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December 22, 2017

Ms. Jeanine Townsend
Clerk to the Board
State Water Resources Control Board
1001 I Street, 24th Floor
P.O. Box 100
Sacramento, CA 95812-0100

Via email: commentletters@waterboards.ca.gov

Re: Comments to A-2239(a)-(c)

Dear Ms. Townsend, Chair Marcus, Board Members and Staff:

Thank you for the opportunity to comment on the 2017 Proposed Order for the East San Joaquin (ESJ Order). The following comments are submitted on behalf of The Otter Project, our water quality program, Monterey Coastkeeper, and our 2000 members and board of directors. Our interest in the ESJ Order is based on several factors including the importance of the ESJ watersheds feeding into the Delta and ultimately coastal California, and because the State Water Resources Control Board has stated the ESJ Order will be precedential to agricultural orders throughout the State, including the Central Coast. The Otter Project and its members place high value on the variety of beneficial uses supported by good water quality and at times threatened by poor regulation of water quality standards meant to support those beneficial uses.

The Otter Project has participated in the preparation and review of the joint comment letter (CCKA / The Otter Project / CRLA), supports those comments, and incorporates those comments by reference herein. This letter supplements those comments and reflects The Otter Project's past decade of experience working on agricultural orders, their strengths and omissions, statewide.

This comment letter will address some of the more technical aspects of the ESJ Order including:

- A and R (applied and removed nitrogen)
- Live organism toxicity testing using EPA approved and SWRCB recommended tests
- Incorporating: aquatic health, a broader range of beneficial uses, and the Central Valley Basin Plan "Biostimulatory Substance" narrative objective into the ESJ Order.

A and R (applied and removed nitrogen)

The 2017 Proposed Order introduces a new metric for nitrogen reporting, Applied (A) and Removed (R). "A" is composed of nitrogen applied as amendments and nitrogen applied that is already present in the irrigation water (which can be a very significant amount because groundwater is already polluted with

nitrogen in many regions). The "A" does not include the nitrogen left over in the soil between planting, which can also be very significant and is a critical omission. "R" is comprised of the nitrogen in the form of yield and any clippings, culls, plants removed, etc.. Growers will only report their yield (which they do not want to do and consider proprietary information). Studies, conducted by the Coalition, which is ill-equipped to conduct such studies and will take many years to accomplish, will then determine the "R" portion comprised of clippings, culls, plants, etc. This portion is very significant in many crops:

- Tree crops such as almonds and citrus are pruned and topped, with the clippings hauled away.
- Diseased culls such as happens in almonds, are hauled away.
- In strawberries, plants are generally removed and hauled away (because of disease concerns) annually, but not always.
- And more.

These numbers are going to be wildly differing estimates, i.e. the prunings of a one year old tree will be very different an eight year old tree.

After years of estimating these numbers and creating the new metric, growers will report their yield, someone (likely the Coalition) will convert the number to determine total "R" and the metric may or may not prove to be useful.

The 2017 Proposed Order next states that there will be a public stakeholder process to determine appropriate target values for each and every crop, and there are hundreds of crops. The goal is to determine how much nitrogen fertilizer does each crop needs to produce a good crop. Growers should be applying somewhere near the crop need.

How useful will the "A" (applied) number, provided by the growers, be? The Central Coast Regional Board, as part of their Ag Order program, has been collecting "applied" data since 2014. We asked for a summary of that data for two crops, lettuce and broccoli.

[continued on next page]

Arithmetic Mean				
Crop	Year	FertComp_LbcCrAc	Irr_LbsCrAc	FertComplrr_LbsCrAc
Broccoli	2014	217	91	307
Broccoli	2015	205	104	309
Broccoli	2016	196	99	295
Lettuce	2014	189	75	264
Lettuce	2015	170	105	275
Lettuce	2016	169	102	271

Median				
Crop	Year	FertComp_LbcCrAc	Irr_LbsCrAc	FertComplrr_LbsCrAc
Broccoli	2014	201	62	293
Broccoli	2015	189	73	271
Broccoli	2016	185	69	272
Lettuce	2014	174	49	246
Lettuce	2015	150	73	246
Lettuce	2016	159	72	247

Weighted Average by Crop Acres				
Crop	Year	FertComp_LbcCrAc	Irr_LbsCrAc	FertComplrr_LbsCrAc
Broccoli	2014	235	84	319
Broccoli	2015	201	104	305
Broccoli	2016	205	105	309
Lettuce	2014	186	71	257
Lettuce	2015	167	94	261
Lettuce	2016	171	102	272

For lettuce and broccoli, following are the aggregated numbers that would be reported under the ESJ Order (as pounds of nitrogen per acre):

- For 2016 lettuce, the (mean) average applied as amendment was 169 pounds nitrogen per acre
- For 2016 lettuce, the average total applied (water plus amendment) was 271 pounds nitrogen
- For 2016 broccoli, the average applied as amendment was 196 pounds
- For 2016 broccoli, the average total applied was 295 pounds.

What is the useful approach? For years, Resource Conservation Districts, U.C. Agricultural Extension, and independent researchers have been determining "Crop Nitrogen Uptake Values" for many crops grown throughout the United States. These values are determined in field trials, on real farms, side-by-side with farm grown crops. The trials determine how much nitrogen the crop needs, before the yield begins to decline. The trials generally create a range of values reflecting various soil types and climates.

Comparing grower reported applications against the research determined crop uptake values for 2016 lettuce and broccoli we see:

Crop	Research determined N Uptake	Total Average N Applied
2016 Lettuce	120-170 pounds per acre	271 pounds per acre
2016 Broccoli	180-337 pounds per acre	295 pounds per acre

This data shows that many growers are applying far too much nitrogen per acre for lettuce crops and are applying towards the top of the acceptable range for broccoli crops.

While the A R approach provides numbers, it provides no context to suggest the amount of nitrogen needed to produce a good crop. The A R approach does not provide useful regulatory guidance. The 2017 Proposed Order ignores an in-use metric, Crop nitrogen uptake, and will spend years developing a new metric that lacks an enforceable measure.

Lessons Learned:

- The Applied and Removed metric will take years to develop.
- The Applied and Removed metric relies upon many estimates.
- The applied and Removed metric does not determine the appropriate amount of nitrogen to apply, but will supply a number without context.
- Some Regions such as the Central Coast have been gathering nitrogen applied data for several years and comparing that data to crop nitrogen uptake values to determine which growers are likely overapplying nitrogen that can leach to groundwater.

Take-away:

- Real-world, field tested, "crop nitrogen uptake values" have been determined for most major crops in California.
- Uptake values are being used by some regions.
- The 2017 Proposed Order should allow for discretion to use whatever scientifically-derived value it chooses.
- The 2017 Proposed Order should allow for the immediate use of best available science in anticipation that A and R values take many years to develop.

Live organism toxicity testing using EPA approved and SWRCB recommended tests

The 2012 ESJ Order required a short-term chronic (three-day) three-species test for water toxicity plus *Hyalella azteca* for sediment. The 2017 Proposed Order perpetuates the same tests (MRP pg 10).

[Note: It is our understanding that the fathead minnow has been eliminated by the Coalition as a test species, which is fine by us. But the disconnect between the State Order and the actual practice of monitoring is troubling.]

Entirely new pesticide classes have been introduced by the agro-chemical industry, sometimes in response to regulatory efforts. The regulation, or banning, of specific chemicals has led to a near constant churning of chemicals and the regulatory response is akin to whack-a-mole. The Department of Pesticide Regulation tracks and reports the use of pesticides and water quality monitoring programs must keep pace with the changes.

Decisions regarding toxicity monitoring for pesticides should be based on pesticide use patterns, and their relative toxicity to different test species and protocols. The tests being specified by the Order are insufficient as the test organisms are not sensitive to in-use chemicals.

Water should not kill the life that is supposed to live in it. It's that simple.

Toxicity testing with live organisms is the application of that premise. The US EPA has created approved protocols and tests for over 40 organisms (generally algae, invertebrates, and small fish) that live in

water or aquatic sediments. Generally, the organisms are placed in a sample of water alongside a clean water "standard." After a standardized period of time in a laboratory control environment the samples are observed, and the number of surviving organisms is counted. Samples can be tested for "acute" (short term) or "chronic" (longer term) exposure. "Growth" and "reproduction" can also be tested. It is important to test both water and sediment as some chemicals adhere to sediment particles and some organisms live in the sediment.

Organisms must be appropriate for the water and sediment being tested and must be sensitive to the suspected cocktail of chemicals; no one test organism is equally vulnerable to all chemicals and therefore, one test organism is seldom adequate. Finally – and especially germane here -- the chemical cocktail is constantly changing, and testing must reflect the chemicals currently in use.

Toxicity testing is critical and expedient. Toxicity testing, while moderately expensive, tests for a broad spectrum of chemicals and avoids expensive chemical analyses to measure the concentrations of each suspect chemical: If the appropriate organisms survive, grow, and reproduce, the water is assumed to be not toxic to aquatic life and further expensive chemical analysis may not be necessary. Aquatic life is important, especially invertebrates, algae, and small fish because these are the organisms that are the base of the food chain. Algae feed invertebrates, that feed fish, that feed endangered species, sport and commercial fish. If water kills the organisms that live in it, the entire aquatic system can collapse and die and weaken the terrestrial systems, people, and communities that depend upon it.

The US EPA toxicity testing manual created under 40 CFR 136.3 can be found at https://www.epa.gov/cwa-methods/acute-toxicity-wet-methods. Importantly, 2015 California Statewide Ambient Monitoring Guidance (SWAMP-TM-2015-0001) recommends the appropriate organisms to use in California and the specific organisms and tests for specific environments and chemicals of concern. SWAMP is a program of the SWRCB. The guidance is readily available, easy to read, and only six pages (and is attached as Attachment One).

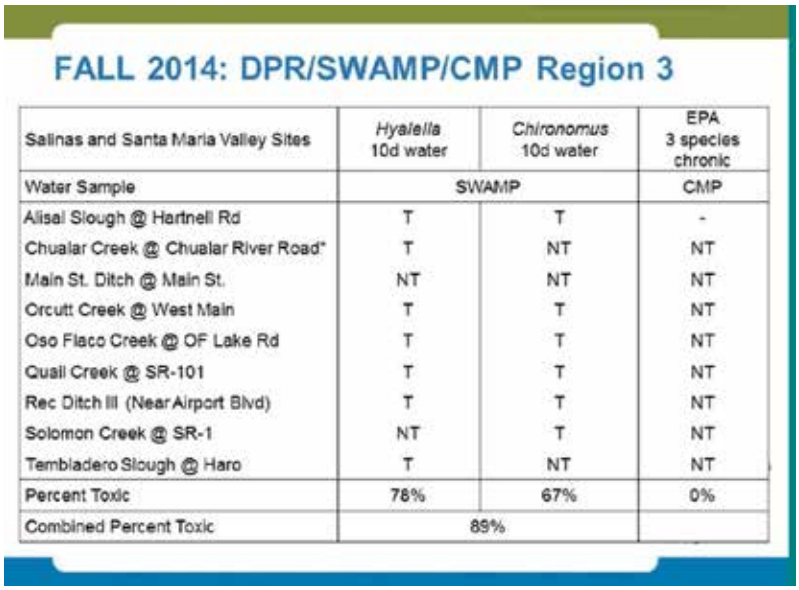
The importance of staying current and picking the appropriate test organisms was illustrated in 2012 and reported in a scientific paper in 2015:

Sample	Ceriodaphnia Survival Percentage	Hyalella Survival Percentage
untreated	80	86
untreated	100	54
untreated	96	98
untreated	96	0
untreated	0	0
untreated	96	50
Samples meeting toxicity standards	**5 of 6**	**2 of 6**

Table comparing toxicity rates based on the 2012 Central Coast Ag Order's testing method and more comprehensive methods that include sediment (Hyalella). Extracted from B.M. Phillips, et al., "The Effects of the Landguard A900 Enzyme on the Macroinvertebrate Community in the Salinas River, California," 69 Arch. Environ. Contam. and Toxicol. 1, 5 (June 29, 2015), available at http://www.ncbi.nlm.nih.gov/pubmed/26118992.

This above figure illustrates that when water alone is tested, with only a single test organism that lives in the water column, five of six samples appear clean. When a sediment test is added, using a small organism that lives on or on top of the sediment, only two of six appear not.

On the Central Coast, a Department of Pesticide Regulation and University of California at Davis research team, conducted follow-up testing at a set of sites monitored by the Central Coast [agricultural] Cooperative Monitoring Program. In this set of tests, another organism was added (a midge) that is sensitive to neonicitinoids, a class of chemicals very quickly gaining in popularity and implicated in pollinating bee population collapse.



FALL 2014: DPR/SWAMP/CMP Region 3

Salinas and Santa Maria Valley Sites	*Hyalella* 10d water	*Chironomus* 10d water	EPA 3 species chronic
Water Sample	SWAMP		CMP
Alisal Slough @ Hartnell Rd	T	T	-
Chualar Creek @ Chualar River Road*	T	NT	NT
Main St. Ditch @ Main St.	NT	NT	NT
Orcutt Creek @ West Main	T	T	NT
Oso Flaco Creek @ OF Lake Rd	T	T	NT
Quail Creek @ SR-101	T	T	NT
Rec Ditch III (Near Airport Blvd)	T	T	NT
Solomon Creek @ SR-1	NT	T	NT
Tembladero Slough @ Haro	T	NT	NT
Percent Toxic	78%	67%	0%
Combined Percent Toxic	89%		

A table comparing toxicity rates based on the 2012 Waiver's testing method and more comprehensive methods. In this table, "T" means "toxic" and "NT" means "not toxic." The fourth column (EPA/CMP) lists the results of the Ag Monitoring Program toxicity test, while the second and third columns represent the results of other EPA-approved and SWRCB Surface Water Ambient Monitoring Program recommended tests methods. Table and Central Coast RWQCB discussion is found at: www.waterboards.ca.gov/centralcoast/board_info/agendas/2015/may/item23/item23_stfrpt.pdf .

In this test, the Ag Monitoring Program (right column and using an old-style testing protocol) found zero toxicity while the recommended testing protocol (sediment test and midge, tested for chronic toxicity) found 89% toxicity.

The 2017 Proposed Order continues to rely upon the old, not recommended, and research discredited tests. As on the Central Coast, there is ample reason to require updated tests. Pesticides change in response to new crops, new bugs, and new regulatory pressures which focus on specific chemicals and catalyze switching to a new toxic chemical instead of solving the toxicity problem with improved management practices.

Very generally, organochlorine pesticides such as DDT à were replaced by organophosphate chemicals such as diazinon and chlorpyrifos à were replaced by pyrethroids such as bifenthrin à were replaced by neonicitinoids and more. Testing protocols must always follow these shifts.

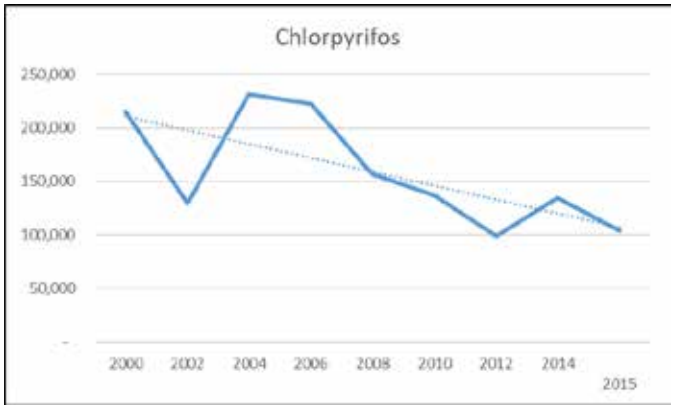
The following table and graphs glean comprehensive pesticide use data for Madera, Merced, and Stanislaus counties and plainly illustrate the changes from pesticide to pesticide. Chlorpyrifos is on the decline, bifenthrin and imidacloprid are on the increase. Total pounds of pesticide applied are also increasing.

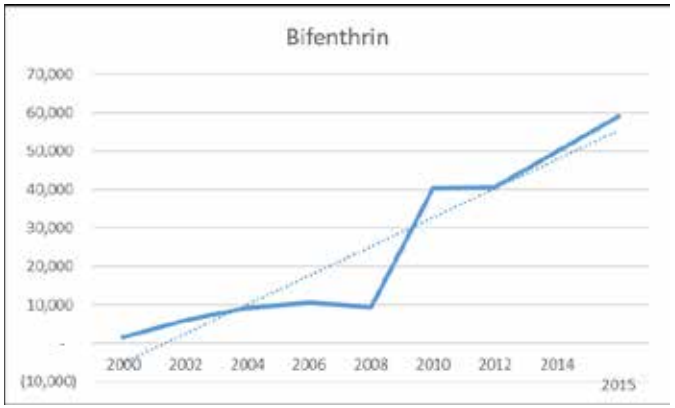
[Note: While not discussed here, the total pounds of copper hydroxide is also shown. Dissolved copper is a recurring and increasing problem in the East San Joaquin].

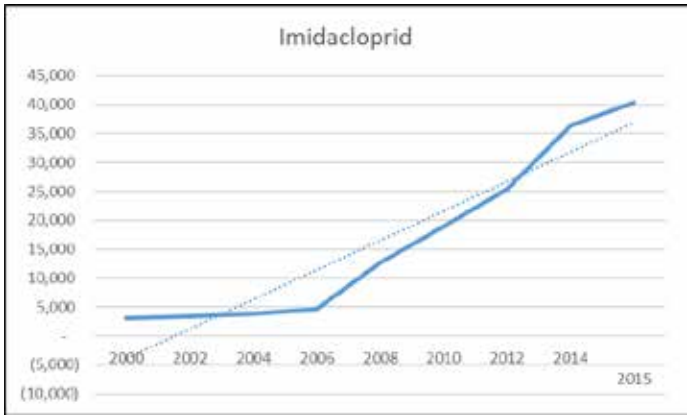
The chemicals listed and illustrated are not cherry-picked by The Otter Project / Monterey Coastkeeper, but are the chemicals called out by the SWRCB SWAMP Technical Report. Increase in imidacloprid use is also called out by numerous DPR reports.

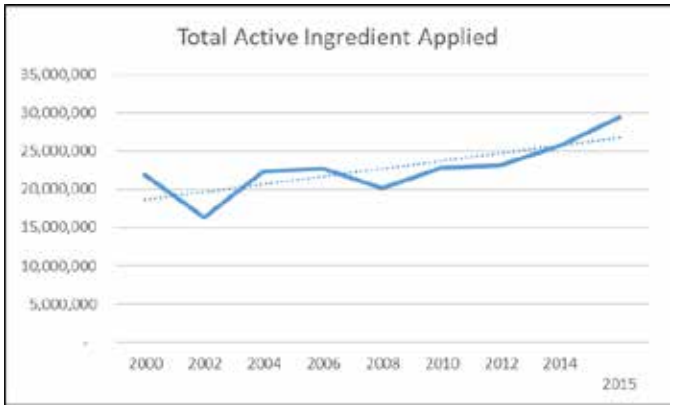
[continued on next page]

	Class	2000	2002	2004	2006	2008	2010	2012	2014	2015 *Most Recent Available*	
Copper hydroxide		539,635	428,220	439,985	553,944	306,962	313,884	281,061	315,810.30	458,368	up & down
Chlorpyrifos	organophosphate	214,929	129,939	231,248	222,598	156,997	136,855	98,700	134,887	103,739	down
Diazinon	organophosphate	118,039	55,862	39,333	15,367	6,860	4,890	5,569	3,751	5,780	down
Bifenthrin	pyrethroid	1,514	5,925	9,226	10,627	9,399	40,291	40,534	49,768	59,089	up
Imidacloprid	neonicitinoid	3,090	3,397	3,825	4,605	12,680	18,917	25,362	36,356	40,332	up
Total Active Ingredient Applied		21,851,223	16,274,100	22,358,683	22,657,555	20,167,845	22,827,954	23,194,710	25,716,476	29,493,910	up



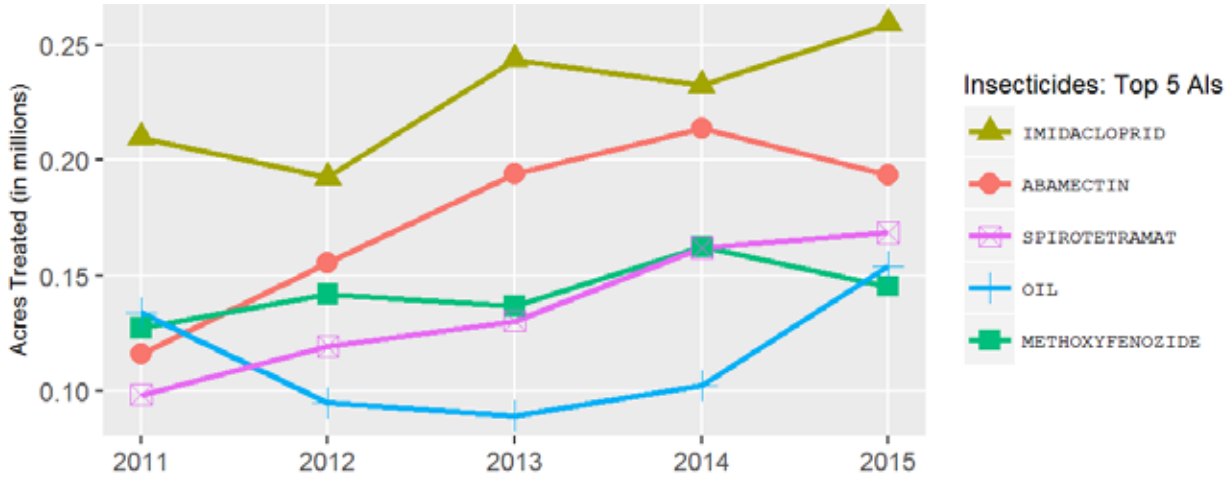






All data represents the sum of pounds of ingredient applied in Madera, Merced, and Stanislaus counties. Note that the trends begin well before the ESJ Order could have any impact. Data is compiled from Department of Pesticide Regulation Annual Pesticide Reports found at:
http://www.cdpr.ca.gov/docs/pur/annual_summaries.htm

Better and more appropriate monitoring of pesticide impacts is essential to protect human health and the environment from harm. The trends of pesticide use are well known and readily available. Grapes (both wine and table) are important crops in the ESJ region and the switch to noenicitinoids/imidacloprid is well documented.



Statewide trend of insecticide use by year and acres treated for wine grapes. Source: http://www.cdpr.ca.gov/docs/pur/pur15rep/comrpt15.pdf pg 107.

A 2016 review of imidacloprid conducted by DPR and entitled "The Environmental Fate of Imidacloprid" (http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/Imidacloprid_2016.pdf) is found at and is attached as Attachment Two. The Report finds:

- Imidacloprid is the largest selling pesticide in the world
- It persists in the environment with a half-life of over 1000 days in some environments
- Degradate products of imidacloprid are also toxic
- In California its number one use (by far) is for wine grapes
- Imidacloprid is highly water soluble and is listed on the DPR Groundwater Protection List

The importance of considering new data annually is especially important given the scale of pesticide application in the ESJ region; Madera, Merced, and Stanislaus counties are ranked 5, 6, an 8 in total pounds of pesticide use statewide (nearby Fresno County is #1).

Lessons Learned:

- In-use pesticides change in response to a variety of factors including regulatory pressures.
- Appropriate live organism toxicity testing is essential as it is our only tool that compensates for pesticide switching and synergistic affects.

Take aways:

- Water should not kill the life that lives in it;
- State ordered water quality monitoring programs must follow the State's own guidance.
- Specifically:
    - Water quality programs must continuously consider the new chemicals being used
    - At this time, testing should be for chronic 10-day exposure and should add the midge, Chiromonus.
- "Success" claims from the ESJ Water Quality Monitoring Coalition are likely exaggerated as chlorpyrifos was declining in use well before the Coalition had any impact.

Incorporating aquatic health, a broader range of beneficial uses, and the Central Valley Basin Plan "Biostimulatory Substance" narrative objective into the ESJ Order

The East San Joaquin Basin Plan includes the narrative objective: "Water shall not contain biostimulatory substances which promote aquatic growths in concentrations that cause nuisance or adversely affect beneficial uses."

And the 2017 Proposed Order states:

> "Implementation of numeric and narrative water quality objectives under the Order involves an iterative process. The Order's MRP establishes management plan trigger limits that are equivalent to the applicable Basin Plan numeric water quality objectives. For constituents that are not assigned Basin Plan numeric water quality objectives, board staff will develop trigger limits in consultation with the Department of Pesticide Regulation (for pesticides) and other agencies as appropriate. Board staff will provide interested parties, including the third-party representing Members, with an opportunity to review and comment on the trigger limits. The Executive Officer will then provide the trigger limits to the third-party. Those trigger limits will be considered the numeric interpretation of the applicable narrative objectives. In locations where trigger limits are exceeded, water quality management plans must be developed that will form the basis for reporting which steps have been taken by growers to achieve compliance with numeric and narrative water quality objectives."

The biostimulatory substance narrative standard has been completely ignored and no trigger limit has been calculated or set. For nutrients, specifically nitrogen, the only trigger limit is the drinking water standard that is far above, perhaps by an order of magnitude, what would be the biostimulatory trigger-limit standard.

Guidance for setting a numeric standard to interpret the narrative is readily available and has been used by other regions.

- In 2000, the US published:

    U.S. EPA, 2000a. Ambient Water Quality Criteria Recommendations: Information Supporting the Development of State and Tribal Nutrient Criteria. Rivers and Streams in Nutrient Ecoregion III. U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA 822-B-00- 016.

U.S. EPA, 2000b. Nutrient Criteria Technical Guidance Manual, Rivers and Streams. U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-822-00-002.

- In 2010, the Central Coast Region used the guidance to interpret their narrative standard. The numeric standard for biostimulatory substances is 1.0 mg/L nitrate (as N). Their methodology was published as a SWRCB SWAMP Technical Report.

  SWAMP, 2010. Interpreting Narrative Objectives for Biostimulatory Substances for California Central Coast Waters. SWRCB SWAMP Technical Report. Found at: https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/reglrpts/rb3_biostimulation.pdf

- And in 2017, the US EPA updated the 2000 guidance. 2017 US EPA Guidance is found at: https://www.epa.gov/nutrient-policy-data/ecoregional-nutrient-criteria-rivers-streams

While at the same time the State Water Board is sending out a press release, "Harmful Algal Bloom Season Beginning in California's Waterways" (found at www.waterboards.ca.gov/press_room/press_releases/2017/pr_05262017_cyanos.pdf), the Central Valley Board is ignoring its Basin Plan standard for biostimulatory substances. The press release includes the guidance:

> "You can help prevent algal blooms in our waters by taking the following measures: Be conservative with your use of water, fertilizers and pesticides on your lawn, garden or agricultural operation."

The biostimulatory standard is an important tool for protecting aquatic life related beneficial uses and recreation uses.

Lessons Learned:

- The Central Valley Basin Plan includes a biostimulatory substances narrative standard.
- The 2017 Proposed Order states that Basin Plan standards will be converted to "trigger limits"
- No trigger limit has been set.
- Guidance is available from both US EPA and SWRCB SWAMP
- The Central Coast Region has applied the guidance and determined an appropriate numeric standard is 1.0 mg/L nitrate as total N.

Take-aways

- The 2017 Proposed Order must include the Biostimulatory standard
- The nitrogen trigger limit of 10 mg/L nitrate (drinking water standard) is entirely inappropriate
- The 2017 Order should use a standard of 1.0 mg/L nitrate as nitrogen in the interim while it develops its own standard.

Thank you for the opportunity to provide comment to the 2017 ESJ Proposed Order, Comments to A-2239(a)-(c). We are available to answer any questions you may have as you consider our comments.

Sincerely,

Steve Shimek
Executive Director

Attachments

Attachment One

Updated recommendations for monitoring current-use pesticide toxicity in water and sediment in the Surface Water Ambient Monitoring Program

Prepared by: Brian Anderson[1], Bryn Phillips[1], Marie Stillway and Linda Deanovic[2], Debra Denton[3], Michael Lyons[4], Mary Hamilton[5]

[1]*University of California, Davis–Granite Canyon*
[2]*University of California, Davis–Aquatic Toxicity Lab*
[3]*U.S. Environmental Protection Agency, Region 9*
[4]*California Regional Water Quality Control Board, Region 4*
[5]*California Regional Water Quality Control Board, Region 3*

SWAMP Technical Memorandum
SWAMP-TM-2015-0001
September 2015

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BACKGROUND – Changing Pesticides

A decade of evidence from the Surface Water Ambient Monitoring Program has indicated that toxicity to invertebrates is most often caused by pesticides (Anderson et al., 2011). As patterns of urban and agricultural pesticide use change in California, the species used to monitor water and sediment toxicity in SWAMP programs should be selected to properly evaluate these variations. While past data showed that much of the surface water toxicity was due to organophosphate pesticides such as diazinon and chlorpyrifos, these have largely been replaced by pyrethroids in most watersheds. In addition, recent data suggest new classes of pesticides are increasing in use, including phenylpyrazoles such as fipronil, and neonicotinoids such as imidacloprid. Decisions regarding toxicity monitoring for these pesticides should be based on their use patterns, and their relative toxicity to different test species and protocols. In addition, the decision to monitor in water and/or sediment depends on the solubility and stability of these pesticides, which dictates their environmental fate. The following discussion provides guidance for application of appropriate test species and protocols to

SWAMP monitoring coordinators interested in incorporating toxicity testing into their monitoring designs. Emphasis is placed on monitoring in freshwater habitats but two protocols are also recommended for marine receiving systems.

RELATIVE SPECIES SENSITIVITY

Four classes of pesticides that continue to be detected at toxicologically relevant concentrations in California streams are organophosphates (e.g., diazinon, chlorpyrifos, malathion), pyrethroids (e.g., bifenthrin, permethrin, cypermethrin), phenylpyrazoles (e.g., fipronil and its degradates), and neonicotinoids (e.g., imidacloprid, clothianidin, thiamethoxam). The relative acute toxicity of selected pesticides from these classes to standard test species is presented as 96-hour median lethal concentrations (LC50s) in Table 1. These data show that at 96 hours, the amphipod *Hyalella azteca* is the most sensitive to pyrethroids such as bifenthrin, the midge *Chironomus dilutus* is most sensitive to fipronil and its degradates, and the cladoceran *Ceriodaphnia dubia* is most sensitive to organophosphates such as chlorpyrifos. Both *C. dubia* (48-hour LC50) and *C. dilutus* have comparable acute sensitivities to imidacloprid, but evidence suggest that *C. dilutus* is more sensitive in chronic exposures. *Hyalella azteca* is also relatively sensitive to the organophosphate pesticide chlorpyrifos. Table 1 also lists a column of fathead minnow (*Pimephales promelas*) LC50 values to demonstrate the lower sensitivities of this vertebrate to current use pesticides. The other component of U.S. EPA three-species testing, the algae *Selenastrum*, does not respond to these pesticides, but could be used for monitoring involving potential toxicity caused by herbicides.

Because pesticides are usually detected in mixtures (U.S.G.S., 2006), the use of more than one toxicity test organism is recommended if multiple pesticides are present or suspected, and if the monitoring budget allows for it. Pesticide mixtures can be additive, synergistic, or antagonistic. Lydy et al. (2004) provides a review of challenges in regulating pesticide mixtures with differing modes of action and relative toxicities to aquatic organisms. Surface waters containing current use pesticides may include mixtures containing the parent compound and its toxic degradates. Phillips et al. (2014) demonstrated that monitoring the single active ingredient of the organophosphate mosquito control pesticide naled did not capture all of the potential impacts to receiving systems because the primary degradate, dichlorvos, was more toxic than the parent compound. This characteristic also applies to fipronil, where the degradates fipronil sulfone and fipronil sulfide are more toxic to *Chironomus dilutus* (Weston and Lydy 2014). Toxicity testing integrates the effects of mixture toxicity from different pesticides, as well as active ingredient and degradates.

Acute tests measure lethality, whereas chronic tests measure sub-lethal effects such as reduced reproduction, growth, or development. The differences between acute and chronic exposures in water column tests are typically defined by the protocol endpoint and test duration. Some pesticides demonstrate greater chronic toxicity to certain species so selection of chronic vs. acute toxicity test protocols should consider this characteristic. For example, there is little difference in 10 day and 28 day sediment exposures of *H. azteca* to the pyrethroid pesticide bifenthrin (Table 2; (Anderson et al., 2015)), but the difference in sensitivity between a 96 hour and 10 day water exposures of *H. azteca* to the neonicotinoid imidacloprid is much greater. The sensitivity of *C. dilutus* to imidacloprid in chronic water exposures is greater than that of *H. azteca*, and even *C. dubia*. Monitoring programs for pyrethroids will be adequately protective using the 96 hour water or 10 day sediment test protocols (note: water vs. sediment monitoring is discussed in the following section). Neonicotinoids, such as imidacloprid, demonstrate greater toxicity in longer term chronic toxicity tests (Table 2; see review (Morrissey et al., 2015)). Therefore, monitoring with longer-term tests using *C. dilutus* is recommended for receiving systems where imidacloprid is of concern (e.g., 10 day and 28 day water test protocols). Recent data by the California Department of Pesticide Regulation suggest that the highest concentrations of imidacloprid

have been measured in agricultural watersheds (Starner and Goh, 2012), so chronic testing in agriculture-dominated watersheds is a current priority. Although the imidacloprid 28 day LC50 for *C. dilutus* is 0.91 µg/L, Morrissey et al., (2015) suggest 0.1 µg/L for chronic sublethal effects. These authors also suggest a long-term chronic protective value based on a probabilistic risk assessment of 0.035 µg/L.

A source of acute and chronic benchmarks for standard test species used for the evaluation of pesticide registration is the U.S. EPA Office of Pesticide Programs (OPP) Aquatic Life Benchmarks Database. The database is maintained by OPP and provides acute and chronic endpoints for over 300 parent pesticide compounds and degradates in surface waters. These benchmarks are developed using data from ecological risk assessments for pesticide registration decisions. The results of toxicity tests using standard species are reported and these species typically include one or more species of fish, invertebrates, and both vascular and non-vascular plants.

Table 1. Acute water toxicity of representative pesticides to standard test species in water.

	96 hour water LC50 (µg/L)			
Pesticide	*Ceriodaphnia dubia*	*Hyalella azteca*	*Chironomus dilutus*	*Pimephales promelas*
Bifenthrin	0.142 [a]	0.0093 [e]	0.069 [i]	1.90 [k]
Fipronil	17.7 [b]	0.728 [f]	0.033 [f]	398 [k]
Imidacloprid	2.07 [c]	65.4 [g]	2.65 [j]	>1,000 [l]
Chlorpyrifos	0.053 [d]	0.086 [h]	0.29 [i]	203 [m]

[a] (Wheelock et al., 2004), [b] (Konwick et al., 2005), [c] 48-hour LC50 (Chen et al., 2010), [d] (Bailey et al., 1997), [e] (Anderson et al., 2006), [f] EC50 (Weston and Lydy, 2014), [g] (Stoughton et al., 2008), [h] (Phipps et al., 1995), [i] (Ding et al., 2012), [j] (LeBlanc et al., 2012), [k] (Beggel et al., 2010)(24-hour LC50), [l] (Lanteigne et al., 2015), [m] (Holcombe et al., 1982)

Table 2. Acute versus Chronic LC50s for bifenthrin and imidacloprid toxicity to *H. azteca* and *C. dilutus*. ND indicates not determined.

	Hyalella azteca			*Chironomus dilutus*	
Pesticide and Matrix	96 hour	10 day	28 day	96 hour	28 day
Bifenthrin in Sediment (ng/g)	ND	9.1 [a]	9.6 [a]	60.2 [c]	Uknown
Imidacloprid in Water (µg/L)	65.4 [b]	7.01 [b]	7.08 [b]	2.65 [d]	0.91 [b]

[a] (Anderson et al., 2015), [b] (Stoughton et al., 2008), [c] (Maul et al., 2008), [d] (LeBlanc et al., 2012)

*Morrissey et al., 2015 suggest 0.1 ug/L for chronic sublethal effects; these authors suggest a long term chronic protective value based on a probabilistic risk assessment of 0.035 ug/L.

WATER and SEDIMENT MATRICES and RECOMMENDATIONS

The environmental fate of current use pesticides largely depends on their relative stability and solubility in water. The octanol water partitioning coefficient (K_{ow}) is a laboratory derived parameter used as a surrogate measure for the potential of organic chemicals to accumulate in tissues; it is also used as an indicator of relative solubility. Pesticides with high log K_{ow} values are hydrophobic and pesticides with lower log K_{ow} values are more soluble. Pyrethroid pesticides like bifenthrin are highly hydrophobic and therefore readily partition to particles in water and accumulate in sediments. Urban stormwater and agriculture monitoring programs also routinely detect pyrethroids in water. Based on this, and the relative sensitivity of test species, the primary environmental compartment and matrix recommended for monitoring pyrethroids would be sediments using the 10-day *H. azteca* protocol (Table 3). Depending on resources, water toxicity testing for pyrethroids also provides useful information and the 96-hour water test with *H. azteca* is appropriate for this application. Fipronil and its degradates have moderate log K_{ow} values and therefore can be expected to accumulate in sediments and be detected in water. As with pyrethroids, they can be monitored in both matrices depending on resources. Toxicity testing should be conducted with the midge *C. dilutus* based on its greater sensitivity to this pesticide. For sediment, the 10-day test is applicable. For water, the 96 hour and 10 day tests are applicable, but the 10 day test is likely more sensitive (Table 3). Since fipronil is not registered for use in agriculture, monitoring for this pesticide should be restricted to urban watersheds. Neonicotinoids are highly soluble and are therefore not expected to accumulate in sediments. Because they are relatively stable and exhibit greater potential for chronic toxicity to chironomids (testing at longer durations), water testing for this pesticide should use the 10-day test with *C. dilutus*.

Table 3. Log K_{ow} partitioning coefficients for selected current use pesticides, likely environmental compartments and recommended monitoring matrices.

Pesticide Class	Representative Compounds	Usage	Solubility (Log Kow)	Primary Recommended Test Species and Test	LC50 for species and exposure
Pyrethroids	Bifenthrin	Urban/Ag	6.4	*H. azteca* - 10-day Sediment	12.9 ng/g
	Cyhalothrin	Urban/Ag	7.1	*H. azteca* - 10-day Sediment	5.6 ng/g
	Cypermethrin	Urban/Ag	6.8	*H. azteca* - 10-day Sediment	14.9 ng/g
	Permethrin	Urban/Ag	6.3	*H. azteca* - 10-day Sediment	201 ng/g
Phenylpyrazoles	Fipronil	Urban	4.1	*C. dilutus* - 10-day Sediment	0.90 ng/g
	Fipronil Sulfide	Urban		*C. dilutus* - 10-day Sediment	1.11 ng/g
	Fipronil Sulfone	Urban		*C. dilutus* - 10-day Sediment	0.83 ng/g
Neonicotinoids	Imidacloprid	Ag/Urban	0.57	*C. dilutus* - 10-day Water	0.91-2.65 ug/L
Organophosphates	Chlorpyrifos	Ag	4.7	*C. dubia* - 96-hour Water	53 ng/L
	Diazinon	Ag	3.8	*C. dubia* - 96-hour Water	320 ng/L
	Malathion	Ag	2.4	*C. dubia* - 96-hour Water	2,120 ng/L

MARINE and ESTUARINE TESTING

The amphipod *H. azteca* is tolerant of a relatively wide range of salinities and can therefore be tested in estuarine systems up to 15‰. Standard U.S. EPA protocols using euryhaline species with high sensitivity to pesticides include the 10-day sediment test with the amphipod *Eohaustorius estuarius,* and the 96-hour acute and 7-day chronic water tests with the mysid *Americamysis bahia.*

STATUS of U.S. EPA PROTOCOLS

The U.S. EPA describes acute toxicity test methods for *C. dubia* in its freshwater acute toxicity test manual (U.S. EPA, 2002). This method allows a range of test durations from 24 to 96 hours. In addition, the manual includes a supplemental list of test species, including the amphipod *H. azteca* and the midge *C. dilutus*.

The U.S. EPA and United State Geological Survey describe 10-day, and 42-day sediment toxicity test protocols for *H. azteca* and *C. dilutus* (U.S. EPA, 2000). The 10-day sediment exposure procedure can be adapted for use as a 10-day water-only static renewal exposure with both *H. azteca* and *C. dilutus* (this is the procedure currently used at the UCD Granite Canyon Lab for water testing with these species).

Long term tests can also be adapted for shorter durations, such as the 28-day exposures with *H. azteca* (measuring growth and survival), and *C. dilutus* (measuring growth, survival and, potentially, emergence). U.S. EPA and USGS are currently in the process of updating the U.S. EPA 2000 sediment toxicity manual, which will include methods for testing both species in water and sediment using different exposure durations that range from 10 to 42 days for *H. azteca*, and 10 to ~50 days for *C. dilutus*. This revision is currently undergoing internal review within these agencies (personal communication, C. Ingersoll, USGS, Columbia, Missouri).

SUGGESTED CITATION

Anderson BS, Phillips BM, Denton D, Stillway M, Deanovic L, Lyons M, Hamilton M. 2015. Updated recommendations for monitoring current-use pesticide toxicity in water and sediment in the Surface Water Ambient Monitoring Program. SWAMP Technical Memorandum. SWAMP-TM-2015-0001.

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Attachment Two

Environmental Fate of Imidacloprid

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September 1, 2016

1. Introduction

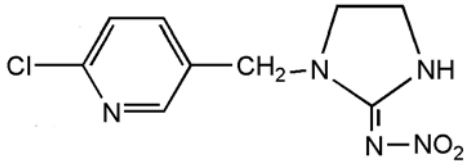
Imidacloprid is the largest selling insecticide in the world (Simon-Delso et al., 2015). Synthesized by Shinzo Kagabu in 1985, this neonicotinoid insecticide was initially manufactured by Bayer CropScience, but has been off patent since 2006 (Tomizawa and Casida, 2011; Kagabu, 1985). While it is used in both urban and agricultural settings, its largest use is in the agricultural sector. Imidacloprid, like the other neonicotinoids, is a systemic insecticide—it is absorbed by the plant at either the roots or leaves and is translocated throughout the plant. Imidacloprid is also found in veterinary and consumer household products (Simon-Delso et al., 2015). Seed treatment is an especially popular method of imidacloprid application in agriculture since the growing plant is protected from pests by incorporating the insecticide as it grows. The application of neonicotinoids as seed treatments were originally marketed as more environmentally friendly than previous generations of insecticides because of the reduced need for foliar applications (Van Dijk et al., 2013). When piercing and sucking pests like aphids feed on treated plants or treated animals, they ingest the insecticide or are exposed via direct contact following foliar application. Neonicotinoids act by modulating post-synaptic nicotinic acetylcholine receptors (nAChRs), thereby disrupting action potential transmission and ultimately leading to death of the exposed organism (Simon-Delso et al., 2015). Imidacloprid is highly water soluble and is relatively stable in the environment. Imidacloprid and other neonicotinoids have come under scrutiny in the last few years as suspects in pollinator bee colony losses associated with colony collapse disorder (CCD). As such, academia, industry, and regulatory agencies have recently conducted extensive reviews of imidacloprid and neonicotinoids to address the role of these insecticides in CCD (USEPA, 2016; Simon-Delso et al., 2015). In this paper, we update the 2000 and 2006 California Department of Pesticide Regulation reviews and discuss recent findings on imidacloprid's effects on nontarget organisms and its environmental fate (Bacey, 2000; Fossen, 2006).

2. Chemistry

Imidacloprid is a chloronicotinyl nitroguanidine insecticide (Fig. 1). It is a solid at room temperature. Among the neonicotinoids, imidacloprid is grouped with those containing a nitro group (along with clothianidin, nitenpyram, thiamethoxam, and dinotefuran) whereas thiacloprid and acetamiprid are grouped separately as those containing a cyano group (Pisa

et al., 2015). Given its low log K_{ow} and high water solubility, imidacloprid is not expected to bind to soils. The physical-chemical properties of imidacloprid are presented in Table 1.

Fig. 1. Molecular Structure:



Chemical Formula: $C_9H_{10}ClN_5O_2$

Table 1. Physical and chemical properties of imidacloprid. All data were submitted in approved studies and obtained from the Pesticide Chemistry Database (California Department of Pesticide Regulation, internal database).

Molecular weight	255.7
Water solubility	514 mg/L (20ºC @ pH 7)
Vapor pressure	1.00 x 10^{-7} mmHg (20ºC)
Hydrolysis half-life	>30 days (25ºC @ pH 7)
Aqueous photolysis half-life	<2 hours (24ºC @ pH 7)
Anaerobic half-life	27.1 days
Aerobic half-life	997 days
Soil photolysis half-life	38.9 days
Field dissipation half-life	26.5–229 days
Henry's constant	6.5 x 10^{-11} atm m^3/mole
(20ºC) Octanol-water coefficient (log K_{ow})	3.7
Soil adsorption coefficient:	
K_d	0.956–4.18
K_{oc}	132–310

3. Chemodynamics

3.1 Soil

Imidacloprid is introduced into soil through direct application or diffusion from treated seeds (Mullins, 1993). Degradation in soil is dependent on characteristics such as soil texture, organic matter content, pH, temperature, sunlight exposure, and sunlight intensity for the region. Imidacloprid is not expected to bind to soils given its high water solubility and low adsorption coefficient (K_d). The US EPA modeled 14 turf insecticides and found that imidacloprid had the highest leaching potential among the modeled insecticides (USEPA, 1993). When sorption was studied in Minnesota-sourced soils, Cox et al. (1997) found that sorption increased with organic carbon content in all soils and at all

concentrations tested (0.05, 1.5, 25, and 250 µg/L). The predominant factor influencing sorption to soil was found to be soil organic matter (Liu et al., 2006). Thus, leaching of imidacloprid to groundwater through soil may be expected in low organic matter soils. The calculated half-life ($t_{1/2}$) with initial imidacloprid concentration of 50 mg/kg under standard laboratory conditions (25 °C, 60% field moisture capacity and darkness) in red brown earth–Natrixeralf soil (1.2 % organic carbon) collected from suburban Adelaide, Australia ranged from 100 to 1,230 days (Baskaran, 1999). Imidacloprid has a shorter half-life when applied to field with cover crops ($t_{1/2}$=48 days) compared to fields without ($t_{1/2}$ = 190 days) (Scholz et al., 1992). In soil, another study found that imidacloprid could be taken up by plants in tandem with natural degradation processes such that concentrations in soil rapidly decrease over time (Horwood, 2007). Studying degradation rates of various termiticides in soil *in situ,* Horwood (2007) found that "products may degrade more rapidly *in situ* than indicated by laboratory experiments." Taken together, these varying values and ranges suggest that persistence of imidacloprid in soil is highly dependent on field and environmental conditions like soil type, organic matter content, clay content, and emergent vegetation.

3.2 Water

Contamination of surface water can occur during and following many of the methods of application. Dust can settle into surface water following drilling of dressed seeds, spray droplets can drift into nearby water, runoff from treated fields can be contaminated, coated seeds can leach into soil water and ground water, and systemically treated plants can decompose and reintegrate the insecticide back into the soil and soil water (Kreutzweiser et al, 2007). Detections of imidacloprid in surface water (described below) have increased as sales and use have increased. Given the physico-chemical properties of imidacloprid, contamination of groundwater is also possible. Groundwater contamination is likely through similar routes as surface water contamination, yet is a larger concern through seed treatment since the pesticide is placed under the soil surface upon initial treatment. In fact, imidacloprid has a Groundwater Ubiquity Score (GUS) leaching potential index of 3.76, which is classified as high (Bonmatin et al., 2015).

3.3 Air

Imidacloprid has low volatility given its low vapor pressure (1.00×10^{-7} mmHg) and Henry's law constant (6.5×10^{-11} atm m^3/mol). Given the properties of the insecticide, the Air Monitoring Network of CDPR (California Department of Pesticide Regulation) does not monitor for imidacloprid. If imidacloprid is ever present in the air, it will likely be for a brief period following spray application. Another possibility is contaminated, volatilized dust from abrasion and dispersion from mechanical blowers on seed sowing machines during planting of treated seeds (Bonmatin et al., 2015). In this scenario, mechanical abrasion associated with planting coated seeds using a mechanical planter could loosen some of the pesticide coating on treated seeds and the blower on the planter would

subsequently disperse the particulate pesticide coating into the air (Greatti et al., 2003), ultimately landing on the soil where it may be incorporated or transported to surface or groundwater.

4. Environmental Degradation

4.1 Biotic

Phugare et al., (2013) reported that imidacloprid degraded up to 78% within 7 days at 30 °C using the bacteria *Klebsiella pneumoniae* strain BCH1. A soil degradation study performed in a laboratory setting (25 °C, 60% field moisture capacity and darkness) found that imidacloprid degraded via first-order kinetics (Baskaran et al., 1999). The 24-month long study found that 37–40% of applied imidacloprid degraded in the red brown earth–Natrixeralf soil. Here, soil moisture content had little to no effect on the rate of imidacloprid degradation. Another study found that in the absence of light, soil degradation half-lives varied between 130 and 160 days (Tisler et al., 2009).

4.2 Abiotic

Hydrolysis

Hydrolysis of imidacloprid is dependent on pH, with increases in alkalinity corresponding to increases in the rate of degradation (Zheng and Liu, 1999). Water with low or neutral pH (pH=3, 5, or 7, respectively) slowly degrades imidacloprid, with one study reporting 1.5% of the pesticide degraded after 3 months (Zheng and Liu, 1999). In pH 9 water, however, original concentrations of imidacloprid decreased by 20% after 3 months. Furthermore, at pH 10.80 and 11.80, the hydrolysis data fit a first-order kinetics equation, with degradation at the higher pH occurring more rapidly. Liu et al., (2006) compared photodegradation and hydrolysis in the dark with intermittent shaking in a 20 mg/L clay-free solution and clay suspension and found that hydrolysis occurred more slowly than photodegradation due to the higher activation energy required by hydrolysis. Zheng and Liu (1999) also reported detection of only one main hydrolysis product, 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidone (imidacloprid urea)—a finding also confirmed by Liu et al., (2006).

Photolysis

Imidacloprid degrades via aqueous photolysis following a first-order reaction rate in a matter of hours, with a reported half-life of 43 minutes in HPLC grade water (Wamhoff and Schneider, 1999). Moza et al. (1998) reported that 90% of imidacloprid in aqueous solution (deionized water) degrades after being irradiated (290 nm) for 4 hours - with a half-life of 1.2 hours. More importantly, degradation of the insecticide in this study did not occur when the aqueous solution was kept in the dark. Using GC-MS, Liu et al. (2006) detected similar photoproducts as Moza et al. (1998) (Fig. 2).

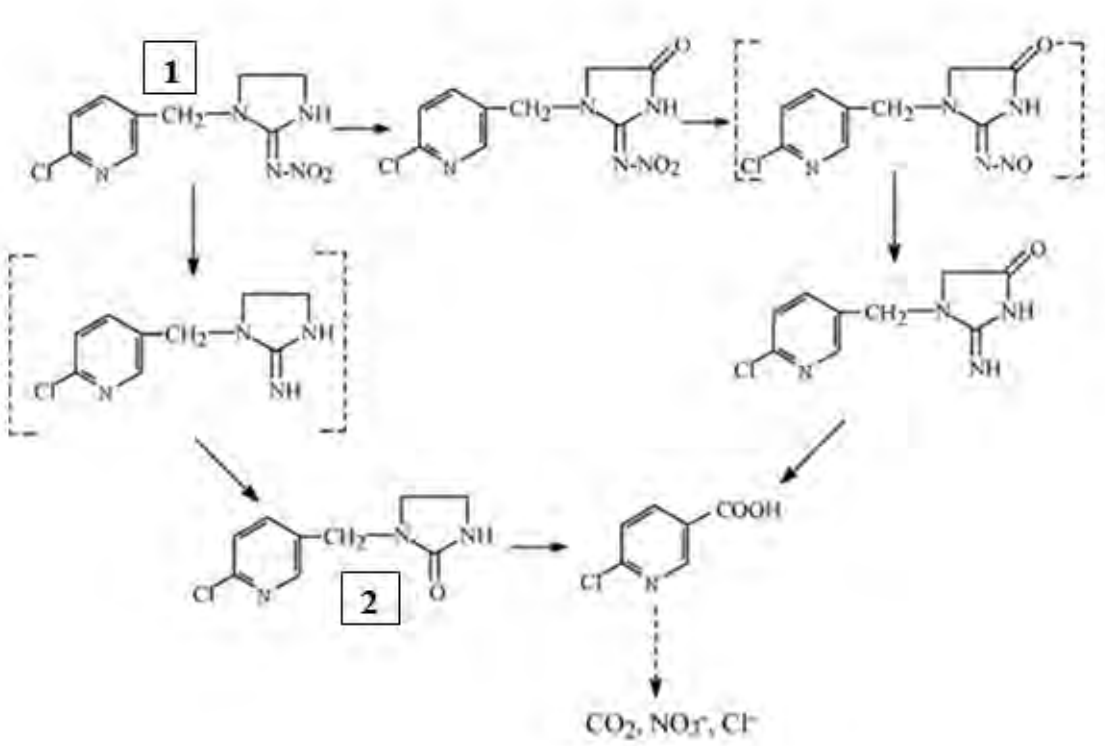


Fig. 2: Proposed pathway for photolysis of imidacloprid in water, adopted from Liu et al. (2006). Dashed brackets represent degradate intermediates. Compound 2, imidacloprid-urea, was the most abundant degradate from the parent imidacloprid, compound 1.

4.3 Use and Detections

Imidacloprid monitoring data, including detections, in California surface water are available beginning in 2000 in the CDPR Surface Water Database (SURF). Unfortunately, there is no data on imidacloprid in the CDPR SURF database for 2006–2009. In 2005, there were 9 detections of imidacloprid (52.9% of the 17 samples analyzed) in California surface water (according to the CDPR SURF Database), but none of the detections exceeded the US EPA chronic invertebrate aquatic life benchmark of 1.05 µg/L (US EPA 2015). However, in 2010, 32 detections (37.2% of the 86 samples analyzed) were recorded with one US EPA benchmark exceedance. By 2014, there were 82 detections of imidacloprid (71.3% of the 115 analyzed samples) in surface water by studies cited in the CDPR SURF database (CDPR, 2016). The newest data for 2015 contain 113 analyzed samples with 78 detections (69.0% detection frequency) and 16 benchmark exceedances. Of the 841 samples stored in the SURF database since records for imidacloprid monitoring became available in 2000, 65 were above the US EPA benchmark (CDPR SURF Database).

Reported use in agricultural settings in California derived from the Pesticide Use Reporting (PUR) database, which does not include seed treatments, in 2014 (the year for which the most-current data is available) totaled 374,061 pounds (CDPR, 2015). The top three sites that were treated with imidacloprid were wine grapes, structural pest control, and grapes (Table 2). Reported imidacloprid agricultural use more than tripled from 2003 to 2013 (Fig. 3). This trend comes as no surprise given the previously reported sales and use figures for imidacloprid (Simon-Delso et al., 2015). Linear regressions were performed between existing benchmark exceedance frequency and imidacloprid use data from PUR for the same year and one year prior. Analysis with PUR of one year prior (i.e., use one year prior chosen to capture all runoff into surface water from previous applications) can give insight into exceedances of the current year and their correlation to product applications from the previous year. The results suggest that benchmark exceedance is correlated with PUR (correlation coefficient=0.708 and 0.859 for PUR of the same year and one year prior, respectively) (Fig. 4, Fig. 5).

Table 2. Top ten use sites for imidacloprid in California in 2014, according to PUR

Site	Pounds imidacloprid
Grape, Wine	56,254
Structural Pest Control	44,093
Grape	36,939
Tomato, Processing	35,344
Orange	22,160
Broccoli	15,970
Landscape Maintenance	15,084
Tangerine	14,244
Pistachio	12,643
Lettuce, Head	12,471

A monitoring study focusing on three agricultural regions in California in 2010 identified the potential for imidacloprid to move off-site and contaminate surface water (Starner and Goh, 2012). This study reported that 14 water samples (19% of total samples) exceeded the US EPA chronic invertebrate aquatic life benchmark. Pursuant to section 13145(d) of the California Food and Agricultural Code, imidacloprid is on the CDPR Groundwater Protection List—a list of pesticides identified by CDPR that have the potential to pollute groundwater. However, a 2009 study by CDPR that monitored for imidacloprid in groundwater did not detect it in any of the 34 wells sampled (Bergin and Nordmark, 2009).

In a study focused on urban surface water monitoring in Southern California, imidacloprid was detected in 73% of the 40 samples analyzed during the July 1, 2014–June 30, 2015 sampling period (Budd, 2016). The Northern California branch of the same monitoring

program detected imidacloprid during the same sampling period in 6 of the 36 samples analyzed (Ensminger, 2016).

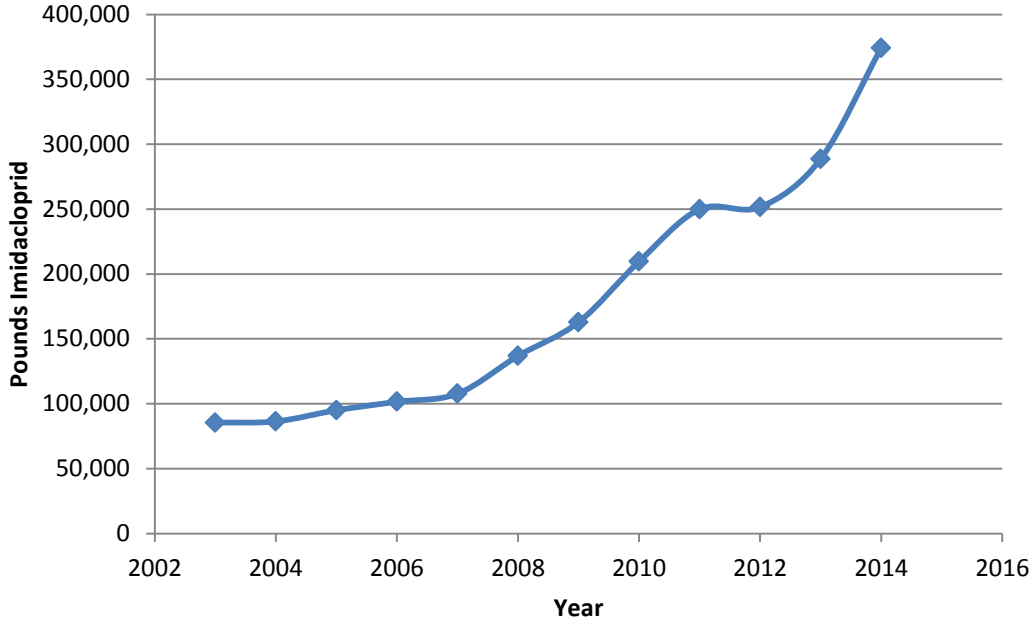


Fig. 3. Imidacloprid pesticide use, California, 2003–2014.

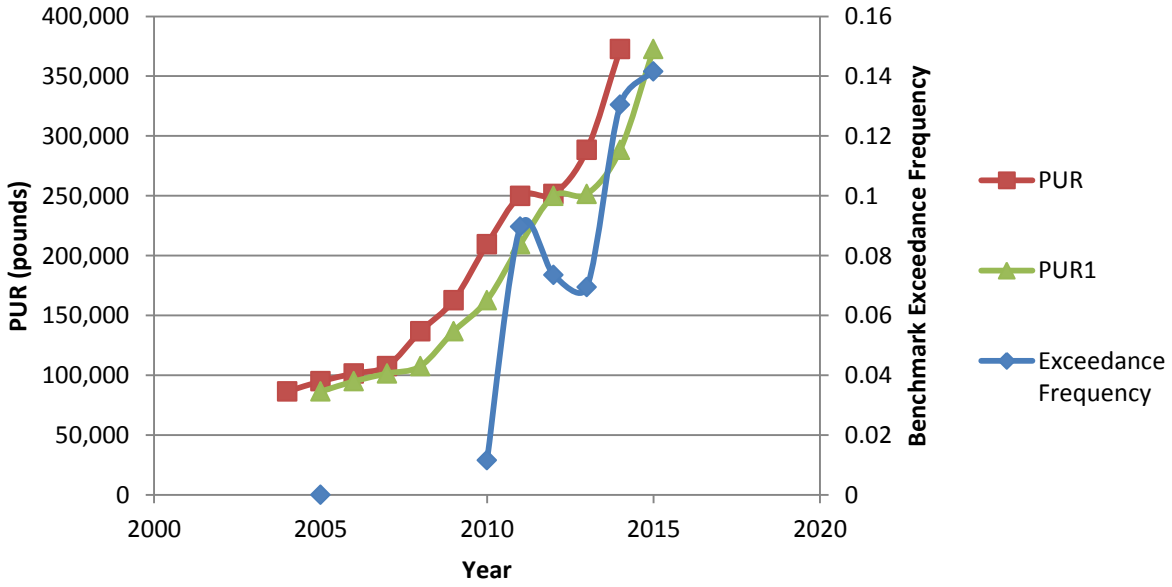


Fig. 4. Imidacloprid pesticide use (PUR) and use one year prior (PUR1) versus chronic aquatic life benchmark exceedance frequency.

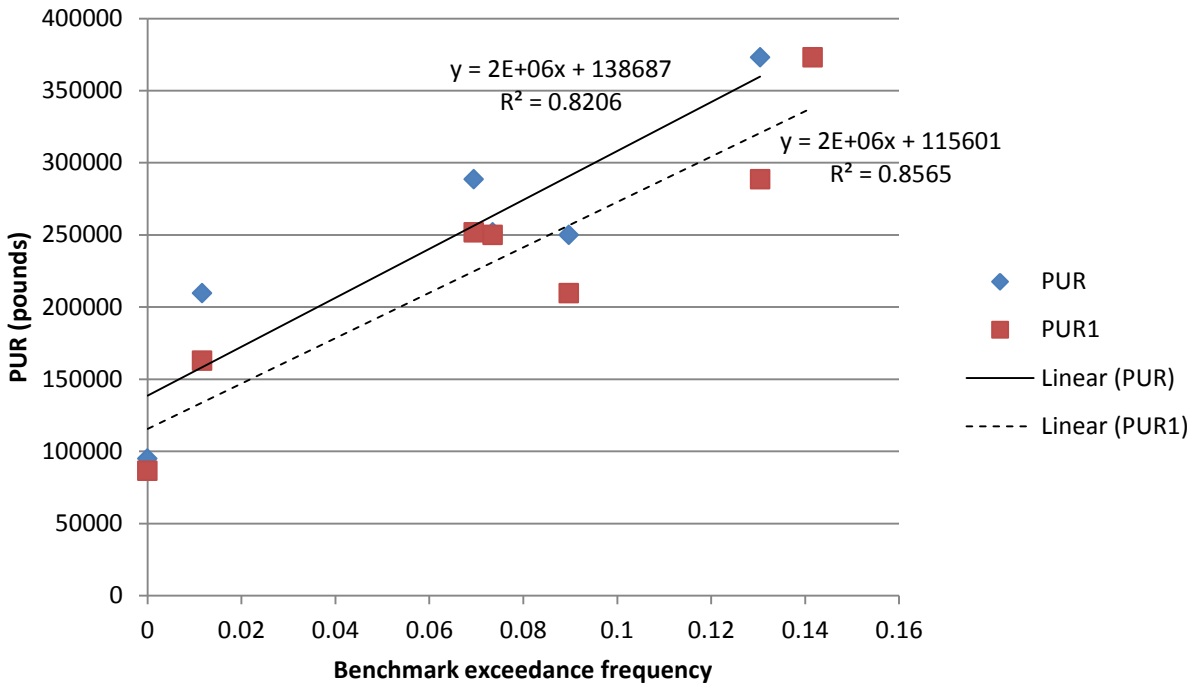


Fig. 5. Linear regressions of pesticide use (PUR) and use one year prior (PUR1) vs benchmark exceedance frequency.

5. Toxicology

5.1 Mode of Action

Imidacloprid acts at the insect nicotinic acetylcholine receptor (nAChR; Liu and Casida, 1993). The insecticide mimics the activity of neurotransmitters by agonistically binding and sending unwarranted neural transmissions. Ultimately, receptors and cells involved in neural transmission become exhausted and fail to function, which results in paralysis (Nishiwaki et al., 2003). Nicotinic receptors with affinity for imidacloprid and other neonicotinoids occur in lower numbers in vertebrates than invertebrates. Thus neonicotinoid toxicity, including imidacloprid, is generally higher in invertebrates than vertebrates (Simon-Delso et al., 2015).

5.2 Aquatic organisms

A large body of published literature exists that addresses the effects of imidacloprid on aquatic macrofauna and other nontarget organisms (Table 3). These studies include lab toxicity tests to stream mesocosm studies to field studies. Fish are less sensitive than invertebrates to the toxic effects of imidacloprid. The LC_{50} values of fish species tested, according to Gibbons et al. (2015), range from 1.2 mg/L for rainbow trout fry to 241 mg/L

for zebrafish. These fish sensitivities are orders of magnitude higher than ambient concentrations detected by CDPR. Thus, it is unlikely that mortality from direct exposure to imidacloprid will affect fish species at current ambient concentrations. Investigating effects to more sensitive invertebrates, Stoughton et al. (2008) conducted a 28-day chronic exposure using the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca.* Growth and survival as measured by the Lowest Observed Effect Concentration (LOEC) were inhibited in *C. tentans* at concentrations >1.14 µg/L. Likewise, *H. azteca* had a 28-d LOEC of 11.46 µg/L. The reported 28-day LC_{50} for *C. tentans* in this same study was 0.91 µg/L. Sanchez-Bayo et al. (2006) reports that ostracods, a class of crustaceans, (48-hour LC_{50}=301–715 µg/L) are orders of magnitude more sensitive to acute imidacloprid exposure than cladocerans, an order of crustaceans (48-hour LC_{50}=65–133 mg/L). Chen et al. (2010) reported a 48-hour LC_{50} of imidacloprid to *Ceriodaphnia dubia* as 2.1 µg/L. The same study found that 19% of the exposed population survived (relative to the control) following chronic exposure at a concentration of 0.3 µg/L. The US EPA chronic invertebrate aquatic life benchmark for imidacloprid is 1.05 µg/L (US EPA, 2015). However, this benchmark was developed in 2008 and there are recent calls for the benchmark value to be lowered drastically in an effort to reflect newer data (Morrissey et al., 2015; Smit et al., 2015). Morrissey et al., (2015) and Smit et al., (2015) agree that the acute threshold should be 0.2 µg/L in order to avoid chronic effects on the most sensitive invertebrate species, but each realizes a different chronic threshold—0.035 µg/L and 0.0083 µg/L, respectively. Nevertheless, concentrations of imidacloprid, especially in agricultural areas of California, are reported in the SURF database (CDPR, 2016) at levels capable of causing short- and long-term impacts on aquatic invertebrate species.

Table 3. Range of LC_{50} values for different taxa

Taxon	96-hr LC_{50} range	Reference
Mammal	131–475 mg/kg	SERA, 2005
Bird	13.9–283 mg/kg	SERA, 2005; Fossen, 2006; Anon 2012
Fish	1.2–241mg/L	SERA, 2005; Cox, 2001
Amphibia	82–366 mg/L	Feng et al., 2004; Nian 2009
Coccinellid	17.25–364 mg/kg	Khani et al., 2012; Youn et al., 2003
Hemiptera	0.3–5,180 mg/kg (residual contact)	Delbeke et al., 1997; Prabhaker et al., 2011
Branchiopoda	.0021–10.4 mg/L	Song et al., 1997; Chen et al., 2010

5.3 Mammals and Birds

Much of the focus in toxicology research has been on invertebrates, especially pollinators (discussed below). Nevertheless, a number of studies have focused on effects to birds and mammals. Imidacloprid can affect birds and mammals directly through toxicity or indirectly through effects to the food chain (Gibbons et al., 2015; Mineau and Palmer, 2013). While imidacloprid is more toxic at lower concentrations to invertebrates than vertebrates, the latter still experiences toxicity from imidacloprid (Gibbons et al., 2015). The 96-hour LC_{50} for different vertebrate taxa varies greatly (Table 2). The LD_{50} for the range of bird species tested spans from 13.9 mg/kg bodyweight for the gray partridge to 283 mg/kg bodyweight for the mallard (Gibbons et al., 2015). While direct exposure is a concern, the indirect effects like growth, development, and reproduction on vertebrate wildlife pose unique challenges as well. One hypothesized indirect effect is the relationship between sensitive invertebrate prey and the vertebrate wildlife that depend on them as a food source. The evidence is not clear as to whether there is a link between pesticide use resulting in decreased invertebrate prey and a decline in vertebrate wildlife populations (Gibbons et al., 2015). Given that indirect effect endpoints like growth and development are difficult to assess, more research is needed to characterize the potential role of imidacloprid to cause sublethal effects.

5.4 Pollinators

Honeybees (*Apis mellifera*) have been widely studied and discussed in recent years since pollinators responsible for a large portion of food crop pollination have seen steady population declines associated with CCD (Pisa et al., 2015). Given the high toxicity of imidacloprid and other neonicotinoids to bees and non-target invertebrates, studies have recently focused on the relationship between neonicotinoid use, CCD, and the health of the global bee population. Mullin (2010) reported an average bee LD_{50} of 280 ng/g bee despite other values ranging from 4 to 104 ng/honeybee (Johnson and Pettis, 2014). Bonmatin et al. (2005) reported that imidacloprid has an acute LD_{50} to bees of 3.7 ng/bee. To put this in perspective, the LD_{50} for DDT is 27,000 ng/bee. Other reported values for the LD_{50} of imidacloprid are higher. Risk assessments focusing on bees reported the LD_{50} to be 490 ng/bee (DEFRA, 2007; 2009). This large discrepancy in reported values may be explained by the differences between oral and contact toxicity, with oral ingestion serving as the more sensitive route of exposure (Pisa et al., 2015).

Sublethal effects of imidacloprid on bees have also been studied. Blanken et al. (2015) studied the relationship between imidacloprid and the parasitic mite *Varroa destructor* with respect to flight capacity of forager bees. Previous studies found that imidacloprid and neonicotinoids could reduce homing of forager bees by altering orientation abilities (Henry et al., 2012). Blanken et al. (2015) found that exposure to *V. destructor* reduced flight distance but the effect increased when bee colonies were exposed to both *V. destructor* and imidacloprid. Despite the increased focus of research efforts on neonicotinoids and

honeybees, as Pisa et al., (2015) point out, "No single cause for high losses has been identified, and high losses are associated with multiple factors including pesticides, habitat loss, pathogens, parasites, and environmental factors."

An extensive risk assessment was released in January 2016 by the US EPA that analyzed the risk imidacloprid poses to bees on different crops (US EPA 2016). This assessment found that imidacloprid sprayed on citrus and cotton posed a risk to bee colony health. A no-observable adverse effect concentration (NOAEC) was set to 25 µg/L for nectar with a lowest-observable adverse effect concentration (LOAEC) at 50 µg/L. Citrus and cotton were identified as risks in the study given the pollen and nectar exposure routes for bees. In these two crops, nectar and pollen may contain imidacloprid above the NOAEC. Other studied crops like corn, which do not contain nectar, are not serious risks to bees for imidacloprid exposure.

6. Summary

Imidacloprid, the predominant neonicotinoid and largest selling insecticide in the world, was initially synthesized in 1985. It is a systemic insecticide applied predominantly in agriculture as a seed treatment to protect against crop damage from biting-sucking pests. Following ingestion, imidacloprid disrupts action potential transmission in the pest by agonistically binding to post-synaptic nAChR receptors. The predominant environmental route for breakdown of imidacloprid is through aqueous photolysis, which has a half-life of <2 hours. The insecticide is highly water soluble (514 mg/L) with a Henry's Law constant of 6.5×10^{-11} atm m^3/mole. Thus, volatilization is not a major dissipation pathway. While not a concern in air, imidacloprid remains a threat to sensitive species in surface water—prompting calls for a reduced chronic aquatic life benchmark. Imidacloprid is on the CDPR Groundwater Protection List, but CDPR studies monitoring for imidacloprid have not detected it in the state.

The science behind the effect of imidacloprid on honey bees and other pollinators, especially with respect to CCD, is still not settled. The recently published US EPA risk assessment on imidacloprid identified cotton and citrus as the only two crops which, when treated with imidacloprid, could introduce bees to toxic concentrations. It is important to note that other stressors like the *V. destructor* mite, habitat loss, and nutrition quality are factors in the reported decline of pollinators nationwide. More research and analysis of existing data is needed in order to decisively identify the relationships between pollinator stressors and CCD.

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