PACIFICORP ENERGY
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July 29, 2008

Jennifer Watts
Water Resources Control Engineer
State Water Resources Control Board
P.O. Box 2000
Sacramento, CA 95812-2000

Subject: Responses to State Water Resources Control Board Letter of June 20, 2008, on Comments on PacifiCorp's 2008 Water Quality Study Plan.

Dear Ms. Watts:

PacifiCorp Energy (PacifiCorp) welcomes the comments the State Water Resources Control Board provided in your letter dated June 20, 2008, regarding PacifiCorp's 2008 Water Quality Study Plan (Study Plan). This Plan is an important component of PacifiCorp's on-going assessment of Reservoir Management Plan (RMP) actions in support of PacifiCorp's application for water quality certification for the Klamath Hydroelectric Project (Project) from the California State Water Quality Control Board (State Water Board) and the Oregon Department of Environmental Quality (ODEQ).

PacifiCorp began implementing the 2008 Study Plan in April 2008 and has begun sharing the data (specifically related to phytoplankton and microcystin toxin) collected that that is available with the Klamath Blue Green Algae Working Group (KBGAWG), which includes State Water Board staff. In addition, PacifiCorp started collecting continuous measurements of temperature, pH, dissolved oxygen, conductivity, and blue green algae below the Iron Gate Powerhouse tailrace in mid-June 2008. PacifiCorp plans on posting this data on our relicensing website (www.pacificorp.com/Article/Article1152.html) on a monthly basis beginning in the summer of 2008. Notification of posting this data will be communicated through the KBGAWG.

PacifiCorp's response to your comments is provided below and is organized so that the comments in your letter are first summarized, and then followed by PacifiCorp's responses. As needed, each section concludes with follow-up questions posed to the State Water Board regarding the comments.

Periphyton Sampling

State Water Board comment

Your June 20 letter states that periphyton are an important indicator of environmental conditions in the Klamath River that provides habitat for the polychaete host for the fish parasite *Ceratomyxa shasta*. Your comments also state that there is a disease node just

below Iron Gate dam which the Federal Energy Regulatory Commission's (FERC) Final Environmental Impact Statement (FEIS) has linked to decreased flushing of periphyton. The State Water Board notes that high abundance of periphyton in a river is associated with the presence of excess nutrients and that the entire length of the Klamath River from the source in Oregon all the way to the Pacific Ocean is 303(d) listed as impaired for nutrients. Your letter also points out that the Water Quality Control Plan for the North Coast Regional Water Quality Control Board addresses the effects of periphyton rather than regulating its presence directly, and that the Hoopa Tribal Water Quality Control Plan specifies a periphyton standard of 150 mg of chlorophyll *a* per square meter of streambed. The State Water Board staff requests that PacifiCorp amend the 2008 Study Plan to include periphyton sampling at multiple sampling sites between Iron Gate dam and the confluence with the Trinity River. The sampling methodology should be done in consultation with the North Coast Regional Water Quality Control Board (NCRWQCB), the lower Klamath tribes, and the State Water Board.

PacifiCorp Response

PacifiCorp understands and appreciates the role of periphyton (herein defined as plant attached to rocks or other submerged surfaces) in the Klamath River. In fact, PacifiCorp studies, in cooperation with the Yurok Tribe and the North Coast Regional Water Quality Control Board, have provided valuable insight into the role of periphyton distribution, species, and seasonal variation. Additional studies included monitoring dissolved oxygen to assess bulk photosynthesis and respiration rates and modeling impacts of benthic algae dynamics on riverine reaches. Other studies have characterized the importance of the weakly buffered status of the Klamath River throughout its length and the role this largely geological phenomenon plays on water quality. This work has been presented in various meetings associated with the FERC relicensing, the ad hoc water quality monitoring group, and at other meetings and venues in the basin. However, periphyton alone does not constitute the benthic flora in the system. While periphyton in the lower river may be the predominate form of benthic flora as perilithic films, in the upper reaches there is considerable diversity including extensive growths of filamentous forms and rooted aquatic vegetation (e.g., *Potemogeton sp.*).

PacifiCorp agrees with the State Water Board that abundant growth is often associated with excess nutrients. The Klamath River is eutrophic, as are some of the tributaries (e.g., Shasta River). The principal driver for the eutrophic conditions of the Klamath River is the large nutrient and organic load entering the river from Upper Klamath Lake and Keno reservoir. PacifiCorp has no control of these large upstream loads of nutrients and organic matter, and any such control will need to occur from implementing total maximum daily loads (TMDLs) that are currently being developed in the Upper Klamath Basin. As the State Water Board staff is aware, the Klamath River TMDLs are being implemented by the Regional Board, in conjunction with the Oregon Department of Environmental Quality (ODEQ), and USEPA Regions 9 and 10. PacifiCorp is hopeful that a comprehensive TMDL for the entire Klamath River system will result in measures to bring about meaningful reductions in nutrient and organic matter from upstream sources

and real improvements in water quality flowing in the Klamath River. The TMDL is a critical process to address the upstream nutrient sources in the Klamath River.

PacifiCorp is concerned that the State Water Board statement regarding the fish disease node just below Iron Gate dam is misleading. Research regarding the prevalence of *C. shasta*, its associated fish mortality, and the distribution of the polychaete host in the Klamath River Basin has been conducted for several years by Oregon State University (OSU) staff and the U.S. Fish and Wildlife Service (USFWS). A fish disease "hot spot" has been identified at the Beaver Creek sampling site which is approximately 30 miles below Iron Gate dam, not immediately below the dam. In fact, recent summaries on OSU's 2007 studies indicate that the areas which experienced the lowest fish disease mortality were the areas above (the Keno Eddy reach) and below (the R-Ranch reach) Iron Gate dam. Furthermore, fish disease mortality was the lowest in the reach just below the Iron Gate dam (R-Ranch) compared with the other sites below the dam The OSU summaries can be found on the USFWS, Arcata office website: www.fws.gov/arcata/

The State Water Board notes that FERC suggested in their FEIS that there may be a link between fish disease and decreased flushing of periphyton. The State Water Board staff is aware that PacifiCorp does not regulate flows from Iron Gate dam that may facilitate "flushing" of periphyton; rather, U.S. Bureau of Reclamation is responsible for the flow regime downstream of the dam (NMFS 2002)[1]. In addition, there does not appear to be a clear relationship between infection rates and elevated flows (e.g., flood event of December 30, 2005).

Follow-Up Questions

PacifiCorp acknowledges the roles of nutrients and periphyton in the Klamath River and has been involved in the Klamath River TMDL process from the beginning. PacifiCorp would like to meet with State Water Board staff to discuss the adequacy of the previous periphyton sampling that has been done in the Klamath River and the role of the Klamath TMDL in controlling nutrients, to formulate a study objective and representative approach to sampling prior to embarking on a sampling program. PacifiCorp would also like to discuss with SWRCB staff how nutrients and periphyton will be dealt with in the water quality certification process, and the implications and relevance of the Hoopa water quality standards.

Increased Monitoring For Microcystis

State Water Board Comment

Your June 20, 2008, letter states that PacifiCorp's 2007 Water Quality Study Plan included monitoring for the cyanobacteria, *Microcystis aeruginosa* (MSAE) from Upper Klamath Lake downstream to River Mile 6 from July through September. Since the Klamath River below Iron Gate dam was posted with recreational contact warning signs in 2007, the State Water Board staff requests that the four locations downstream of

[1] National Marine Fisheries Service. 2002. Biological Opinion—Ongoing Klamath Project Operation.

Walker Bridge that were included in the 2007 Water Quality Study Plan be included in the 2008 Study Plan.

PacifiCorp Response

The 2007 sampling that the State Water Board is referring to was a field study of blue-green algae presence and distribution in the Klamath River system. The purpose of this study was to provide synoptic surveys of blue-green algae presence and distribution in the Klamath River system to provide a clear context for and understanding of their distribution and abundance throughout the system. PacifiCorp did supplemental sampling for MSAE and microcystin in the lower river in the fall of 2007 in response to elevated levels of high cell counts and microcystin concentrations noted in the river by participants in the KBGAWG.

Starting in 2008, the KBGAWG, which includes State Water Board staff, began discussing with participants their 2008 sampling plans. The purpose of these discussions was to inform the group on what type of monitoring was being done, where sample sites were, and the frequency of monitoring to avoid duplicating efforts and identify any possible data gaps.

The Karuk and Yurok tribes are currently sampling the lower river for MSAE to inform the appropriate entities if and when recreational contact warning signs are needed in the lower Klamath River. (Please let us know if you need copies of their sampling plans). PacifiCorp sees no reason to duplicate this effort. PacifiCorp's 2007 basic water quality monitoring stopped at the Klamath River at the I-5 rest area (RM 176). Our 2008 basic water quality monitoring extends approximately 30 miles below the Iron Gate Dam, ending at Walker Bridge (RM 159). PacifiCorp believes the sampling sites identified for the basic water quality monitoring outlined in the 2008 Study Plan are adequate to describe water quality conditions in the Project vicinity and to monitor water quality during the performance of other field studies listed in the plan.

Fish Tissue Sampling for Cyanotoxins

State Water Board Comment

Your June 20, 2008 letter expresses concern that the presence of MSAE in the Klamath River system may lead to the bioaccumulation of cyanotoxin in aquatic organisms with the potential consequences for human health if these aquatic organisms are consumed. The letter cites the fish tissue and mussel sampling that was done under the direction of State Water Board staff in 2007, and the preliminary evaluation of the sampling results that was prepared for the Karuk Tribe (Kann 2008)[2]. The State Water Board staff acknowledges that the Office of Environmental Health Hazard Assessment (OEEHA) is currently reviewing the 2007 laboratory results to determine whether any action to limit the ingestion of fish and mussels is advised. The State Water Board requests that

[2] Kann, J. 2008. Technical Memorandum. Microcystin Accumulation in Klamath River Fish and Freshwater Mussel Tissue: Preliminary 2007 Results. Prepared for the Karuk Tribe of California. April 2008.

PacifiCorp conduct monitoring activities for cyanobacterial toxins in fish and mussels collected in and downstream of Project reservoirs. The State Water Board also recommends that this sampling take place not only during the summer, when bloom conditions are present in the reservoirs, but also year-round to better understand the duration of potential impacts.

PacifiCorp Response

PacifiCorp is also concerned about any potential for any bioaccumulation of cyanotoxin in aquatic organisms with the potential consequences to human health. PacifiCorp did review the technical memorandum prepared for the Karuk Tribe and forwarded our concerns to the State Water Board and OEEHA. In our comment letter, PacifiCorp identified concerns in the analysis that could result in inappropriate or incorrect conclusions regarding the potential for and hazard from microcystin (See attached letter).

Because of these concerns, PacifiCorp has initiated a resident fish tissue sampling program in the Project vicinity (see the sampling and analysis plan in Attachment A). In May 2008, PacifiCorp collected fish (mostly yellow perch and a few crappie) from Iron Gate and Copco reservoirs, and rainbow trout in the stretch of the Klamath River between Iron Gate dam and the Klamathon Bridge (an approximately 4- mile section). This collection in May 2008 occurred before any blue-green algae blooms were present in either the reservoirs or the river. Based on past data collection conducted by both PacifiCorp and the Karuk Tribe on these reservoirs; algae blooms are typically present from mid-July through October. PacifiCorp plans to sample for fish in Copco and Iron Gate reservoirs in July and September to capture bloom conditions and then sample again in November for post bloom conditions. PacifiCorp feels that this sampling frequency will capture the duration of any potential bioaccumulation of cyanotoxins.

Continuous Temperature Monitoring

State Water Board Comment

The State Water Board recommends that the continuous temperature monitoring sites listed in PacifiCorp's 2007 Water Quality Study plan be included in the 2008 Study Plan.

PacifiCorp Response

PacifiCorp regrets the omission of the continuous temperature monitoring in the 2008 Study Plan. The temperature data loggers from 2007 are for the most part still in place (we are still trying to locate two of them), and PacifiCorp plans to continue to monitor the same sites. In addition, as noted above, PacifiCorp is now monitoring water temperature continuously below the Iron Gate powerhouse tailrace.

Effectiveness Testing of Sodium Carbonate Peroxyhydrate (PAK™27)

State Board Comment

Your June 20, 2008, letter states that any study of the use of chemical algaecides should also identify the water quality monitoring needed for an ecological risk assessment to identify any risks to the aquatic community that may result from the use of the chemical.

PacifiCorp Response

Environmental effects of algaecides and associated treatments will be explored by PacifiCorp during 2008. Several inter-related tasks include:

- Review algaecide treatment history and chemistry, wherein the background and impetus for algae treatment in reservoirs, as well as methods of treatment and chemistry of algaecide-algae interaction will be explored.
- Investigate current technologies with respect to (a) chemistry and environmental documentation (e.g. material safety data sheets, which include ecological information), (b) general application technologies and strategies, (c) reservoir applications, and (d) other approaches (alternative or non-algaecide).
- Identify potentially appropriate algaecides for Iron Gate and Copco reservoirs based on findings of previous task. Identify appropriate products that may be used at small, intermediate, and large scales in these reservoirs. Determine permitting requirements of local and state agencies. Estimate treatment levels and costs for the reservoirs.
- Complete small scale bench tests on algaecides such as PAK-27. Test efficacy on small containers of reservoir water on impacts to cyanobacteria, and byproducts of treated algal populations.

Application of algaecides to reservoirs or Klamath River reaches is not considered in this initial phase of algaecide studies.

Pilot Testing of Solar-Powered Circulators in Copco Reservoir

State Water Board Comment

Your June 20, 2008 letter states that it is difficult to address the adequacy of PacifiCorp's plan to study the use of solar-powered circulators in Copco reservoir due to the lack of detail provided in the 2008 Study Plan. The State Water Board would like information on the lateral and vertical extent over which the solar-powered circulators affect water quality. The State Water Board recommends multiple measurements of water quality parameters that extend out away from the circulators to delineate circulator effectiveness in improving water quality.

PacifiCorp Response

PacifiCorp initiated pilot-scale testing of solar-powered circulators in Copco reservoir to gain better reliability and effectiveness information for this technology. This testing will assess operational consistency and reliability in field conditions, and assess water quality improvement in the "treated" area relative to other "untreated" areas, particularly in

controlling blooms of blue-green algae such as MSAE. PacifiCorp consulted with Solar Bee, the manufacturer of the solar-powered units, and 12 solar-powered circulators were placed in Copco Reservoir in April 2008, per the manufacturer's recommendation to meet the study objectives. The intent of this study is not to measure individual unit performance but rather to assess the use of solar-power circulator technology to control blue-green algae blooms. To meet these study objectives, PacifiCorp, in consultation with Joe Eilers of Solar Bee established five sampling points positioned both in and out of "treatment zone". At these sampling locations, PacifiCorp is taking vertical profiles at 1-meter intervals for temperature, pH, DO, and conductivity. In addition, PacifiCorp is taking surface and depth-integrated samples for phytoplankton and microcystin analysis. This sampling is done on a bi-weekly basis. PacifiCorp believes that this sampling addresses the State Water Boards concerns regarding the lateral and vertical extent of the solar-powered circulators effectiveness in improving water quality conditions.

PacifiCorp welcomes the opportunity to meet with the State Water Board staff to discuss these comments or any concerns that you may have regarding the 2008 Study Plan activities. PacifiCorp provided technical memorandums (dated July 1 and July 14, 2008) to the KBGAWG that provide the most recent available data on MSAE and microcystin. PacifiCorp has also placed this information on the Klamath relicensing website for water quality data and documents (www.pacificorp.com/Article/Article82802.html). PacifiCorp plans on releasing these memos regarding MSAE and microcystin in a similar fashion on a recurring basis when new data is available.

If you have any questions, please contact Linda Prendergast at (503) 813-6625.

Sincerely,

Cory E. Scott

Cory Scott
Klamath Licensing Manager

Attachment A: CH2MHill Technical Memorandum, July 18, 2008: Sampling and Analysis Plan for Investigation of Microcystins in Tissues of Resident Fish in the Vicinity of the Klamath Hydroelectric Project

Attachment B: PacifiCorp Letter of May 13, 2008, to OEHHA: Information Related to the Occurrence of Microcystin in the Tissues of Klamath River Biota

Attachment A

Sampling and Analysis Plan for Investigation of Microcystins in Tissues of Resident Fish in the Vicinity of the Klamath Hydroelectric Project

PREPARED FOR: John Sample (PacifiCorp)

PREPARED BY: Ken Carlson/PDX
Tim Hamaker/RDD
Dennis Shelton/CVO

DATE: July 18, 2008

Introduction

This sampling and analysis plan (SAP) addresses the investigation of blue-green algae (Cyanobacteria) toxins, known as microcystins, in tissues of resident fish in the vicinity of the Klamath Hydroelectric Project (Project) facilities in northern California. The objective of this investigation is to gather information on the possible presence of microcystin in resident fish residing in or near Project reservoirs in California, and evaluate the potential human health risks from consumption of these fish. This SAP describes the specific task elements and activities, field and laboratory methods, and data assessment and reporting procedures to be followed for this investigation.

The basic approach of the investigation is to analyze for microcystin compounds in fillet tissues from edible-sized resident fish from Copco and Iron Gate reservoirs and the Klamath River upstream and downstream of these reservoirs. Copco and Iron Gate reservoirs are a focus of this investigation because of the recent occurrences of summertime blooms of the blue-green algae (BGA) species *Microcystis aeruginosa* (MSAE), which is capable of producing the toxin microcystin. The analytical results from this investigation will be used to estimate potential human exposure from consumption of microcystin compounds that may be contained in edible tissues of commonly-caught resident sport fish from these reservoirs and the Klamath River upstream and downstream.

Field Sampling Plan

Sampling Area

The field sampling effort will focus on collecting representative numbers of fish from the following four reservoir and river segments in the Project vicinity:

- Iron Gate reservoir (located on the Klamath River from about River Mile [RM] 190.5 to RM 196.3)

- Copco reservoir (located between RM 198.6 to RM 204)
- Klamath River downstream of the Iron Gate dam to the I-5 freeway crossing (RM 176.7 to RM 190.5)
- Klamath River upstream of the Copco reservoir to the Stateline (RM 204 to RM 209)

Target Species and Life-Stages

Iron Gate and Copco Reservoirs

Yellow perch (*Perca flavescens*) and crappie (*Promoxis* sp.) will be targeted in the reservoirs to represent resident sport fish in the reservoirs that are typically captured and consumed. Adult yellow perch larger than 6 inches (15 cm) total length (TL) and crappie larger than 6 inches (15 cm) TL will be targeted in the two reservoirs. Smaller fish sizes or alternate species may also be kept based on field decisions, particularly if only insufficient numbers of samples of the target species can be obtained.

Klamath River

Rainbow trout (or steelhead) (*Oncorhynchus mykiss*) will be targeted in the river segments to represent resident sport fish in the river that are typically captured and consumed. Adult rainbow trout greater than approximately 10 inches (25 cm) TL will be targeted for collection in the mainstem Klamath River in the segment downstream of Iron Gate dam and the segment upstream of Copco reservoir. In consultation with the California Department of Fish and Game (CDFG)[3] and under a Scientific Collectors Permit, specimens collected will be from either wild trout (identified by determining that the adipose fin is present) or trout of hatchery origin (adipose fin is clipped). Smaller fish sizes or alternate species may also be kept, based on field decisions, particularly if only insufficient numbers of samples of the target species can be obtained.

Sampling Schedule

Sample collection during 2008 will occur during the summertime period of BGA algal blooms in the reservoirs. Sample collection also will occur before and after the summertime period of BGA algal blooms in the reservoirs to provide information on microcystin presence in resident fish tissues before and after the period of blooms. The following tentative sampling schedule will be implemented:

- Spring. Sampling before the BGA bloom period (week of May 26, 2008)[4]
- Summer 1. First sampling during algal bloom period (week of July 14, 2008)
- Summer 2. Second sampling during algal bloom period (week during mid-August to mid-September, 2008)
- Fall. Sampling after the BGA bloom period (week during October to mid-November, 2008)

[3] Pers. Comm. between Tim Hamaker (CH2M HILL) and Larry Hanson/Jim Whelan (CDFG).
[4] As of the date of this SAP, the Spring sampling has been completed.

Fish Sample Numbers

At least three and up to 10 rainbow trout (or steelhead) specimens will be collected from each of the river segments when practicable. Trout, regardless of sex or age, will be collected for tissue sampling. As indicated above, either wild trout (adipose fin is present) or trout of hatchery origin (adipose fin is clipped) will be obtained for tissue samples.

At least 10 and up to 20 adult yellow perch will be targeted for collection from each reservoir as practicable. Similarly, at least 3 to as many as 10 adult crappies will be targeted for collection from the reservoirs. Adults of these species, regardless of sex or age, will be collected for tissue sampling.

Fish Collection Methods

The most practical and effective method of capturing the fish targeted for this program will be by hook and line sampling ("angling"). The May 2008 sampling has been conducted and demonstrated this as an efficient method for obtaining the targeted species and life-stages. PacifiCorp and/or CH2M HILL boats and personnel will be used for fish collections. Fishing guides and boats also may be utilized if needed to assist in the fish collection efforts.

Fish Tissue Sample Preparation and Handling

Methods for tissue sample preparation, preservation, and handling will generally follow those outlined in the USFWS National Wild Fish Health Survey Manual (FWS 2004), and also as directed Dr. Gregory Boyer (State University of New York-SUNY Great Lakes Science Consortium).

Immediately following field collection, each fish specimen will be placed into a clean zip-lock bag. The bag will be labeled using a permanent marking pen with a unique identification number and immediately placed on wet ice in an insulated cooler. At the end of each day's sampling activity, individual fish specimens will be photographed[5], weighed to the nearest gram, and a total length will be obtained and recorded. Each fish specimen will then be examined and noted for any abnormal external conditions (e.g., lesions, parasites).

Each fish specimen will be dissected to obtain a skinless fillet. From a skinless fillet, a sub-sample of approximately 2-10 grams will be obtained and placed into a new pre-labeled 50-ml Blue Max® polyethylene sampling bottle. For quality assurance purposes, a duplicate sample will be obtained from a skinless fillet from the opposite side of every twentieth fish specimen processed. The sample label on each bottle will identify a unique sample number assigned to the fish, provide the time and date of capture, provide the species common name, and the collector's initials.

Each completed sample bottle will be placed on dry ice and frozen for preservation. All tissue samples for analytical determination of microcystin concentrations will be flash frozen on dry ice in the field and held in a freezer until shipped to the analytical lab. During shipment to the analytical laboratory, the samples will be contained in an

[5] Only selected and representative fish will be photographed.

insulated cooler containing dry ice to insure all tissue samples remain frozen during shipment.

Laboratory Analyses

Analytical determination of tissue concentrations of microcystin compounds in fish tissue samples will be directed by Dr. Gregory Boyer, State University of New York College of Environmental Science and Forestry, Syracuse New York. Frozen samples will be shipped under Chain-of-Custody procedures using overnight courier service to the SUNY laboratory in Syracuse, New York. On receipt at the SUNY laboratory samples will be held in an ultra-cold freezer until preparation for analysis of microcystins is initiated.

Method for Determination of Tissue Concentrations of Microcystin

To prepare the samples for analysis, the SUNY laboratory lyophilizes the frozen samples to dryness and homogenizes the dried samples using a mortar and pestle. Approximately 50 mg (0.05 g dry weight) of the homogenized tissue sample will be mixed with 1 ml of water and 4 µg of internal standard (7cys-S-propyl microcystin LR), and allowed to stand at 4°C for 60 minutes.

After incubation, 5 ml of 50 percent methanol, acidified with 1 percent glacial acetic acid, will be added and the samples sonicated on ice for 1 minute at 21 W. The samples will be evaporated, reconstituted in 100 percent methanol and lipids removed using a Bligh-Dyer extraction. After clarification by centrifugation, the supernatants will be taken to dryness and reconstituted in 50 percent acidified methanol. Samples then will be sealed in autosampler vials and stored frozen until further analysis.

Following tissue sample preparation, the concentrations of microcystin compounds will be quantified by high performance liquid chromatography with mass spectral detection (LCMS). This LCMS assay measures the molecular weight of microcystin congeners within the tissues using a ZQ4000 single quad instrument and a 0.02 percent trifluoroacetic acid (TFA) acetonitrile gradient. The instrument will be standardized using microcystin-RR, -LR, -tLR and -LF congeners. The analysis will focus on unbound (largely free toxins within the tissue) concentrations of microcystin compounds.

The analysis will determine the “free” fractions of microcystin congeners that are not bound to proteins. The mechanism of toxic action by microcystins involves covalent binding to proteins. Once bound, this fraction is no longer accessible or “bioavailable” for toxicity (Ibelings and Chorus 2007). Also, of the various congeners, the vast majority of reported research on microcystin toxicity is focused on the –LR congener, and the –LR congener is generally regarded as the most toxic congener (Funari and Testai 2008).

This LCMS assay will obtain spectra with a specific mass-to-charge ratio (m/z) between m/z 800 and 1200 atomic mass units (amu), and ions of interest corresponding to known microcystin congeners will be extracted out of the total ion current. Microcystins will be identified on the basis of their ultraviolet (UV) signatures, liquid chromatography retention times relative to microcystin standards, and comparison of their molecular weights against a database of approximately 70 known microcystin congeners.

Results will be reported on a weight basis in units of μg/g dry weight of tissue. The instrument detection limit is approximately 1 ng microcystin-LR on column. Method detection limits will be determined from the recovery of the internal standard and are expected to be generally less than <0.15 μg/g dry weight of tissue.

Data Reporting

The laboratory results of microcystins tissue analyses will be summarized and a report of the procedures, analytical results and summary interpretation of these results will be prepared and submitted to PacifiCorp. In addition, a technical memorandum will be prepared and submitted to PacifiCorp that describes the field sampling efforts, including photographs, field notes, and a summarization of the fish specimen lengths, weights, and condition. The estimated schedule for final completion of these reports is February 2009.

Human Health Risk Evaluation

The overall objective of the risk assessment will be to estimate the magnitude and probability of potential harm to public health from consumption of fish that have accumulated microcystins from the samples as described above. The risk evaluation will be comprised of the following components:

- Human Exposure Assessment. This component will describe the methodology used to assess potential human exposures, and evaluates the magnitude, frequency, and duration of these exposures.
- Toxicity Assessment for Human Health. This component will summarize the literature-based toxicological data and studies of microcystins and the relationship between the magnitude of exposure and the potential occurrence of adverse health effects.
- Human Health Risk Characterization. This component will integrate information from the exposure and toxicity assessments to characterize the risks to human health posed by potential exposure to microcystins via fish consumption.
- Uncertainties and Assumptions Associated with the Risk Assessment. This component will discuss the uncertainties and assumptions associated with the human health risk assessment.

Tolerable Daily Intake (TDI) values for microcystins in food items have been recommended by Ibelings and Chorus (2007). These include a Lifetime TDI, a Seasonal TDI, and an Acute TDI. The Lifetime TDI is the intake that can be tolerated from daily ingestion on an ongoing, perennial basis over a full lifetime. The Seasonal TDI reflects what is tolerable over a single season, and the Acute TDI reflects what is tolerable from a single exposure. As a practical matter, it is unrealistic to assume lifetime daily exposure from fish consumption for a toxin that is produced by the cyanobacteria *Microcystis* that blooms only seasonally during the year.

A reliable and accurate estimation of such seasonally-variable exposure should be based on the trend of tissue concentrations over time (e.g., seasonally), so that exposure levels are appropriately pro-rated on an annual basis. For example, there is no evidence to indicate that yellow perch from Copco and Iron Gate reservoirs are consumed on a perennial, daily basis throughout the year over a lifetime. Rather, the Seasonal TDI – defined as intake that can be tolerated from daily ingestion over several weeks during the cyanobacterial season – is the more realistic and appropriate TDI value to use.

The four fish sampling events described in this sampling plan (i.e., before, during, and after the summertime algal blooms) are intentionally scheduled to provide data that will realistically characterize the potential seasonal exposure to microcystins. This sampling scheme and exposure estimation approach will allow for a more accurate evaluation of the potential for risk posed by fish consumption.

The procedures used for the human health risk assessment will be consistent with standard procedures described in state and federal guidance documents (for example, Cal-EPA 1996, EPA 1989). The results of the risk assessment will be presented in a clear and consistent fashion in the risk assessment report, allowing for effective communication of the results. The report will clearly consider the nature and weight of evidence supporting the risk estimates, as well as the magnitude of uncertainty surrounding such estimates. The estimated schedule for final completion of this report is February 2009.

References

Cal-EPA. 1996. Supplemental Guidance for Human Health Multimedia Risk Assessments of Hazardous Waste Sites and Permitted Facilities. California Environmental Protection Agency, Department of Toxic Substances Control.

EPA. 1989. Risk Assessment Guidance for Superfund (RAGS), Volume I: Human Health Evaluation Manual. Interim Final. U.S. Environmental Protection Agency. March 1989.

Funari, E. and E. Testai. 2008. Human Health Risk Assessment Related to Cyanotoxins Exposure. Critical Reviews in Toxicology, 38:97-125.

Ibelings, B.W., and I. Chorus. 2007. Accumulation of Cyanobacterial Toxins in Freshwater “Seafood’’ and its Consequences for Public Health: A Review. Environ. Pollut. 150: 177-192.

Attachment B

PACIFICORP ENERGY
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825 NE Multnomah, Suite 1500
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May 13, 2008

George Alexeeff, Deputy Director
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
1001 I Street
P.O. Box 2815
Sacramento, CA 95812-2815

Subject Information Related to the Occurrence of Microcystin in the Tissues of Klamath River Biota

Dear Mr. Alexeeff:

PacifiCorp Energy operates the Klamath Hydroelectric Project (Project) on the Klamath River in California and Oregon. In the California portion of the Project area, Project facilities include Iron Gate reservoir (located between about River Mile [RM] 190 and 196.8) and Copco reservoir (located between about RM 198.6 and 203.2). In the last few years, blooms of the blue-green algae *Microcystis aeruginosa* (MSAE) have occurred during the summer in Iron Gate and Copco reservoirs and other areas of the watershed. MSAE can produce microcystin – a peptide substance that in large quantities can have adverse health effects on animals including humans. As a result of the occurrence of these recent MSAE blooms, PacifiCorp and other entities have monitored MSAE and microcystin levels in the reservoirs and elsewhere in the Klamath River. This information has been used to facilitate decisions regarding California's guidance for voluntary posting of health advisories in recreational waters related to blue-green algae (SWRCB 2007)[6].

Recently, the Karuk Tribe issued a technical memorandum by Kann (2008)[7] that describes the results of sampling in 2007 for the presence of microcystin in the tissues of 42 resident yellow perch (*Perca flavescens*) from Iron Gate and Copco reservoirs, six fall Chinook salmon (*Oncorhynchus tshawytscha*) yearlings collected from the Iron Gate Hatchery, and 20 samples of freshwater mussel (*Gonidea angulata*) collected from the Klamath River at six locations between Orleans (around RM 58) and I-5 (around RM 176). A copy of the memorandum by Kann (2008) in included in Attachment A to this letter. PacifiCorp recommends that the Office of Environmental Health Hazard Assessment (OEHHA) review this memorandum, along with other information in this letter, to evaluate whether a Fish Consumption Advisory with Safe Eating Guidelines may be appropriate for Iron Gate and Copco reservoirs and the Klamath River.

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[6] SWRCB. 2007. Cyanobacteria in California Recreational Water Bodies: Voluntary Guidance about Harmful Algal Blooms, Their Monitoring, and Public Notification. (Document by the Blue-green Algae Work Group of SWRCB and OEHHA).

[7] Kann, J. 2008. Technical Memorandum. Microcystin Accumulation in Klamath River Fish and Freshwater Mussel Tissue: Preliminary 2007 Results. Prepared for the Karuk Tribe of California. April 2008.

Regarding the Kann (2008) memorandum, PacifiCorp offers several comments described below that OEHHA should consider when reviewing the results and recommendations presented in the memorandum.

Comments on the Kann (2008) Memorandum's Discussion of Potential Toxicity

The Kann (2008) memorandum provides analytical results based on concentrations of eight separate congeners of microcystin detected in liver and fillet tissue samples as analyzed by the California Department of Fish and Game (CDFG) Water Pollution Control Laboratory (Rancho Cordova, CA). The eight analyzed congeners included MCYST-LA, -LF, -LR, -LW, -RR, -YR, and the demethylated forms of –LR and –RR (denoted as –LR-DM and –RR-DM). Of the eight congeners, the vast majority of reported research on microcystin toxicity is focused on the –LR congener, and the –LR congener is generally regarded as the most toxic congener (Funari and Testai 2008)[8].

Kann (2008) acknowledges that microcystin congener toxicity is variable, but assumes that the toxicity of the -LR-DM and –LA congeners are equal to that for the -LR congener. This is a questionable assumption, as there are no reliable studies or data supporting the assumption that the toxicities of the -LR-DM and –LA congeners are equal to that for the -LR congener. The stated basis by Kann (2008) for assuming equal toxicity are citations of two studies that apparently reported similarity of intraperitoneal LD50 doses in mice for these congeners (Höger 2003[9], Sivonen and Jones 1999[10]). However, extrapolating potency ratios via the intraperitoneal route to the ingestion route has no basis, since it ignores potential differences in uptake (hence toxicity) following ingestion exposure. EPA (2006)[11] warns about such differences in uptake in their *Toxicological Reviews of Cyanobacterial Toxins: Microcystins LR, RR, YR and LA*, which states:

> "Wolf and Frank (2002)[12] proposed toxicity equivalency factors (TEFs) for the four major microcystin congeners based on LD50 values obtained after i.p. administration. The proposed TEFs, using MCLR as the index compound (TEF=1.0) were 1.0 for MCLA and MCYR and 0.1 for MCRR. The application of TEFs based on i.p. LD50 values to assessment of risk from oral or dermal exposure is questionable given that differences in liphophilicity and polarity of

[8] Funari, E. and E. Testai. 2008. Human Health Risk Assessment Related to Cyanotoxins Exposure. Critical Reviews in Toxicology, 38:97-125.
[9] Höger S.J. 2003. Problems during drinking water treatment of cyanobacterial loaded surface waters: consequences for human health. Doctoral Thesis Constance: Universität Konstanz. (Cited in Kann 2008).
[10] Sivonen K, G. Jones. 1999 Cyanobacterial Toxins. In Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management, eds. Chorus, I, and Bartram, J, London: E & FN Spon. 41–111. (Cited in Kann 2008).
[11] U.S. Environmental Protection Agency (EPA). 2006. Toxicological Reviews of Cyanobacterial Toxins: Microcystins LR, RR, YR and LA. National Center for Environmental Assessment, Office of Research and Development, Cincinnati, OH. External Draft Report 45268 NCEA-C-1765. November 2006.
[12] Wolf, H.-U. and C. Frank. 2002. Toxicity assessment of cyanobacterial toxin mixtures. Environ. Toxicol. 17(4): 395-399. (Cited in EPA 2006).

> the congeners may lead to variable absorption by non-injection routes of exposure."

Supporting this, Ibelings and Chorus (2007)[13] state that:

> ".. minor structural changes, characteristic of the different MCYST congeners, may have major effects on uptake, organ distribution and excretion of these toxins. Possibly the affinity of the organic ion transporters which are responsible for the transport of MCYST across cell membranes differs for respective microcystins."

Given these limitations, tolerable limits for microcystins are best only applied to the –LR congener since this is where toxicological data exist. Of the data reported in Table 1 of Kann (2008), the –LR congener was not detected in any of the fish samples, and only detected in four of the 20 mussel samples.

Comments on the Kann (2008) Memorandum's Analytical Procedures

The Kann (2008) memorandum indicates that all tissue samples were extracted and analyzed using liquid chromatographic mass spectrometric (LC/MS) methodology. The Kann (2008) memorandum does not specify whether the extraction method used by the CDFG laboratory to retrieve microcystins from the biota tissues reported by Kann (2008) included only the "free" fraction of microcystin that is bioavailable, or also included the protein-bound fraction that is not bioavailable. The mechanism of toxic action by microcystins involves covalent binding to proteins. Once bound, this fraction is no longer accessible for toxicity. Ibelings and Chorus (2007) state:

> "Is covalently bound MCYST in fish muscle tissue still (equally) toxic to humans that eat the fish? Presumably not. Microcystin that is covalently bound to protein phosphatases in ingested food will not be readily bioavailable, nor is it likely that our digestive tract would release it, so that the bound microcystin fraction in food would not be toxic to the consumer (Humpage and Lawton, pers. commun.), and the fraction of free microcystin that is extractable with the typically applied methanolic procedures would indeed be the relevant fraction to quantify in food."

There is no mention in Kann (2008) of the extraction method used. However, in response to an email from Linda Prendergast (PacifiCorp) inquiring about the method, Jacob Kann stated that the "…CDFG Lab uses sonification with a methanol extraction…" and further stated that the "…methanolic extraction does not extract covalently bound MCYST in tissue". As such, if the microcystin tissue concentrations reported include only the free fraction, then these concentrations are appropriate for use in estimating toxicity exposure and risk. Nonetheless, whether the microcystin tissue concentrations reported include only the free fraction should be verified with the CDFG laboratory.

[13] Ibelings, B.W., and I. Chorus. 2007. Accumulation of cyanobacterial toxins in freshwater "seafood'' and its consequences for public health: A review. Environ. Pollut. 150: 177-192. (Cited in Kann 2008).

Another question related to CDFG analytical methods concerns the meaning of a footnote in the analytical data sheets in Appendix I of Kann (2008), which states "dimethyl analogue quantified based on parent compound". This suggests that no calibration standard was used for the dimethyl analogue, but rather the calibration for the -LR congener was used. If this is the case, it needs to be further demonstrated that this extrapolation approach results in reliable concentration estimates for the –LR-DM congener. This is an important issue since the –LR-DM congener was the overwhelmingly dominant congener reported by Kann (2008), particularly for the yellow perch samples.

Comments on the Kann (2008) Memorandum's Analysis With Respect to Potential Health Risk

The Kann (2008) memorandum concludes that:

> "Evaluation of bioaccumulation in yellow perch fillets and freshwater mussels with respect to public health guidelines indicates that all TDI guideline levels as defined by Ibelings and Chorus (2007) were exceeded to varying degrees in tested Klamath River organisms, including several observations of values exceeding Acute TDI thresholds. Current public health advisories for toxic cyanobacteria in the Klamath River system are primarily for recreational contact and do not warn against ingestion of fish or freshwater mussels. In light of these bioaccumulation data, public health advisories should include warnings for ingestion of fish and freshwater mussels."

PacifiCorp is committed to assisting as appropriate in informing users of the Project reservoirs about consumption advisories and safe eating guidelines that may be determined necessary by OEHHA. However, it is important for OEHHA to carefully consider whether the Kann (2008) memorandum's analysis and conclusions accurately state the true human health risk associated with ingestion of fish and mussel from the Klamath River system.

First, there are some important uncertainties associated with the Acute Tolerable Intake (TI) values that Kann (2008) used for comparison against the tissue data. The Acute TI represents the intake that can be tolerated from a single exposure event (e.g., ingestion of catch during a week-end fishing trip). For example, Kann (2008) used the Acute TI recommended by Ibelings and Chorus (2007) based on a single LD50 study by Fawell et al. (1999)[14] on mice exposed to microcystin using intraperitoneal injection. As previously mentioned, making inferences about the ingestion route from intraperitoneal injection is inappropriate. Even Ibelings and Chorus (2007) acknowledge that the oral LD50 is 100-fold higher (less toxic) than that for intraperitoneal injection; i.e., Ibelings and Chorus

[14] Fawell, J.K., Mitchell, R.E., Everett, D.J., and Hill, R.E. 1999. The toxicity of cyanobacterial toxins in the mouse: I. Microcystin–LR. Hum. Exp. Toxicol. 18:162–67. (Cited in Ibelings and Chorus 2007, and Funari and Testai 2008).

(2007) state that "the acute LD50 for oral exposure in fact is 100 times higher than that for i.p. exposure."

In a review of human health risks related to cyanotoxins, Funari and Testai (2008) also state the following:

> "The MC-LR acute toxicity after intraperitoneal (ip) administration to mice results in a LD50 = 50 μg/kg bw; when given by the oral route, MC-LR is less toxic (LD50 = 5000 μg/kg bw and even higher in the rat) (Fawell et al., 1994, 1999a)."

These results indicate that the Acute TI assumed by Kann (2008) based on intraperitoneal injection likely substantially overstates acute risks from single event exposure by ingestion.

Second, it is unrealistic for Kann (2008) to compare the tissue microcystin concentrations reported by the CDFG lab against the Lifetime Tolerable Daily Intake (TDI) values recommended by Ibelings and Chorus (2007). Kann (2008) reports that the tissue microcystin concentrations reported by the CDFG lab "often exceeded the Lifetime TDI values". The Lifetime TDI is the intake that can be tolerated from daily ingestion on an ongoing, perennial basis. However, as a practical matter, it is unrealistic to assume lifetime daily exposure for a toxin that is produced by the cyanobacteria MSAE, which blooms only seasonally during the year (if at all). A reliable and accurate estimation of such seasonally-variable exposure should be based on the trend of tissue concentrations throughout the year (e.g., monthly or seasonally), so that exposure levels are appropriately pro-rated on an annual basis.

Also, it is likely unrealistic to assume lifetime daily consumption of 100 grams per day for these particular species from the Klamath River as assumed by Kann (2008) for both a 10 kg child and a 75 kg adult. There is no evidence to indicate that yellow perch or mussels from the Klamath River are consumed on a perennial, daily basis throughout the year. The Seasonal TDI – defined as intake that can be tolerated from daily ingestion over several weeks during the cyanobacterial season – is in concept the more appropriate TDI value to use. However, while a fish consumption rate of 100 grams per day assumed in the Seasonal TDI rate used by Kann (2008) may be appropriate for adults, it likely substantially overstates daily consumption for a child. For example, the longer-term ingestion rate for a 1-yr old tribal child, as reported by Columbia River Inter-Tribal Fish Commission (CRITFC) was 19.6 grams per day[15].

Another issue not addressed by the Kann (2008) memorandum is what proportion of the total fish/shellfish consumed is actually comprised of invertebrates such as mussels. The TDI values used for evaluation of mussel concentrations should be pro-rated downward

[15] Columbia River Inter-Tribal Fish Commission (CRITFC). 1994. A Fish Consumption Survey of the Umatilla, Nez Perce, Yakama, and Warm Springs Tribes of the Columbia River Basin, Technical Report 94-3. Columbia River Inter-Tribal Fish Commission, Portland, Oregon.

to account for the fraction that mussels comprise of the total consumption rate of 100 grams per day.

Lastly, as previously described in this letter, the vast majority of reported research on microcystin toxicity indicates that the –LR congener is the most toxic form, yet Kann (2008) assumes that the toxicity of the -LR-DM and –LA congeners are equal to that for the -LR congener. There are no reliable studies or data supporting the assumption that the toxicities of the -LR-DM and –LA congeners are equal to that for the -LR congener. The data reported in Kann (2008) shows that the –LR congener was not detected in any of the fish samples, and only detected in four of the 20 mussel samples. As such, the TDI thresholds are best applied to the –LR congener since this is where toxicological data exist, and if so applied leads to a conclusion different than provided in the Kann (2008) memorandum.

Analysis of Microcystin in Salmon and Steelhead Fish Tissues in the Klamath River

The Kann (2008) memorandum describes the results of sampling in 2007 for the presence of microcystin in the tissues of six fall Chinook salmon yearlings collected from the Iron Gate Hatchery. Microcystin was detected in the "liver composite" sample for the –LA congener, but was non-detect for all other congeners. Microcystin was non-detect for all congeners in the other yearling samples (i.e., "stomach composite", "fillet composite"). These results, combined with the fact that salmon yearlings are not consumed by humans, indicate no risk to human health from ingestion.

Two other sources of information are available on testing for microcystin in the tissues of adult Chinook salmon and steelhead (*Oncorhynchus mykiss*) in the Klamath River. The first source includes tissue samples collected from Chinook salmon and steelhead in the river by the Yurok Tribe in September and October 2005 (Fetcho 2007)[16]. The Chinook salmon samples included three liver tissue samples and two filet samples from the Klamath River below Weitchpec (about RM 42.5), and two liver tissue samples and two filet samples from the Iron Gate Hatchery. All Chinook salmon tissue samples were non-detect for microcystin. The steelhead samples included two liver tissue samples and two filet samples from the Klamath River at Weitchpec (about RM 43.5). The steelhead filet samples were non-detect for microcystin. Small amounts of microcystin were reported for the two steelhead liver samples (i.e., "trace" and 0.54 ng/g, respectively). The results indicate no risk to human health from ingestion of these fish.

The second source includes tissue samples for microcystin analysis collected by PacifiCorp from Chinook salmon and steelhead in October 2007. The samples included liver and muscle tissues from four Chinook salmon and two steelhead specimens collected from the Klamath River near Klamath Glen (about RM 5.7), one steelhead specimen from the Klamath River near Somes Bar (about RM 65), and one Chinook salmon specimen from the Klamath River near Seiad Valley (about RM 129). The

[16] Fetcho, K. 2006. Klamath River Blue-Green Algae Bloom Report. Water Year 2005. Prepared for the Yurok Tribe Environmental Program. January 2006.

samples also included liver and muscle tissues from six Chinook salmon and five steelhead specimens collected from the Iron Gate Hatchery.

The PacifiCorp samples are being analyzed by the laboratory of Dr. Greg Boyer at the State University of New York (SUNY) in Syracuse. The SUNY laboratory employs a state-of-the-art method for analysis of microcystins in tissues. Samples are lyophilized and sonicated, and lipids removed using a Bligh-Dyer extraction. Microcystins are determined by LC/MS on the basis of their UV signatures, high performance liquid chromatography (HPLC) retention times relative to microcystin standards, and comparison of their molecular weight against a database of approximately 70 known microcystin congeners. Method detection limits are determined from the recovery of the internal standard and are generally less than <0.15 µg/g dry weight of tissue.

The SUNY laboratory recently reported to PacifiCorp that all samples were non-detect for un-bound or "free" microcystin (see Attachment B). These results indicate that these fish pose no risk to human health from ingestion. Results from the laboratory of bound (non-toxic) microcystin in the samples are still pending. PacifiCorp plans to issue a report on these samples following completion of the laboratory analysis, and will make this report available promptly to OEHHA.

Please contact me by phone at (503) 813-6011 or by e-mail at cory.scott@pacificorp.com if you have any questions or need additional information. PacifiCorp appreciates OEHHA's interest in this matter, and we look forward to coordinating with OEHHA on appropriate responses and actions as may be needed.

Sincerely,

/s/ Mark Sturtevant for Randy Landolt

Randy Landolt
Managing Director

Attachments

Cc: Terry Barber, Siskiyou County
Gail Louis, EPA
Catherine Kuhlman, NCRWQCB
Matt St. John, NCRWQCB