, by protecting our waters. Please do not continue to allow fireworks to pollute the water any further. You will be doing our children a great favor. To the best of my knowledge, there is nothing intrinsically patriotic about fireworks.

Warm regards,

Dr. Lorri Greene
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Cardiff-by-the-Sea, CA 92007
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