PacifiCorp's 2008 Sampling and Analysis Plan for Investigation of Microcystins in Tissues of Resident Fish in the Vicinity of the Klamath Hydroelectric Project

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)[1] 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)[2]
- 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)

[1] Pers. Comm. between Tim Hamaker (CH2M HILL) and Larry Hanson/Jim Whelan (CDFG).

[2] 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[3], 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 insulated cooler containing dry ice to insure all tissue samples remain frozen during shipment.

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

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.