Occurrence of Microcystin in Tissues of Chinook Salmon and Steelhead in the Klamath River in 2007

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Table of Contents

Executive Summary **1**
Introduction **3**
Methods **5**
Field Procedures 5
Locations Sampled 5
Specimen Capture and Processing 5
Laboratory Procedures 8
Analysis of Microcystin in Fish Tissues 8
Histopathological Examination of Fish Livers 9
Results **12**
Specimens Obtained 12
Specimens from the Lower Klamath River Sampling Area 12
Specimens from the Middle Klamath River Sampling Areas 13
Specimens from the Iron Gate Hatchery 14
Analysis of Microcystin in Fish Tissues 15
Histopathological Examination of Fish Livers 17
Discussion **20**
Potential Effects on Salmon and Steelhead in the Klamath River 20
Analysis with Respect to Public Health Guideline Values 22
References **25**

List of Figures and Tables

Figure 1. Map of Klamath River showing locations that demark areas from which fish were collected during October 2007. 7
Table 1. Histologic lesion types and characteristics. 10
Table 2. Summary of fish caught and processed in the lower Klamath River (near Klamath Glen) on October 10, 2007. 12
Table 3. Summary of fish caught and processed as samples on the middle Klamath River-Lower (upstream of Somes Bar) on October 16, 2007. 13
Table 4. Summary of fish caught and processed as samples on the middle Klamath River-Lower (near Seiad Valley and the Scott River) on October 26, 2007 13
Table 5. Summary of fish obtained and processed at the Iron Gate Hatchery on October 31, 2007. 14
Table 6. Microcystin laboratory analysis results for fish tissue samples obtained on October 10, 2007 from the lower Klamath River (near Klamath Glen). 15
Table 7. Microcystin laboratory analysis results for fish tissue samples obtained on October 16 and 26, 2007 from the middle Klamath River sampling areas. 16
Table 8. Microcystin laboratory analysis results for fish tissue samples obtained on October 31, 2007 from Iron Gate Hatchery. 16
Table 9. Number of Chinook salmon and steelhead specimens with observed liver lesions, October 2007. 18

Figure 2. Microcystin data obtained in water samples at various river sites in the Klamath River below Iron Gate dam, June through October 2007 (Source: various data sources as listed in Appendix F). 22
Table 10. Guideline values for freshwater fish consumption derived from Ibelings and Chorus (2007). 23

List of Appendices

Appendix A: Photographs of Specimens from the Lower Klamath River Sampling Area

Appendix B: Photographs of Specimens from the Middle Klamath River Sampling Areas

Appendix C: Photographs of Specimens from the Iron Gate Hatchery

Appendix D: SUNY-CESF Laboratory Report on Analysis of Microcystin in Fish Tissues

Appendix E: Report on Histopathological Examination of Fish Livers

Appendix F: Microcystin Data Obtained in Water Samples below Iron Gate Dam, June through October 2007

Executive Summary

This report provides the results of PacifiCorp's October 2007 sampling for the presence of microcystin in the tissues of salmon and steelhead specimens taken from the Klamath River downstream of Iron Gate dam in California. This report includes descriptions of the collection of the specimen samples in October 2007 by CH2M HILL, and describes the results of fish tissue laboratory analysis of microcystin on the samples conducted by the State University of New York (SUNY) College of Environmental Science and Forestry (CESF) Laboratory in Syracuse, NY under the direction of Dr. Greg Boyer. This report also describes the results of histological examination of liver tissues (on the salmon and steelhead samples) conducted by Dr. Swee Teh of the University of California at Davis (UC-Davis).

Field sampling collected a total of eleven (11) adult Chinook salmon and eight (8) adult steelhead from the Klamath River during their fall migration period. Four Chinook salmon and two steelhead were obtained from angling in the lower Klamath River below the Trinity River from about River Mile (RM) 6 to RM 36. One steelhead and one Chinook salmon were obtained from angling in the middle Klamath River from about RM 75 to RM 143. Six Chinook salmon and five steelhead were obtained from collection at the Iron Gate Hatchery (near RM 189).

The SUNY-CESF Laboratory determined that un-bound or "free" microcystin was not detected in any of the muscle or liver samples at the specified Method Detection Limit (MDL). The MDL varied with sample type and recovery from 0.09 to 0.24 µg/g on a dry weight[1] (dw) basis, with an average MDL of 0.13 µg/g dw. Correcting for sample moisture content (75 percent), the equivalent MDL on a wet weight[2] (ww) basis varied by sample from 0.02 to 0.06 µg/g ww, with an average MDL of 0.03 µg/g ww[3]. The non-detection of free microcystin in the samples for the specimens in this study is likely explained by three main factors: (1) low microcystin levels in the river; (2) period of potential exposure to microcystins was short; and (3) minimal or no food ingestion by the migrating Chinook salmon and steelhead adults.

Histological examination of liver tissues determined that lesions were present in the liver tissues from both species. The presence of liver lesions indicates that the specimens likely were exposed to contaminants, toxicants, or other stress-inducing factors that can affect migrating and spawning salmon and steelhead, such as inanition, disease, or prolonged over-exertion. The absence of detectable levels of free microcystin in the samples suggests that microcystin likely was not the determinative factor affecting the livers of these Chinook salmon and steelhead specimens. A range of liver histopathological effects in fish have been described in the research literature as a result of exposure to a variety of inorganic and

[1] Dry weight is the weight of microcystin found in subsequent analysis divided by weight of the dried tissue which once contained it.

[2] Wet weight is the weight of microcystin found in analysis divided by weight of the tissue before water is removed by drying.

[3] To convert dry-weight concentrations to wet-weight concentrations, the dry-weight concentration is multiplied by a factor of 1 minus the percentage of moisture content expressed as a decimal.

organic contaminants. Various liver histopathological effects are also described in the research literature for migrating and spawning salmon and steelhead due to severe glycogen depletion and progressive loss of liver function during spawning migrations.

Although free microcystin was not detected in filet (muscle) samples at the specified MDL, the sample MDLs are above the guideline value proposed by Ibelings and Chorus (2007) for what they considered the Tolerable Daily Intake (TDI) of microcystin over a lifetime ("Lifetime TDI"). Therefore, if microcystin is present at levels below the MDL but above this guidance value, the filet (muscle) samples could pose a potential for exposures exceeding this guideline if daily consumption occurred over a lifetime. However, daily consumption exposure to microcystin throughout each year over a lifetime (as the Lifetime TDI assumes) is not a probable scenario since microcystin occurs seasonally during the cyanobacteria "bloom" season, and Chinook salmon and steelhead adults are only present seasonally in the river for capture by potential consumers.

The filet (muscle) sample MDLs are all less than the Seasonal TDI guidance value for an adult, indicating that daily consumption over several weeks poses no unacceptable health risk to an adult. MDLs for 14 of the 17 filet (muscle) samples are less than the Seasonal TDI guidance value for a child, leaving three samples with MDLs that are above the Seasonal TDI guidance value for a child. This suggests some potential for exposures exceeding this guideline for a 15 kg child if daily consumption occurred over several weeks and if microcystin is present at levels below the MDL, but above the guidance value.

The filet (muscle) sample MDLs are all substantially less than (i.e., below) the Acute TDI guidance value for an adult or child, indicating that single-event, single-meal consumption poses no unacceptable health risk.

Introduction

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. MSAE has the capability to produce microcystin – a peptide substance that in sufficient quantity 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 voluntary guidance for posting health advisories in recreational waters related to blue-green algae (SWRCB 2007).

In recent years, sampling has also been conducted by PacifiCorp and others related to the occurrence of microcystin in the tissues of Klamath River biota (Fetcho 2006, Kann 2008, PacifiCorp 2008a, PacifiCorp 2008b). Related to anadromous salmonids, Fetcho (2006) collected liver and muscle tissue samples from five Chinook salmon and two steelhead specimens taken from the Klamath River at or near Weitchpec (near RM 43) and from Iron Gate Hatchery (at RM 189) during September and October 2005. All Chinook salmon tissue samples (liver and muscle) collected by Fetcho (2006) did not contain detectable levels of microcystin. The two steelhead muscle samples collected by Fetcho (2006) also did not contain detectable levels of microcystin. The two steelhead liver samples (obtained from the river at Weitchpec) did contain detectable levels of microcystin of 0.17 and 0.54 µg/g, respectively (based on a method detection limit of 0.15 µg/g[4]).

In October 2007, PacifiCorp collected samples for microcystin analysis of tissues from Chinook salmon and steelhead in the Klamath River. The samples included liver and muscle tissues from specimens collected from the Klamath River near Klamath Glen (about RM 5.7), near Somes Bar (about RM 65), near Seiad Valley (about RM 129), and from the Iron Gate Hatchery. Following receipt of the laboratory results of the October 2007 fish tissue sample analyses, PacifiCorp reported the results to the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA), Siskiyou County Department of Health, and the North Coast Regional Water Quality Control Board (PacifiCorp 2008a, PacifiCorp 2008b).

This report provides detailed discussions of the methods and results of PacifiCorp's October 2007 sampling of salmon and steelhead tissues for the presence of microcystin. This report includes descriptions of the approach and methods of the October 2007 field collection of the salmon and steelhead samples, and presents the results of fish tissue laboratory analysis

[4] Although not discussed by Fetcho (2006), it is assumed that this reported analytical detection limit of 0.15 µg/g is on a wet weight basis, whereby the weight of microcystin found in the analysis is divided by weight of the tissue, including the fraction of weight made up of the tissue's original water content.

of microcystin on the samples conducted by the State University of New York (SUNY) College of Environmental Science and Forestry (CESF) Laboratory in Syracuse, NY under the direction of Dr. Greg Boyer. This report also presents and assesses the results of histological examination of liver tissues (on the salmon and steelhead samples) conducted by Dr. Swee Teh of the University of California at Davis (UC-Davis).

Methods

Field Procedures

Field methods used and results of sampling activities during October 2007 were directed at obtaining tissue samples from adult Chinook salmon and adult steelhead migrating back into the Klamath River during their fall migration period. An attempt was made to collect fish along the entire length of the migration corridor; i.e., from the mouth of the Klamath River to the terminus at Iron Gate dam. This field collection obtained a total of eleven (11) adult Chinook salmon and eight (8) adult steelhead as described below.

Locations Sampled

Field sampling effort was applied in four areas of the Klamath River downstream of Iron Gate dam (Figure 1). The four areas included:

1. the lower Klamath River below the Trinity River from approximately Klamath Glen to near the confluence of Metah Creek (about RM 6 to RM 36)
2. the middle Klamath River above the Trinity River from approximately to Stuart's Bar to Ti Bar (RM 74.7 to RM 81.5)
3. the middle Klamath River from about Seiad Valley to near the confluence of the Scott River (RM 130 to RM 143)
4. fish in the Klamath River below Iron Gate dam (RM 189) were obtained at the Iron Gate Hatchery in cooperation with hatchery staff.

Specimen Capture and Processing

Adult Chinook salmon and steelhead specimens were obtained from Klamath River sites by angling from a boat. Adult Chinook salmon and steelhead specimens were obtained from Iron Gate Hatchery staff immediately following capture and processing of the fish in the hatchery's spawning building.

The processing of samples from each fish was conducted immediately following capture to minimize elapsed time between death and sample acquisition. Three types of tissue samples were obtained from each specimen for analyses of microcystin by the SUNY-CESF laboratory: (1) muscle tissue; (2) skin tissue; and (3) liver tissue. In addition, liver tissue slices were also obtained for histological analyses by Dr. Swee Teh of UC-Davis.

The following is a description of the steps followed to process each specimen fish for both Chinook salmon and steelhead:

1. Upon capture and identification, specimens were sacrificed and processing begun immediately;

2. Specimens were externally examined. A photograph of the whole fish was taken, and total length and weight was recorded;
3. For histopathological samples, the liver was dissected and four representative tissue slices were obtained, placing each slice into individual sample jars containing 10 percent neutral buffered formalin;
4. Histopathological samples were placed in bottles labeled with unique sample identification numbers, sample matrix type, time and date, and sampler's initials;
5. For microcystin laboratory samples, the residual liver tissue was placed into a zip-lock plastic bag containing a label affixed on the bag with a unique sample identification number, sample matrix type, time and date, and sampler's initials. These samples were then placed on dry ice;
6. For microcystin laboratory samples, a small wedge (of at least 10 grams) of muscle tissue was excised from the upper body musculature (above the lateral line immediately posterior to gill operculum) from left side of the body;
7. For microcystin laboratory samples, skin from muscle tissue was removed;
8. Muscle and skin samples were each placed into separate zip lock plastic bags containing labels affixed on the bags with unique sample identification numbers, sample matrix type, time and date, and sampler's initials. These samples were then placed on dry ice t.

This process was repeated for each fish taken and processed at each sampling location.

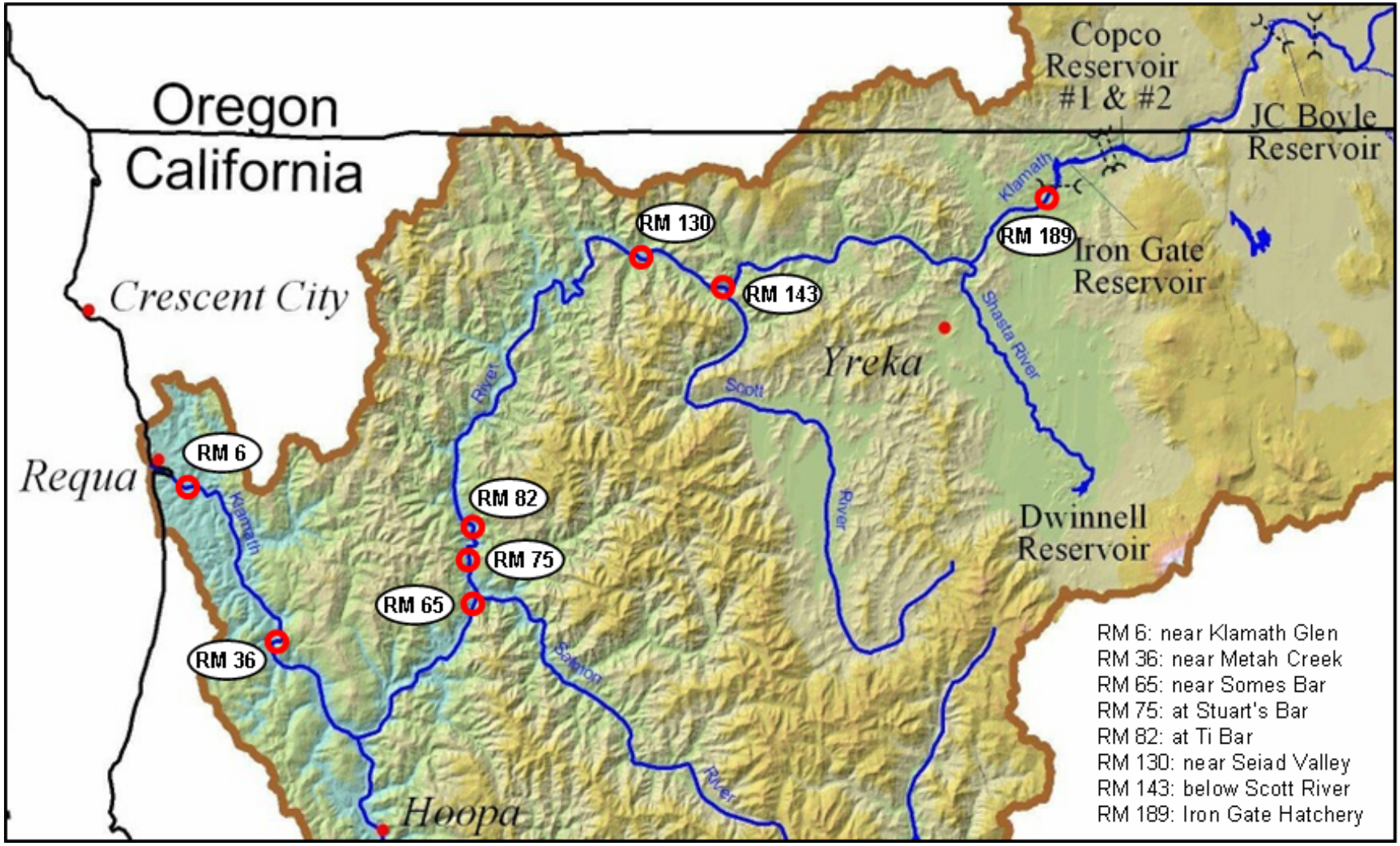

Figure 1. Map of Klamath River showing locations that demark areas from which fish were collected during October 2007.

Laboratory Procedures

Analysis of Microcystin in Fish Tissues

The analysis of microcystin in Chinook salmon and steelhead tissue samples was conducted by the SUNY-CESF Laboratory in Syracuse, NY under the direction of Dr. Greg Boyer. Frozen samples were shipped under Chain-of-Custody procedures using overnight courier service to the SUNY-CESF Laboratory in Syracuse. Upon receipt at the laboratory, samples were held in an ultra-cold freezer until analysis.

As described below, the basic analytical approach to analyzing microcystins in the samples involved liquid chromatography-mass spectrometry (LC-MS)[5] for detection of ultraviolet (UV) signatures, high performance liquid chromatography (HPLC)[6] retention times relative to microcystin standards, and comparison of their molecular weight against a database of known microcystin congeners.

Sample Preparation

To prepare the samples for analysis, the frozen samples were lyophilized (i.e., freeze-dried) to dryness at SUNY-CESF and the lyophilizate was vortexed (i.e., mixed by whirlpool effect) to ensure uniformity. A 100 mg (0.1 g dry weight) subsample was mixed with 1 ml of water containing 4 µg of the internal standard 7cys-S-propyl-microcystin-LR (per the methodology of Smith and Boyer 2008). Five ml of 50 percent aqueous methanol was added and the samples were sonicated (21 watts power) on ice for 1 minute. Following sonication, the samples were allowed to stand for 30 min at -20°C, centrifuged to settle debris, and the clarified supernatant decanted into a clean glass tube. The solvent was removed in vacuo and the dry material reconstituted in 1 ml of 80 percent aqueous methanol. The sample was again allowed to stand for 30 minutes at -20°C, clarified by centrifugation, and the supernatant transferred to an autosampler vial, which was sealed and stored at -20°C for subsequent analysis.

Analysis of Total Free Microcystins

Following tissue sample preparation, the concentrations of microcystin compounds were quantified by high performance liquid chromatography with mass spectral detection (LCMS). This LCMS assay measures the molecular weight of microcystin congeners within

[5] Liquid chromatography-mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS has very high sensitivity and specificity for the specific detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture). Mass spectrometry (MS) is an analytical technique for the determination of the elemental composition of a sample or molecule. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios. MS instruments consist of three modules: an ion source, which can convert gas phase sample molecules into ions (or, in the case of electrospray ionization, move ions that exist in solution into the gas phase); a mass analyzer, which sorts the ions by their masses by applying electromagnetic fields; and a detector, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present.

[6] High-performance liquid chromatography (HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

the tissues using a ZQ4000 single quad instrument and a 0.02 percent trifluoroacetic acid (TFA) acetonitrile gradient. The instrument was standardized using microcystin-RR, -LR, -tLR and -LF congeners.

The analysis determined 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).

This LCMS assay obtained 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 were extracted out of the total ion current. Microcystins were 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 were reported on a weight basis in units of µg/g dry weight of tissue. The Instrument Detection Limit (IDL)[7] is approximately 1 ng microcystin-LR on column[8] in the full scan mode and 0.01 ng on column in the SIM mode. The Method Detection Limit (MDL)[9] relative to the LCMS spectra scan was determined for each sample from the recoveries of the internal standard (7cys-S-propyl microcystin LR) in full scan mode, and are generally less than <0.15 µg/g dry weight of tissue.

Histopathological Examination of Fish Livers

Histological examination of liver tissues on the Chinook salmon and steelhead samples was conducted by Dr. Swee Teh of UC-Davis.

Sample Preparation

Upon receipt at UC-Davis, liver slices from individual fish were assigned a random alpha-numeric identification code (e.g., 07ST9-1-07ST9-19). To prepare the samples for analysis, liver slices were routinely paraffin processed and paraffin blocks sectioned at 3-5 microns. Sections were mounted on glass slides and stained with hematoxylin and eosin.

Histopathological Examination

Following tissue sample preparation, the stained tissue sections were screened under a microscope for lesions (i.e., any abnormal tissue growth or damage) and subjected to detailed, semi-quantitative histopathologic analysis. The type and condition of observed lesions were categorized based on criteria listed in Table 1. Liver lesion severity scoring was based on a scale of 0 = not present, 1 = mild, 2 = moderate, and 3 = severe.

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[7] Instrument Detection Limit (IDL) is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise by a particular instrument. The IDL is similar to the "critical level" and "criterion of detection" as defined in the literature. (Standard Methods, 18th edition).

[8] On-column detection occurs when analytes are detected on the analytical column (LCMS Ace C18) over which the injected sample flows.

[9] Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, and is determined from analysis of a sample in a given matrix containing the analyte. MDLs are statistically determined values that define how easily measurements of a substance by a specific analytical protocol can be distinguished from measurements of a blank (background noise).

Table 1. Histologic lesion types and characteristics.

Type	Definition and Characteristics
1. GD = Glycogen depletion	Glycogen depletion occurs when the glycogen stores in the muscles and liver are depleted, and the blood glucose level begins to fall, usually following physical exhaustion. Characterized by decreased size of hepatocytes, loss of the 'lacy', irregular, and poorly demarcated cytoplasmic vacuolation typical of glycogen, and increased cytoplasmic basophilia (i.e., blue coloration).
2. CI= Cytoplasmic inclusions	Cytoplasmic inclusions are circumscribed masses of abnormal foreign (e.g., viruses) or extracellular proteins within the cytoplasm or nucleus of a cell. Characterized by accumulation of foreign and eosin-staining materials within the cytoplasm of liver cells.
3. LIP = Lipid or fatty vacuole	Large lipid or fatty vacuoles can accumulate in liver cells via the abnormal retention of lipids within a cell. It reflects an impairment of the normal processes of synthesis and elimination of triglyceride fat that can occur from several stressors, including malnutrition and toxins. Characterized by lipid droplets appearing as clear, round, well-demarcated, cytoplasmic vacuoles.
4. FPCVL = Focal or multifocal parenchymal, perivascular, and/or pericholangial lymphocytes	This category of lesions includes inflammations in response to infection or toxin exposure. Characterized by focal to multifocal aggregates of lymphocytes (small white blood cells), occasionally mixed with other inflammatory cells, that infiltrates the connective tissue around bile ducts, blood vessels, or other liver tissue.
5. MA = Macrophage aggregates	Macrophage aggregates are structures sometimes present in the liver of fishes which store and detoxify cellular wastes and externally-derived substances. Changes in MA density, size and pigment content can be indicators of contaminant exposure. However, MA number and structure can also be affected by other factors, including general stress or nutritional status of fish. Characterized by lesion of the liver tissue that are pigmented yellow-brown to green-brown, and often mixed with lymphocytes.
6. SCN = Single cell necrosis	Cellular necrosis can be induced by a number of external sources, including injury, infection, cancer, infarction, poisons, and inflammation. Necrosis typically begins with cell swelling, disruption of the plasma membrane and organelle membranes, leading to organelle breakdown and cell lysis. Characterized by lesion of liver cells having eosin-staining (i.e., pink coloration) cytoplasm with condensation or fragmentation of the nucleus.
7. SC = Sinusoidal congestion or hemorrhagic lesions	Sinusoidal dilatation and congestion in the liver is often the result of blood vein outflow impairment. Venous outflow impairment can be a sign of severe stress or exposure to contaminants. Characterized by dilation of sinusoidal spaces due to vascular hemorrhage.
8. AMY= Amyloidosis	Amyloidosis occurs when amyloid proteins are abnormally deposited in the liver. Amyloid proteins are a particular aggregated insoluble protein form. The cause of amyloidosis is not well understood but appears associated with chronic infection and inflammatory states. Characterized by vascular disease lesion with deposition of amyloid-like proteins in the spaces between sinusoidal lining cells and hepatocytes.
9. PAR = Helminthes and myxosporean parasites.	Helminthes and myxosporean parasites are internal parasites associated with fish disease. Helminths are worm-like organisms that live and feed off living hosts. Myxosporea are a class of microscopic parasites, with a complex life cycle that comprises vegetative forms in fish and another host, generally an aquatic invertebrate.

Table 1. Histologic lesion types and characteristics.

Type	Definition and Characteristics
10. FCA = Foci of cellular alteration	Foci of cellular alteration represent the earliest stage in the progression of a cancerous liver tumor. Cells in the foci are composed of hepatocytes which are variable in size. Classes of FCA include amphophilic basophilic, clear cell, eosinophilic, vacuolated or mixed. Because of the importance of foci in the progression of fish liver cancer, these lesions are enumerated rather than scored by severity.

Results

Specimens Obtained

A total of 19 adult salmonids, consisting of eleven (11) Chinook salmon and eight (8) steelhead were obtained from the Klamath River in October 2007. Multiple specimens of both species were obtained from the lowermost sampling area in the Klamath River (near Klamath Glen) and from Iron Gate Hatchery. Only one adult hatchery steelhead and one adult Chinook salmon were obtained from the middle sampling areas of the Klamath River during this sampling effort. Less-effective angling in the middle areas likely was due to rainfall and resulting high and turbid water conditions.

Specimens from the Lower Klamath River Sampling Area

Four Chinook salmon and two steelhead were obtained during angling on October 10, 2007 in the lower Klamath River below the Trinity River from approximately Klamath Glen to near the confluence of Metah Creek (about RM 6 to RM 36). Table 2 summarizes the fish catch of October 10, 2007. Photographs of the fish are found in Appendix A.

All four Chinook salmon and two steelhead appeared to be normal in appearance and healthy. No physical abnormalities or signs of disease or parasites were observed. Both steelhead were hatchery marked (adipose fin-clipped). It was noticeable that the second fish caught (i.e., LKR-SH-A-2) appeared to have both ovaries and testes. Neither of these reproductive organs was gravid at the time of capture.

Table 2. Summary of fish caught and processed in the lower Klamath River (near Klamath Glen) on October 10, 2007.

Species[a]	Fish I.D.	Sex	Length (in.)	Weight (lbs.,oz.)	Photo Nos.	Remarks
CS	LKR-CS-A-1	Male	27.0"	8 lbs. 9 oz.	1	bright fish
CS	LKR-CS-A-2	Female	25.5"	7 lbs. 5 oz.	2	bright fish
SH	LKR-SH-A-1	Female	23.5"	4 lbs. 3 oz.	5	hatchery mark
CS	LKR-CS-A-3	Female	26.0"	7 lbs. 0 oz.	3	bright fish
SH	LKR-SH-A-2	Female/ male	26.5"	6 lbs. 10 oz.	6	hatchery mark
CS	LKR-CS-A-4	Male	25.0"	7 lbs. 0 oz.	4	bright fish
Totals	CS	SH				
	4	2				

[a] CS = Chinook Salmon; SH = Steelhead

Specimens from the Middle Klamath River Sampling Areas

One adult hatchery steelhead was obtained during angling on October 16, 2007 in the middle Klamath River above the Trinity River from approximately to Stuart's Bar to Ti Bar (RM 74.7 to RM 81.5). Table 3 summarizes the fish catch of October 16, 2007. Photographs of the fish are found in Appendix B. No Chinook salmon caught were caught or seen on this day.

The captured steelhead was an adult male caught near Kissing Rock upstream of Sandy Bar (Figure 1) and was hatchery marked (i.e., adipose fin-clipped). There were no signs of external or internal parasites, disease, or abnormal appearances this fish. Several additional wild "half-pounder" steelhead were caught, landed and released during the day. No other fish were caught or observed.

One adult female Chinook salmon was obtained during angling on October 26, 2007 in the middle Klamath River from about Seiad Valley to near the confluence of the Scott River (RM 130 to RM 143). Table 4 summarizes the fish catch of October 26, 2007. Photographs of the fish are found in Appendix B.

This fish had previously spawned and was in very poor condition. This fish was alive but approaching death as a post spawner. There were numerous lamprey scars on both sides of the body and many of these scars were infected with fungus. Internally the organs seemed to be normal with the exception of empty ovaries.

Table 3. Summary of fish caught and processed as samples on the middle Klamath River-Lower (upstream of Somes Bar) on October 16, 2007.

Species[a]	Fish I.D.	Sex	Length (in.)	Weight (lbs.,oz.)	Photo Nos.	Remarks
SH	SB-SH-A-1	Male	25.0"	6 lbs. 5 oz.	7	hatchery mark
Totals	CS	SH				
	0	1				

[a] CS = Chinook Salmon; SH = Steelhead

Table 4. Summary of fish caught and processed as samples on the middle Klamath River-Lower (near Seiad Valley and the Scott River) on October 26, 2007

Species[a]	Fish I.D.	Sex	Length (in.)	Weight (lbs.,oz.)	Photo Nos.	Remarks
CS	SV-CS-A-1	Female	26.0"	6 lbs. 1 oz.	8	Post spawner; lamprey scars
Totals	CS	SH				
	1	0				

[a] CS = Chinook Salmon; SH = Steelhead

Specimens from the Iron Gate Hatchery

Six Chinook salmon and five steelhead were obtained on October 31, 2007 from the Iron Gate Hatchery. Table 5 summarizes the fish obtained from the hatchery of October 31, 2007. Photographs of the fish are found in Appendix C.

The six Chinook salmon consisted of three males and three females. All showed signs of external lamprey scars and some skin fungus, but otherwise appeared normal for spawning fish. Internally, these six Chinook salmon showed no obvious signs of disease or parasites.

The five adult steelhead consisted of two males and three females. Four of the five steelhead were relatively bright and appeared normal for spawning fish. One of the steelhead (i.e., IGH-SH-A-5) appeared to be much darker (exterior pigment coloration) (see Photo 17, Appendix C) and its liver had the appearance of blood (hemorrhagic) on the surface of the liver tissue.

Table 5. Summary of fish obtained and processed at the Iron Gate Hatchery on October 31, 2007.

Species[a]	Fish I.D.	Sex	Length (in.)	Weight (lbs.,oz.)	Photo Nos.	Remarks
SH	IGH-SH-A-1	Female	12.0"	0 lbs. 13 oz.	13	small adult
SH	IGH-SH-A-2	Female	21.0"	3 lbs. 8 oz.	14	full sized adult
SH	IGH-SH-A-3	Male	13.0"	0 lbs. 15 oz.	15	small adult
CS	IGH-CS-A-1	Female	25.0"	6 lbs. 0 oz.	9	roe removed in hatchery
CS	IGH-CS-A-2	Female	30.0"	10 lbs. 8 oz.	10	full of roe
CS	IGH-CS-A-3	Male	29.0"	10 lbs. 2 oz.	11	lamprey ulcers
CS	IGH-CS-A-4	Female	26.0"	7 lbs. 9 oz.	12	lamprey ulcers
SH	IGH-SH-A-4	Male	12.0"	1 lb. 0 oz.	16	small adult
SH	IGH-SH-A-5	Female	16.0"	1 lb. 15 oz.	17	liver appears hemorrhagic
CS	IGH-CS-A-5	Male	29.0"	10 lbs. 3 oz.	No photo	good shape
CS	IGH-CS-A-6	Male	29.5"	9 lbs. 5 oz.	No photo	good shape
Totals	CS	SH				
	6	5				

[a] CS = Chinook Salmon; SH = Steelhead

Analysis of Microcystin in Fish Tissues

The SUNY-CESF Laboratory determined that un-bound or "free" microcystin was not detected in any of the muscle or liver samples at the specified Method Detection Limit (MDL). Tables 6, 7, and 8 summarize the analytical results for all muscle and liver samples obtained from Chinook salmon and steelhead specimens in the lower Klamath River area, the middle Klamath River areas, and Iron Gate Hatchery, respectively. The SUNY-CESF Laboratory report is contained in Appendix D.

The SUNY-CESF Laboratory was not able to analyze the samples of skin due to matrix effects produced by analytical interference from other non-target substances. The presence of non-target compounds can be a common matrix interference (Nicholson and Burch 2001). However, the SUNY-CESF Laboratory indicated that the skin would be expected to contain even less microcystin relative to the liver or muscle tissues (which were all non-detect).

The MDL varied with sample type and recovery from 0.09 to 0.24 µg/g on a dry weight[10] (dw) basis, with an average MDL of 0.13 µg/g dw. Correcting for sample moisture content (assumed at 75 percent per Clark and Maret [1998]), the equivalent MDL on a wet weight[11] (ww) basis varied by sample from 0.02 to 0.06 µg/g ww, with an average MDL of 0.03 µg/g ww[12].

Table 6. Microcystin laboratory analysis results for fish tissue samples obtained on October 10, 2007 from the lower Klamath River (near Klamath Glen).

Species[a]	Fish I.D.	Sample Type	Free Microcystin Levels	MDL (µg/g dry wt)
Chinook	LKR-CS-A-1	Muscle	Below detection	< 0.13
		Liver	Below detection	< 0.09
Chinook	LKR-CS-A-2	Muscle	Below detection	< 0.13
		Liver	Below detection	< 0.11
Chinook	LKR-CS-A-3	Muscle	Below detection	< 0.24
		Liver	Below detection	< 0.10
Chinook	LKR-CS-A-4	Muscle	Below detection	< 0.15
		Liver	Below detection	< 0.13
Steelhead	LKR-SH-A-1	Muscle	Below detection	< 0.18
		Liver	Below detection	< 0.11
Steelhead	LKR-SH-A-2	Muscle	Below detection	< 0.24
		Liver	Below detection	< 0.11

[10] Dry weight is the weight of microcystin found in subsequent analysis divided by weight of the dried tissue which once contained it.

[11] Wet weight is the weight of microcystin found in analysis divided by weight of the tissue before water is removed by drying.

[12] To convert dry-weight concentrations to wet-weight concentrations, the dry-weight concentration is multiplied by a factor of 1 minus the percentage of moisture content expressed as a decimal.

Table 7. Microcystin laboratory analysis results for fish tissue samples obtained on October 16 and 26, 2007 from the middle Klamath River sampling areas.

Species[a]	Fish I.D.	Sample Type	Free Microcystin Levels	MDL (μg/g dry wt)
Steelhead	SB-SH-A-1	Muscle	Below detection	< 0.15
		Liver	Below detection	< 0.11
Chinook	SV-CS-A-1	Muscle	Below detection	< 0.12
		Liver	Below detection	< 0.10

Table 8. Microcystin laboratory analysis results for fish tissue samples obtained on October 31, 2007 from Iron Gate Hatchery.

Species[a]	Fish I.D.	Sample Type	Free Microcystin Levels	MDL (μg/g dry wt)
Chinook	IGH-CS-A-1	Muscle	Below detection	< 0.13
		Liver	Below detection	< 0.11
Chinook	IGH-CS-A-2	Muscle	Below detection	< 0.13
		Liver	Below detection	< 0.11
Chinook	IGH-CS-A-3	Muscle	Below detection	< 0.13
		Liver	Below detection	< 0.15
Chinook	IGH-CS-A-4	Muscle	Below detection	< 0.13
		Liver	Below detection	< 0.11
Chinook	IGH-CS-A-5	Muscle	Below detection	< 0.12
		Liver	Below detection	< 0.10
Chinook	IGH-CS-A-6	Muscle	Below detection	< 0.10
		Liver	Below detection	< 0.11
Steelhead	IGH-SH-A-1	Muscle	Below detection	< 0.10
		Liver	Below detection	< 0.19
Steelhead	IGH-SH-A-2	Muscle	Below detection	< 0.12
		Liver	Below detection	< 0.10
Steelhead	IGH-SH-A-3	Muscle	Below detection	< 0.11
		Liver	Below detection	< 0.09
Steelhead	IGH-SH-A-4	Muscle	Below detection	< 0.13
		Liver	Below detection	< 0.14
Steelhead	IGH-SH-A-5	Muscle	Below detection	< 0.11
		Liver	Below detection	< 0.14

Histopathological Examination of Fish Livers

Histological examination of liver tissues was performed by Dr. Swee Teh at UC-Davis on the eleven Chinook salmon and eight steelhead samples obtained from the Klamath River in October 2007. The examination determined that lesions were present in the liver tissues from both species. The types of liver lesions and average histologic scores for the lesions are given by specimen sample in Table 2 of Dr. Teh's report contained in Appendix E.

The most prevalent of the liver lesion types were the single cell necrosis (SCN) and focal parenchymal, perivascular, and/or pericholangial lymphocytes (FPCVL). Each of these two types was found in 18 of the 19 specimen samples (Table 9). The SCN lesion type includes liver cell swelling, membrane disruption, and lysis that can be induced by a number of external sources, including injury, infection, cancer, infarction, poisons, and inflammation. The FPCVL lesion type includes aggregates of lymphocytes (small white blood cells), occasionally mixed with other inflammatory cells, that infiltrates liver tissue in response to infection or toxin exposure. Both the SCN and FPCVL lesion types were observed to have mild severity scores on average (Table 9). The maximum score for the SCN lesion type was 2.75 (moderate-to-severe) observed in one of the steelhead specimens from Iron Gate Hatchery (IGH-SH-A-2). The maximum score for the FPCVL lesion type was 1.50 (mild-to-moderate) observed in one of the Chinook salmon specimens from Iron Gate Hatchery (IGH-CS-A-2).

The next-most prevalent of the liver lesion types was glycogen depletion (GD) found in 15 of the 19 specimen samples (Table 9). The GD lesion type occurs when the glycogen stores in the liver are depleted, usually following chronic physical exhaustion. The GD lesion type was observed to have moderate severity scores on average, with a maximum score of 3.00 (severe) observed in four of the specimens, including a Chinook salmon from the lower Klamath River area (LKR-CS-A-2), and one Chinook salmon and two steelhead from Iron Gate Hatchery (IGH-CS-A-1, IGH-CS-A-1, and IGH-SH-A-4) (Table 9).

The helminthes and myxosporean parasites (PAR) lesion type was found in 12 of the 19 specimen samples, including specimens of both species taken in the lower Klamath River area, the middle Klamath River areas, and Iron Gate Hatchery (Table 9). Helminthes and myxosporean parasites are internal parasites associated with fish disease. The PAR lesion type was observed to have mild severity scores, both on average and as the maximum score.

The macrophage aggregates (MA) lesion type was found in 11 of the 19 specimen samples, including specimens of both species taken in the lower Klamath River area, the middle Klamath River areas, and Iron Gate Hatchery (Table 9). Macrophage aggregates in the liver of fishes can be indicators of general stress, contaminant exposure, or nutritional status of fish. The MA lesion type was observed to have mild severity scores on average, with a maximum score of 1.25 (mild-to-moderate) observed in one of the steelhead specimens from Iron Gate Hatchery (IGH-SH-A-2) (Table 9).

Table 9. Number of Chinook salmon and steelhead specimens with observed liver lesions, October 2007.

Lesion Type	Number of Specimens with Lesion Type			Severity Score	
	Total	Chinook salmon	Steelhead	Average [1]	Maximum
SCN = Single cell necrosis	18	10	8	1.07	2.75
FPCVL = Focal or multifocal parenchymal, perivascular, and/or pericholangial lymphocytes	18	10	8	0.99	1.50
GD = Glycogen depletion	15	9	6	2.13	3.00
PAR = Helminthes and myxosporean parasites.	12	7	5	0.50	1.00
MA = Macrophage aggregates	11	6	5	0.58	1.25
SC = Sinusoidal congestion or hemorrhagic lesions	8	5	3	0.64	1.25
CI= Cytoplasmic inclusions	5	5	0	1.30	3.00
LIP = Lipid or fatty vacuole	5	3	2	1.15	2.25
FCA = Foci of cellular alteration	4	1	3	NA	NA
AMY= Amyloidosis	2	2	0	1.25	2.00

[1] Average severity scores were calculated including only specimens observed with lesions; that is, scores of 0.0 (lesion type absent) were not included in the calculation of averages.

The sinusoidal congestion (SC) lesion type was found in 8 of the 19 specimen samples, including specimens of both species taken at Iron Gate Hatchery (Table 9). Sinusoidal dilatation and congestion in the liver is often the result of blood vein outflow impairment, and can be a sign of physiological stress or exposure to contaminants. The SC lesion type was observed to have mild severity scores on average, with a maximum score of 1.25 (mild-to-moderate) observed in one of the Chinook salmon and one of the steelhead from Iron Gate Hatchery (IGH-CS-A-2 and IGH-SH-A-5) (Table 9).

The cytoplasmic inclusions (CI) and lipid or fatty vacuole (LIP) liver lesion types were each found in 5 of the 19 specimen samples (Table 9). The CI lesion type are circumscribed masses of abnormal foreign (e.g., viruses) or extracellular proteins within liver cells that are caused by the accumulation of foreign (exogenic) materials in the body. The presence of the LIP lesion type indicates abnormal retention of lipids within the liver that can occur from several stressors, including malnutrition and toxins. Both the CI and LIP lesion types were observed to have mild-to-moderate severity scores on average (Table 9). The maximum score for the CI lesion type was 3.00 (severe) observed in one of the Chinook salmon specimens from Iron Gate Hatchery (IGH-CS-A-1). The maximum score for the LIP lesion

type was 2.25 (moderate-to-severe) observed in one of the Chinook salmon specimens from the lower Klamath River (LKR-CS-A-1).

The foci of cellular alteration (FCA) lesion type was found in four of the 19 specimen samples, including in one Chinook salmon and three steelhead specimens from Iron Gate Hatchery (Table 9). The FCA lesion type represents the earliest stage in the progression of a cancerous liver tumor. Because of the importance of foci in the progression of fish liver cancer, these lesions were enumerated rather than scored by severity (Appendix E).

The amyloidosis (AMY) lesion type was found in 2 of the 19 specimen samples, including two Chinook salmon specimens from Iron Gate Hatchery (IGH-CS-A-1 and IGH-CS-A-2) (Table 9). Amyloidosis occurs when amyloid proteins are abnormally deposited in the liver, resulting in vascular disease lesions. The cause of amyloidosis is not well understood but appears associated with chronic infection and inflammatory states.

Discussion

Potential Effects on Salmon and Steelhead in the Klamath River

As described above, free microcystin was not detected in any of the muscle or liver samples for Chinook salmon and steelhead specimens obtained for this study in October 2007. However, histological examination of liver tissues determined that lesions were present in the liver tissues from both species. The presence of liver lesions indicates that Chinook salmon and steelhead specimens likely were exposed to contaminants, toxicants, or other stress-inducing factors that can affect fish, including migrating and spawning salmon and steelhead, such as inanition, disease, or prolonged over-exertion (Ibelings and Havens 2007, Malbrouck and Kestemont 2006, Wolf and Wolf 2005, Carruth et al. 2002, Fischer et al. 2000, Hinton and Lauren 1990, Trams 1969, Robertson and Wexler 1960). The absence of detectable levels of free microcystin in the samples, suggests that microcystin likely was not a determinative factor affecting these Chinook salmon and steelhead specimens.

Research has demonstrated that there are three primary exposure routes by which microcystins can affect fish: (1) uptake directly from the water; (2) via food ingestion; and (3) direct acute exposure. Most studies where fish are exposed to microcystins have been in the laboratory using purified microcystin toxins (Ibelings and Havens 2007). These studies have proven valuable in finding the mechanisms through which fish are affected by microcystins but are less informative about the importance of uptake of dissolved toxins in the ecosystem. Ibelings and Havens (2007) maintain that direct acute exposure of fish to high concentrations of dissolved microcystins are unlikely because processes like mixing, adsorption to clay particles, photolysis and bacterial degradation rapidly reduce the availability of dissolved microcystins. Moreover, Ibelings and Havens (2007) point out that aquatic biota may not be particularly sensitive to dissolved microcystins via direct uptake because microcystins tend to be quite water soluble and polar, and do not readily pass the lipid bilayer of membranes (Best et al. 2001, Karjalainen et al. 2005, Lurling and van der Grinten 2003).

Feeding seems to be the most important route for exposure of aquatic biota to cyanobacterial toxins (Martins and Vasconcelos 2009, Ibelings and Havens 2007, Malbrouck and Kestemont 2006, Smith and Haney 2006). This exposure route is particularly germane for organisms that directly feed on seston that includes cyanobacteria, such as zooplankton, filter feeding bivalves and phytoplanktivorous fish (unless they manage to avoid toxic cyanobacteria). For those biota that do not feed directly on cyanobacteria, toxins must reach them via the food web (Ibelings and Havens 2007, Malbrouck and Kestemont 2006). The risk of being exposed to toxins via the food web is much increased if biomagnification takes place. This is commonly found for persistent lipophilic toxicants like polychlorinated biphenyls (PCBs), but is less likely for hydrophilic compounds like microcystins (Ibelings and Havens 2007). In fact, Ibelings and Havens (2007) and Karjalainen et al. (2005) conclude that rather than biomagnification, microcystins may be subject to biodilution in the foodweb whereby microcystin concentrations are diluted at steps through the food chain.

The non-detection of free microcystin in the samples for the specimens in this study is likely explained by three main factors: (1) relatively low microcystin levels in the river; (2) short periods of potential exposure to microcystins in the river; and (3) minimal or no food ingestion by the migrating Chinook salmon and steelhead adults. The upstream migration of Chinook salmon and steelhead adults in the Klamath River overlapped with the presence of microcystin in 2007, but primarily occurred when microcystin levels in the river were low and declining. Peak concentrations of microcystin in the waters of the Klamath River in 2007 occurred in July and August, and declined to non-detectable levels by early October (Figure 2). Fall Chinook salmon adults enter the Klamath River to begin the upriver migration starting in mid-August and early September (NRC 2004, FERC 2007). Fall Chinook salmon reach their upstream spawning grounds within 2 to 4 weeks after they enter the river, after which they spawn and die. Spawning normally peaks during mid-October, and is complete by the middle of November (NRC 2004, FERC 2007). Winter steelhead are reported to enter the Klamath River from late August to February, and spawn during the period January through April (NRC 2004, FERC 2007). Summer steelhead enter the Klamath River from May to July and migrate upstream to deep pools of cooler larger tributaries where they hold until becoming sexually mature. Summer steelhead spawn primarily in December, usually in waters upstream of where winter steelhead spawn (NRC 2004, FERC 2007). There is little or no potential microcystin exposure via food ingestion, since Chinook salmon do not feed during their upstream migration (Moyle et al. 1995) and steelhead do not feed in fresh water, with possible rare exceptions (Shapovalov and Taft 1954).

As noted above, the presence of liver lesions indicates that Chinook salmon and steelhead specimens likely were exposed to contaminants, toxicants, or other stress-inducing factors that can affect liver structure and function. Research has shown that exposure to microcystins can cause severe histopathological effects in the liver (Malbrouck and Kestemont 2006, Andersen et al. 1993). However, the non-detectable levels of free microcystin in muscle and liver tissue samples for the specimens in this study suggests that the observed liver lesions likely were not caused from potential microcystin exposure in this case.

Dr. Teh's histological examination report (contained in Appendix E) states that while histopathology can be supportive of a link between contaminants and effects, it is not contaminant-specific. Dr. Teh notes that other contaminants can exert similar histological effects as known for microcystins. A range of liver histopathological effects in fish have been described in the research literature as a result of exposure to a variety of inorganic and organic contaminants (Wolf and Wolf 2005, Rudolph et al. 2002, Collier et al. 1998, Hinton and Lauren 1990, Eisler 1985) and disease or parasitic infections (Racicot et al. 2006, Wolf and Wolf 2005, Schmidt-Posthaus et al. 2001, Arkoosh et al. 1998). Moreover, various liver histopathological effects are also described in the research literature for migrating and spawning salmon and steelhead due to severe glycogen depletion and progressive loss of liver function during spawning migrations (French et al. 1983, Trams 1969, Robertson and Wexler 1960, Robertson and Wexler 1959).

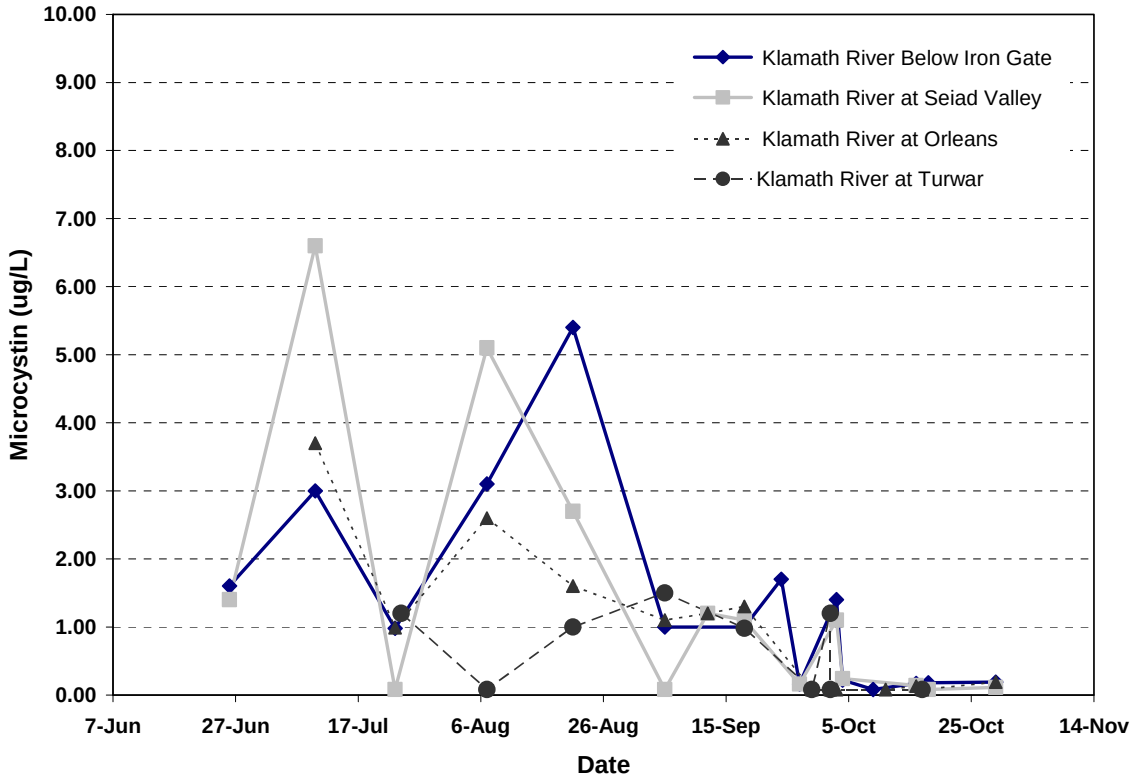

Figure 2. Microcystin data obtained in water samples at various river sites in the Klamath River below Iron Gate dam, June through October 2007 (Source: various data sources as listed in Appendix F).

Analysis with Respect to Public Health Guideline Values

Ibelings and Chorus (2007) evaluated cyanotoxin doses that may occur through human consumption of freshwater fish, and proposed guideline values for tolerable microcystin concentrations in freshwater fish tissues subject to consumption. The guideline values proposed by Ibelings and Chorus (2007) for freshwater "seafood" included a "Lifetime TDI" derived based on the Tolerable Daily Intake (TDI) of microcystin-LR of 0.04 µg/kg-day proposed by the World Health Organization (WHO). This TDI was defined by WHO as an estimate of the daily tolerable intake of microcystin-LR over a lifetime (WHO 2006), and is likewise referred by Ibelings and Chorus (2007) as the "Lifetime TDI".

The No Observed Adverse Effects Level (NOAEL)[13] assumed by WHO (2006) equals 40 mg/kg body weight (bw), based on slight effects in liver histopathology and serum enzyme level changes detected in a three-month study by Fawell et al. (1999) using chronic oral exposure of mice to pure microcystin-LR. The NOAEL of 40 mg/kg bw was then divided by a total Uncertainty Factor of 1000 to derive the TDI of 0.04 µg/kg-day. The Uncertainty Factor included multiplication factors of 10 applied twice – one for intra-species variability and one for inter-species variability, which Ibelings and Chorus (2007) note is a common

[13] NOAEL denotes the highest tested dose or concentration at which no adverse effect was found in exposed test organisms where higher doses or concentrations resulted in an adverse effect.

practice in TDI derivation. The Uncertainty Factor assumed by WHO (2006) included a third factor of 10 to account for additional uncertainty assumed because of the extrapolation of the three-month study to lifetime exposure. Ibelings and Chorus (2007) indicate that the use a total Uncertainty Factor of 1000 implies protection in the worst case, but is justified given the limited amount of information available to assess chronic microcystin-LR toxicity.

Ibelings and Chorus (2007) also derived an "Acute TDI" to calculate what they considered a safe dose for a single exposure (consumption) event. The "Acute TDI" derived by Ibelings and Chorus (2007) was the maximum tolerable dose for a single exposure event of 2.5 µg/kg bw determined by Fromme et al. (1999) based on extrapolations from acute toxicity studies of mice exposed to single abdominal injections of microcystin-LR.

Between the Lifetime TDI and Acute TDI, Ibelings and Chorus (2007) derived a "Seasonal TDI" to calculate a safe dose for the more-likely scenario for microcystin exposure from freshwater fish consumption; that is, assuming daily consumption for several weeks during the cyanobacteria "bloom" season. To derive the Seasonal TDI, Ibelings and Chorus (2007) used the NOAEL of 40 mg/kg bw derived by WHO (2006) using the Fawell et al. (1999) study results. However, in this case, Ibelings and Chorus (2007) left out the Uncertainty Factor of 10 that was used for extrapolating from a three-month study to lifetime exposure, leading to a Seasonal TDI of 0.4 mg per kg bw (leaving a residual Uncertainty Factor of 100).

For calculating final guideline values for freshwater "seafood" consumption, Ibelings and Chorus (2007) multiplied the Acute TDI, Seasonal TDI, and Lifetime TDI values by the body weight for an adult person (assumed at a nominal 75 kg) and a child (assumed at a nominal 10 kg), and then divided that product by a daily amount of fish meat ingested (assumed at a nominal 100 g fish per day). The respective guideline values are listed in Table 10. Because the values derived by Ibelings and Chorus (2007) are on a wet-weight (ww) basis, Table 10 also includes values converted to a dry-weight (dw) basis (assuming a fish tissue moisture content of 75 percent per Clark and Maret [1998]) so as to allow easier comparison to the tissue analysis results presented in the Results section of this report.

Table 10. Guideline values for freshwater fish consumption derived from Ibelings and Chorus (2007).

TDI Category	TDI Value (µg/kg)	Guideline Value (µg/kg wet weight)		Guideline Value (µg/kg dry weight)	
		Adult	Child	Adult	Child
Acute	2.5	1900	250	7600	1000
Seasonal	0.4	300	40	1200	160
Lifetime	0.04	30	4	120	16

As presented in the Results section of this report, free microcystin was not detected in any of the Chinook salmon and steelhead filet (muscle) samples at the specified MDL. Free microcystin also was not detected in any of the liver samples, although it is presumed that livers are not subject to human consumption, and are therefore not further addressed in this consumption discussion.

Although free microcystin was not detected in filet (muscle) samples at the specified MDL, it cannot be ruled out that microcystin was present at levels less than the MDL (i.e., below the ability of the analytical method to quantify it). This consideration is relevant if guidance values are less than (i.e., below) the MDL. The MDL of the filet (muscle) samples varied with sample type and recovery from 0.10 to 0.24 µg/g dw, with an average MDL of 0.14 µg/g dw. The equivalent MDL in ***per-kilogram*** units (to match the units of values presented in Table 10) varied by sample from 100 to 240 µg/kg dw, with an average MDL of 140 µg/kg dw[14].

The filet (muscle) sample MDLs are all substantially less than (i.e., below) the Acute TDI guidance value for an adult or child, indicating that single-event, single-meal consumption would pose no unacceptable health risk. The filet (muscle) sample MDLs are above the Lifetime TDI guidance value for an adult or child, indicating a potential for exposures exceeding this guideline if daily consumption occurred over a lifetime ***and*** if microcystin is present at levels below the MDL, but above the guidance value. However, as discussed above, daily consumption exposure to microcystin throughout each year over a lifetime (as the Lifetime TDI assumes) is not a probable scenario since microcystin occurs seasonally during the cyanobacteria "bloom" season, and Chinook salmon and steelhead adults are only present seasonally in the river for capture by potential consumers.

The filet (muscle) sample MDLs are all less than the Seasonal TDI guidance value for an adult, indicating that daily consumption over several weeks poses no unacceptable health risk to an adult. MDLs for 14 of the 17 filet (muscle) samples (as well as the average MDL for all 17 samples) are less than the Seasonal TDI guidance value for a child, leaving three samples with MDLs that are above the Seasonal TDI guidance value for a child. This suggests some potential for exposures exceeding this guideline for a 15 kg child if daily consumption occurred over several weeks and if microcystin is present at levels below the MDL, but above the guidance value.

[14] Values in Table 10 are in per-kilogram units for consistency of units as reported by Ibelings and Chorus (2007), and MDL values (Tables 6 through 8) are in per-gram units for consistency of units as reported by the SUNY-CESF Laboratory (Appendix D).

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Appendix A:
Photographs of Specimens from the Lower Klamath River Sampling Area

Photograph 1. ID LKR-CS-A-1, male Chinook salmon.

Photograph 2. ID LKR-CS-A-2, female Chinook salmon.

Photograph 3. ID LKR-CS-A-3, female Chinook salmon.

Photograph 4. ID LKR-CS-A-4, male Chinook Salmon.

Photograph 5. ID LKR-SH-A-1, female steelhead.

Photograph 6. ID LKR-SH-A-2, female/male steelhead.

Appendix B:
Photographs of Specimens from the Middle Klamath River Sampling Areas

Photograph 7. SB-SH-A-1, Male hatchery steelhead.

Photograph 8. I.D SV-CS-A-1, Female Chinook salmon.

Appendix C:
Photographs of Specimens from the Iron Gate Hatchery

Photograph 9. ID IGH-CS-A-1, female Chinook salmon.

Photograph 10. ID IGH-CS-A-2, female Chinook salmon.

Photograph 11. ID IGH-CS-A-3, male Chinook salmon.

Photograph 12. ID IGH-CS-A-4, female Chinook salmon.

Photograph 13. ID IGH-SH-A-1, female steelhead.

Photograph 14. ID IGH-SH-A-2, female steelhead.

Photograph 15. ID IGH-SH-A-3, male steelhead.

Photograph 16. ID IGH-SH-A-4, male steelhead.

Photograph 17. ID IGH-SH-A-5, female steelhead.

Appendix D:
SUNY-CESF Laboratory Report on Analysis of Microcystin in Fish Tissues

State University of New York
COLLEGE OF ENVIRONMENTAL SCIENCE AND FORESTRY

March 31, 2008

Timothy L. Hamaker
Senior Fisheries Biologist
CH2M Hill
2525 Airpark Drive
Redding, CA 96001

RE: Results from Toxin analysis for 2007:

Here are the results of the un-bound (free) microcystin analysis from your Klamath River tissue samples. All values are expressed as μg of toxin per g of dry tissue. Don't hesitate to call if you have any questions.

Methods:

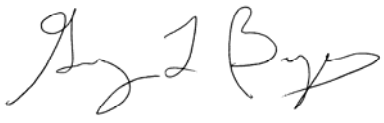
Upon receipt, frozen samples were lyophilized to dryness and homogenized (dry) using a mortar and pestle. Approximately 50 mg (0.05 g dry weight) of the homogenized tissue sample was mixed with 1ml of water and 4 μg of internal standard (7cys-S-propyl microcystin LR), and allowed to stand at 4°C for 60 minutes. After incubation, 5 ml of 50% methanol, acidified with 1% glacial acetic acid, was added and the samples sonicated on ice for 1 minute at 21W. The samples were evaporated, reconstituted in 100% methanol and lipids removed using a Bligh-Dyer extraction. After clarification by centrifugation, the supernatants were taken to dryness and reconstituted in 50% acidified methanol. Samples were sealed in autosamper vials and stored frozen until analysis.

Microcystins were determined by LCMS using a ZQ4000 single quad instrument and a 0.02% TFA acetonitrile gradient. The Instrument was standardized using microcystin RR, LR, tLR and LF. Spectra were obtained between m/z 800 and 1200 amu, and ions of interest corresponding to known microcystin were extracted out of the total ion current. Microcystins were identified on the basis of their UV signatures, HPLC retention times relative to microcystin standards and comparison of their molecular weight against a database of approximately 70 known microcystin congeners. The instrument detection limit is ~ 1 ng microcystin LR on column. Method detection limits were determined from the recovery of the internal standard and were generally less than <0.15 μg/g dry weight of tissue.

Results and Comments:

We tested your liver and muscle tissues for free or unbound microcystins: all of your samples tested negative. Matrix effects and recoveries were determined using the internal standard ^{7}cys-S-propyl microcystin LR added directly to the samples prior to extraction. Recoveries were excellent and ranged between 40-70%. The detection limit varies with sample type and recovery, but was less than 0.15 μg/g for most samples. It is our general policy to not indicate "zero" values, so your detection limits are shown on the next page. Reported concentrations of microcystins in fish and shellfish range from 0.5-20 μg/gdw (Smith et al, submitted and references cited within) with fish tissues tending to be on the lower end of the range. We were unable to extract you skin samples due to interference from the matrix, however the skin should be a small reservoir of toxin relative to the liver or muscle tissues if any was present at all.

Don't hesitate to call if you have any questions.



Greg Boyer
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glboyer@esf.edu (email)

Klamath Fish Samples: free microcystins

ESF number	Sample ID	Date collected	Recovery of internal std	Free Microcystin levels	Method Detection Limit (µg/gdw)
07-1640	LKR-CS-A-1-L, Liver	10/10/2007	62%	Below detection	< 0.09
07-1641	LKR-CS-A-1-M, Muscle	10/10/2007	43%	Below detection	< 0.13
07-1643	LKR-CS-A-2-L, Liver	10/10/2007	54%	Below detection	< 0.11
07-1644	LKR-CS-A-2-M, Muscle	10/10/2007	45%	Below detection	< 0.13
07-1646	LKR-CS-A-3-L, Liver	10/10/2007	56%	Below detection	< 0.10
07-1647	LKR-CS-A-3-M, Muscle	10/10/2007	24%	Below detection	< 0.24
07-1649	LKR-CS-A-4-L, Liver	10/10/2007	47%	Below detection	< 0.13
07-1650	LKR-CS-A-4-M, Muscle	10/10/2007	41%	Below detection	< 0.15
07-1652	LKR-SH-A-1-L, Liver	10/10/2007	52%	Below detection	< 0.11
07-1653	LKR-SH-A-1-M, Muscle	10/10/2007	33%	Below detection	< 0.18
07-1655	LKR-SH-A-2-L, Liver	10/10/2007	53%	Below detection	< 0.11
07-1656	LKR-SH-A-2-M, Muscle	10/10/2007	24%	Below detection	< 0.24
07-1658	SB-SH-A-1-L, Liver	10/16/2007	55%	Below detection	< 0.11
07-1659	SB-SH-A-1-M, Muscle	10/16/2007	39%	Below detection	< 0.15
07-1661	SV-CS-A-1-L, Liver	10/25/2007	60%	Below detection	< 0.10
07-1662	SV-CS-A-1-M, Muscle	10/25/2007	47%	Below detection	< 0.12
07-1664	IGH-CS-A-1-L, Liver	10/31/2007	54%	Below detection	< 0.11
07-1665	IGH-CS-A-1-M, Muscle	10/31/2007	45%	Below detection	< 0.13
07-1667	IGH-CS-A-2-L, Liver	10/31/2007	54%	Below detection	< 0.11
07-1668	IGH-CS-A-2-M, Muscle	10/31/2007	45%	Below detection	< 0.13
07-1670	IGH-CS-A-3-L, Liver	10/31/2007	40%	Below detection	< 0.15
07-1671	IGH-CS-A-3-M, Muscle	10/31/2007	44%	Below detection	< 0.13
07-1673	IGH-CS-A-4-L, Liver	10/31/2007	53%	Below detection	< 0.11
07-1674	IGH-CS-A-4-M, Muscle	10/31/2007	43%	Below detection	< 0.13
07-1676	IGH-CS-A-5-L, Liver	10/31/2007	57%	Below detection	< 0.10
07-1677	IGH-CS-A-5-M, Muscle	10/31/2007	49%	Below detection	< 0.12
07-1679	IGH-CS-A-6-L, Liver	10/31/2007	52%	Below detection	< 0.11
07-1680	IGH-CS-A-6-M, Muscle	10/31/2007	59%	Below detection	< 0.10
07-1682	IGH-SH-A-1-L, Liver	10/31/2007	30%	Below detection	< 0.19
07-1683	IGH-SH-A-1-M, Muscle	10/31/2007	58%	Below detection	< 0.10
07-1685	IGH-SH-A-2-L, Liver	10/31/2007	61%	Below detection	< 0.10
07-1686	IGH-SH-A-2-M, Muscle	10/31/2007	48%	Below detection	< 0.12
07-1688	IGH-SH-A-3-L, Liver	10/31/2007	64%	Below detection	< 0.09
07-1689	IGH-SH-A-3-M, Muscle	10/31/2007	53%	Below detection	< 0.11
07-1691	IGH-SH-A-4-L, Liver	10/31/2007	44%	Below detection	< 0.14
07-1692	IGH-SH-A-4-M, Muscle	10/31/2007	46%	Below detection	< 0.13
07-1694	IGH-SH-A-5-L, Liver	10/31/2007	44%	Below detection	< 0.14
07-1695	IGH-SH-A-5-M, Muscle	10/31/2007	54%	Below detection	< 0.11

Appendix E:
Report on Histopathological Examination of Fish Livers

Final Pathology Report: Histopathological Analysis of Chinook Salmon (*Oncorhynchus tshawytscha*) and Steelhead Trout (*Oncorhynchus mykiss*)

A Data Report

For

CH2MHILL Inc.
PO Box 241329
Denver, CO 80224

In Support Of The

PACIFICORP Klamath Litigation Support Project

January 15, 2008

By

Swee J. Teh, Ph.D

Toxicopathology Consulting
1302 Locust Place
Davis, California 95618

Confidential and Privileged

Executive Summary:

Liver samples were collected by scientists of CH2MILL Inc at necropsy from two species of anadromous fish: Chinook salmon (*Oncorhynchus tshawytscha*) and Steelhead Trout (*Oncorhynchus mykiss*) on October 2007 at various locations along the Klamath River. A total of 11 Chinook salmon (4 from Lower Klamath River, 1 from Selad Valley on Klamath River, and 6 from Iron Gate Hatchery) and 8 Steelhead trout (2 from Lower Klamath River, 1 from Somes Bar on Klamath River, and 5 from Iron Gate Hatchery) were collected. During necropsy, liver of individual fish was dissected longitudinally with clean surgical blades into approximately 8 slices. Slice 2, 4, 6, and 8 were pooled and shipped to Dr. Gregory L. Boyer in Syracuse, New York for microcystins analysis and will be discussed by Dr. Boyer. Slice 1, 3, 5, and 7 were fixed individually in 10% neutral buffered formalin and hand delivered to Dr. Swee Teh in Davis, California. All 76 livers were routinely paraffin processed and paraffin blocks sectioned at 3-5 microns. Sections were mounted on glass slides and stained with hematoxylin and eosin. Stained tissue sections were screened for lesions and subjected to detailed, semi-quantitative histopathologic analysis.

Comparison of prevalence of lesions for Chinook salmon collected from Lower Klamath River (LKR) with those from Iron Gate hatchery (IGH) revealed several significant differences. Higher number of Chinook salmon from IGH had significant average lesion scores for: 1) cytoplasmic inclusions; 2) single cell necrosis; 3) sinusoidal congestion and hemorrhage; and 4) amyloidosis, when compared to LKR fish. Foci of cellular alteration were seen in one fish collected from IGH and none were observed in LKR fish. No significant lesion was observed in SV fish.

Comparison of prevalence of lesions for steelhead trout collected from LKR with those from IGH also resulted in numerous differences. Higher number of steelhead trout from IGH had higher average lesion score for sinusoidal congestion when compared to LKR fish. One steelhead trout from LKR and one trout from IGH had severe single cell necrosis. Foci of cellular alteration were seen in three fish collected from IGH and none were observed in LKR fish. No significant lesion was observed in SB fish.

In summary, results suggest Chinook salmon and steelhead trout collected from IGH have, in general, a greater variety of lesions, higher lesion prevalences, and more severe lesions in liver. Severe single cell necrosis and hemorrhagic lesions in livers strongly suggest the exposure of fish to liver toxicants. In addition, foci of cellular alteration in livers of salmon and trout are indicative of exposure to xenobiotic carcinogen(s) and or promoters. Comparison of histopathology results with water and tissue microcystins analyses and additional research with both species is highly recommended.

Introduction:

In an effort to evaluate the potential adverse effects of microcystins on teleost fish residing in Klamath River in California, a pilot study was conducted surveying two anadromous species of fish for histopathological lesions. The two species examined were: adult Chinook salmon (*Oncorhynchus tshawytscha*) and Steelhead trout (*Oncorhynchus mykiss*). Four Chinook salmon (2 females and 2 males) and two steelhead trout (1 female and 1 male) were sampled from Lower Klamath River (LKR), one Chinook salmon (female) collected from Selad Valley (SV) on Klamath River, and one steelhead trout (male) was sampled from Somes Bar (SB) on Klamath River. For comparison, six salmon (3 females and 3 males) and five trout (3 females and 2 males) were also sampled from the Iron Gate Hatchery (IGH). All fish were sampled in the Fall of 2007. Only livers were sampled from all fish. This report was prepared for CH2MHILL, Inc in support of the PACIFICORP Klamath Litigation Support Project.

Materials and Methods:

Liver of individual fish was dissected longitudinally with clean surgical blades into approximately 8 slices. Slice 2, 4, 6, and 8 were pooled and shipped to Dr. Gregory L. Boyer in Syracuse, New York for microcystins analysis and will be discussed by Dr. Boyer. Slice 1, 3, 5, and 7 were fixed individually in 10% neutral buffered formalin and hand delivered to Dr. Swee Teh in Davis, California. Livers from individual fish were assigned a random alpha-numeric identification code (e.g., 07ST9-1-07ST9-19). In addition, each random number is assigned a letter to identify specific slices of livers (eg. a = slice 1, b = slice 3, c = slice 5, and d =slice 7). All livers were routinely paraffin processed and paraffin blocks sectioned at 3-5 microns. Sections were mounted on glass slides and stained with hematoxylin and eosin. Stained tissue sections were screened for lesions and subjected to detailed, semi-quantitative histopathologic analysis. Liver lesion severity scoring were based on a scale of 0 = not present, 1 = mild, 2 = moderate, and 3 = severe.

Results:

Liver histologic lesion criteria are described in Table 1. Average histologic scores for liver lesions are given in Table 2. Due to small sampling size, no statistical analysis was performed in this study. Significant lesions were seen in both species. Lesions in Chinook salmon included: cytoplasmic inclusions, single cell necrosis, sinusoidal congestion and hemorrhage, amyloidosis, and foci of cellular alteration. Significant lesions in steelhead trout were single cell necrosis, sinusoidal congestion, and foci of cellular alteration.

Comparisons of prevalence of Chinook salmon with significant lesions revealed that IGH had markedly higher number of fish with cytoplasmic inclusion (n= 3 of 6 fish); single cell necrosis (n= 3 of 6 fish); sinusoidal congestion (n= 3 of 6 fish); amyloidosis (n=2 of 6 fish); and foci of cellular alteration (n=1 of 6 fish) when compared to LKR and SV. There was little or no difference in prevalence of lesions between IGH, LKR and SV fish with respect to glycogen depletion, macrophage aggregates, or Focal parenchymal or pericholangial and perivascular leukocytes.

Comparisons of prevalence of significant lesions among steelhead trout revealed that IGH had higher number of fish with sinusoidal congestion (n=3 of 5 fish), and foci of cellular

alteration (n= 3 of 5 fish) when compared to LKR and SB fish. There was little or no difference in prevalence of lesions between IGH, LKR and SB fish with respect to glycogen depletion, macrophage aggregates, or Focal parenchymal or pericholangial and perivascular leukocytes. Severe single cell necrosis was found in one trout collected from LKR (n=1 of 2 fish) and IGH (n=1 of 5 fish). In addition, this LKR steelhead trout had one normal and one atrophic lobes of ovary (gross examination by scientists of CH2MHILL Inc).

Conclusions:

Liver histopathology results revealed that Chinook salmon and steelhead trout collected from LKR, SB, and SV, in general, were healthier than fish collected from IGH. Three Chinook salmon and three steelhead trout from IGH and one steelhead trout from LKR had lesions that signified contaminant etiology.

The most significant lesions observed in Chinook salmon and steelhead trout were sinusoidal congestion, single cell necrosis, amyloidosis, and foci of cellular alteration. Please refer to Table 1 for explanation of each lesion type. Single cell necrosis and sinusoidal congestion as a result of vascular hemorrhage are likely due to the exposure of fish to liver toxicants. Amyloidosis is a vascular disease which is likely due to prolong extensive vascular hemorrhage and sinusoidal congestion because of exposure to the liver toxins. The preneoplastic lesion (FCA) is consistent with exposure to a xenobiotic carcinogen(s) and or promoters. While histopathology can be supportive of a link between contaminants and effects, it is not contaminant-specific. Although microcystins is a known liver toxin and tumor promoter, other contaminants such as heavy metals, pesticides, and organochlorines may have exerted similar histological effects. Therefore, correlation of histopathology result with tissue microcystins analysis performed by Dr. Boyer is necessary to delineate the potential adverse effects of microcystins.

Recommendations:

In this pilot study, average lesion scores were difficult to compare because of the limited number of fish. Cytoplasmic glycogens were low in females and high in males in all locations which may suggest gender differences in liver of Chinook salmon and steelhead trout. However, statistical analysis was impossible due to small number of fish. Therefore, it is recommended that future sampling should sample a minimum of 15 females and 15 males per site for proper statistical analysis. Because of the presence of hemorrhagic and preneoplastic lesion in the liver, sampling of Chinook salmon and steelhead trout are strongly recommended for future studies.

Although future sampling of Chinook salmon and steelhead trout for histopathology could be limited to just liver, it is highly recommended that samples of gill, kidney, and gonads also be collected, fixed, and archived for possible later processing and analyses. Histopathological analysis of multiple major organs is necessary to provide direct assessment of individual fish health. Multiple organs analyses will help to differentiate types of contaminants and to determine whether the contaminants have reproductive effects. For example, fish with severe liver lesions but has normal kidney and ovary morphology may suggest the toxicant is targeting mainly liver and is not a reproductive toxicant. While fish has normal liver but significant kidney and gonad lesions may suggest the exposure to reproductive toxicants such as heavy metals. In addition, due to their long resident time within a location, sampling of resident

fish is also highly recommended.

Finally, I strongly recommended a detail workplan to be implemented in an integrated framework of investigations to discern significant contaminant-related effects. At the minimum, study should include collecting water qualities, fish condition indices, and archiving tissues for biochemical biomarkers approach.

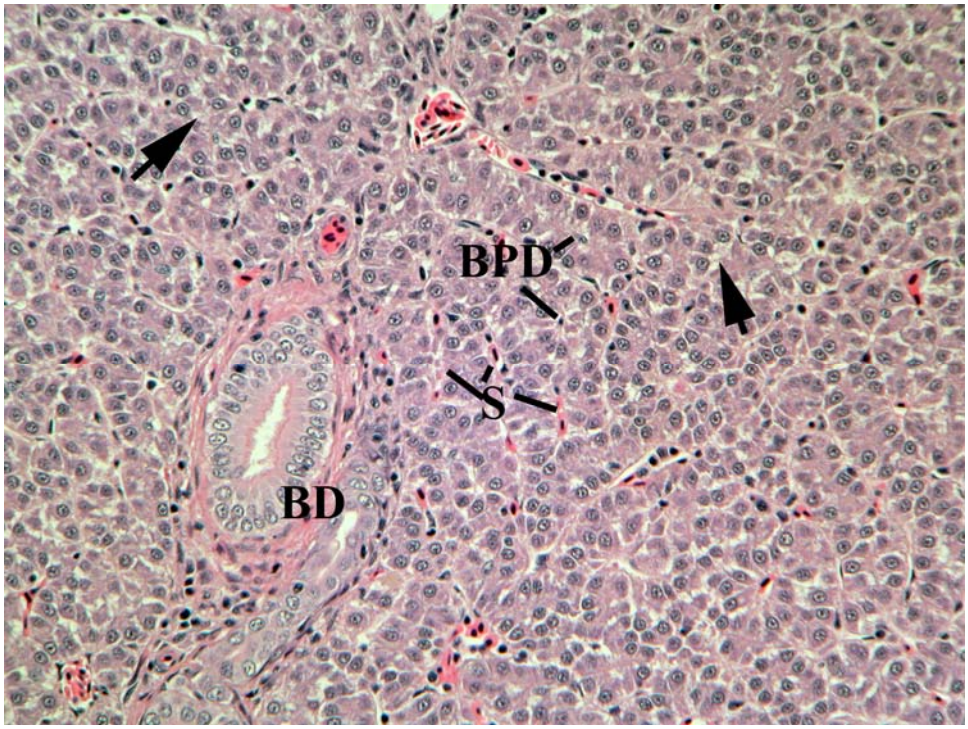

Figure 1 Hematoxylin and eosin (H&E) stained histological sections showing normal liver morphology of a trout (IGH-SH-A-5-L-5) collected from Iron Gate Hatchery. Arrows point to glycogen in the hepatocytes. BD= bile duct, BPD= bile preductular cells, S= sinusoid.

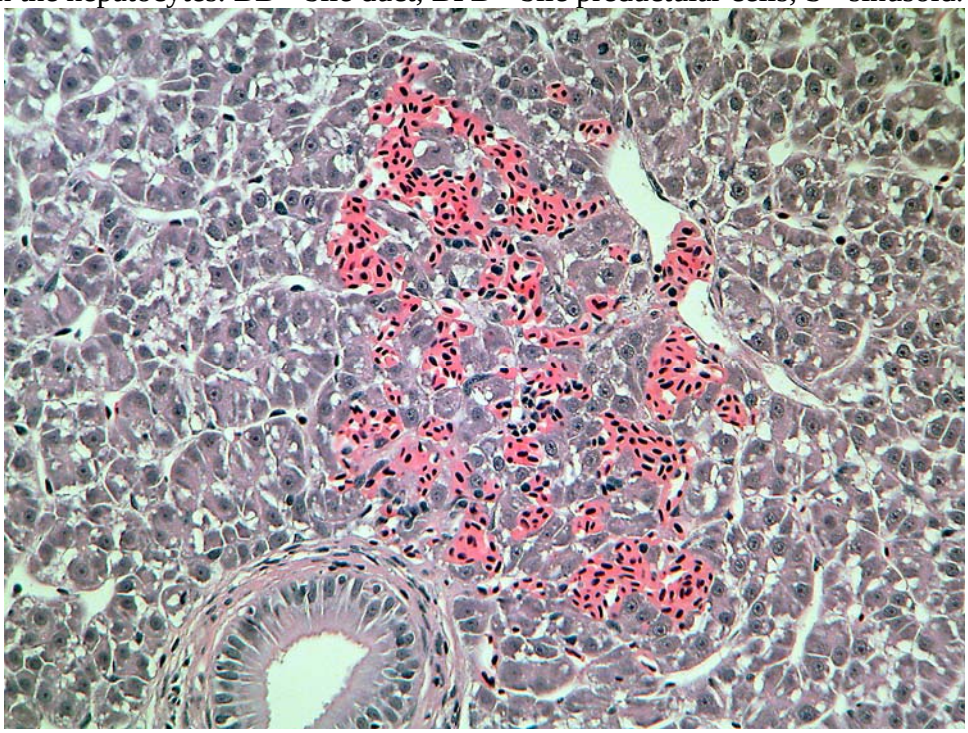

Figure 2 Sinusoidal congestion or hemorrhage in a trout liver (IGH-SH-A-3-L-3) collected from Iron Gate Hatchery. H&E stain.

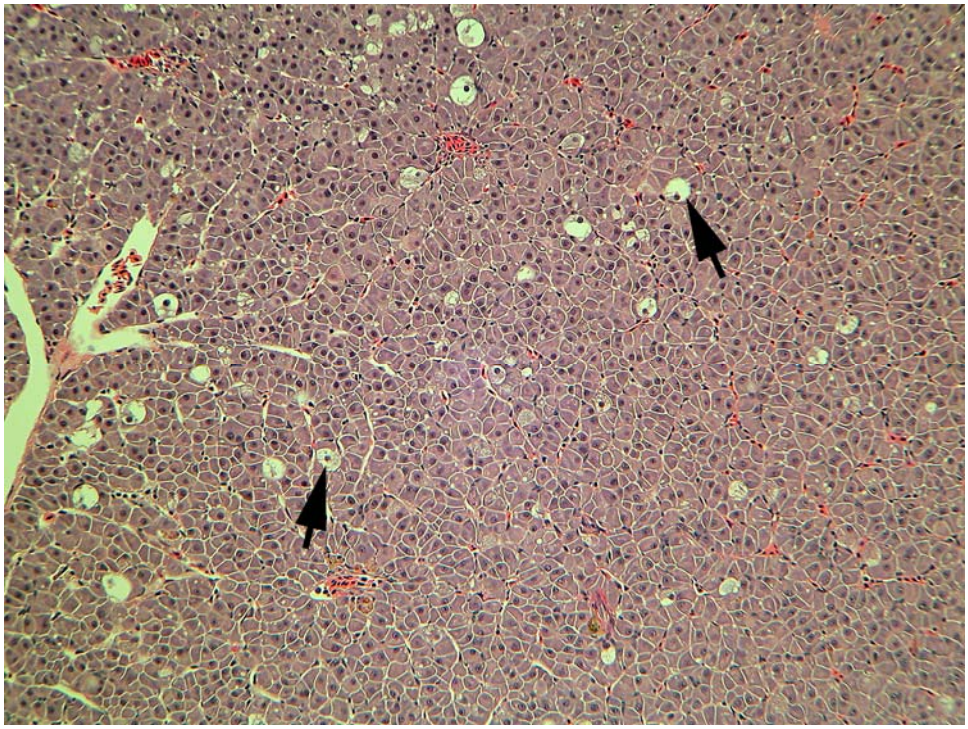

Figure 3 Severe single cell necrosis (arrows) and glycogen depletion in liver of a trout (IGH-SH-A-2-L-2) collected from Iron Gate Hatchery. H&E stain.

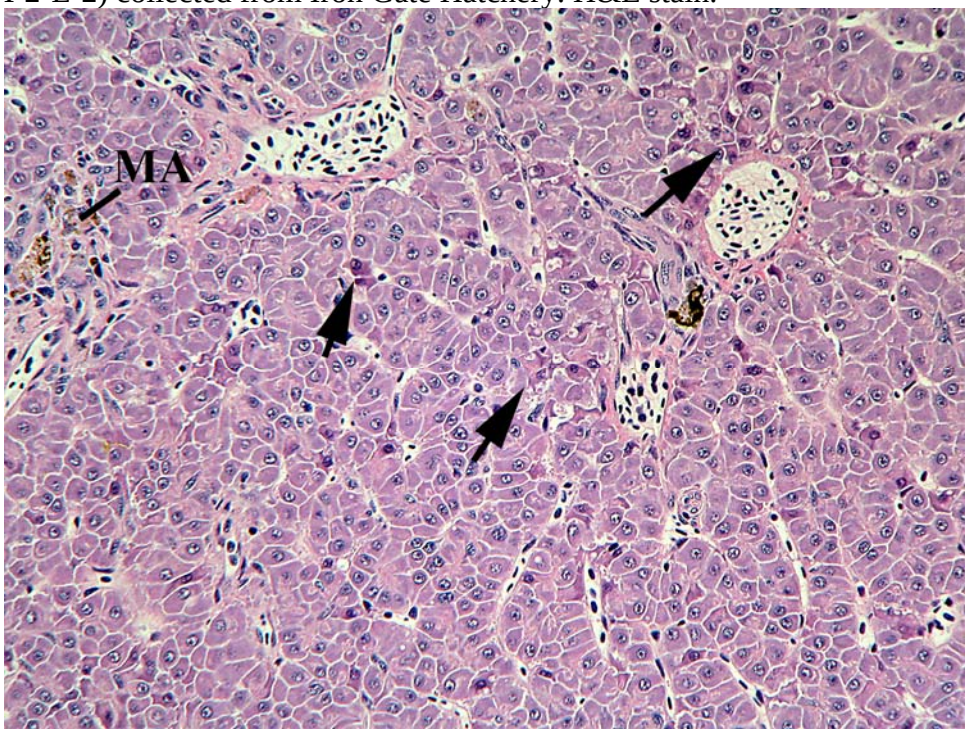

Figure 4 Severe privascular hepatocellualr necrosis (arrows) in liver of a trout (LKR-SH-A-2) collected from Lower Klamath River. MA= macrophage aggregate. H&E stain.

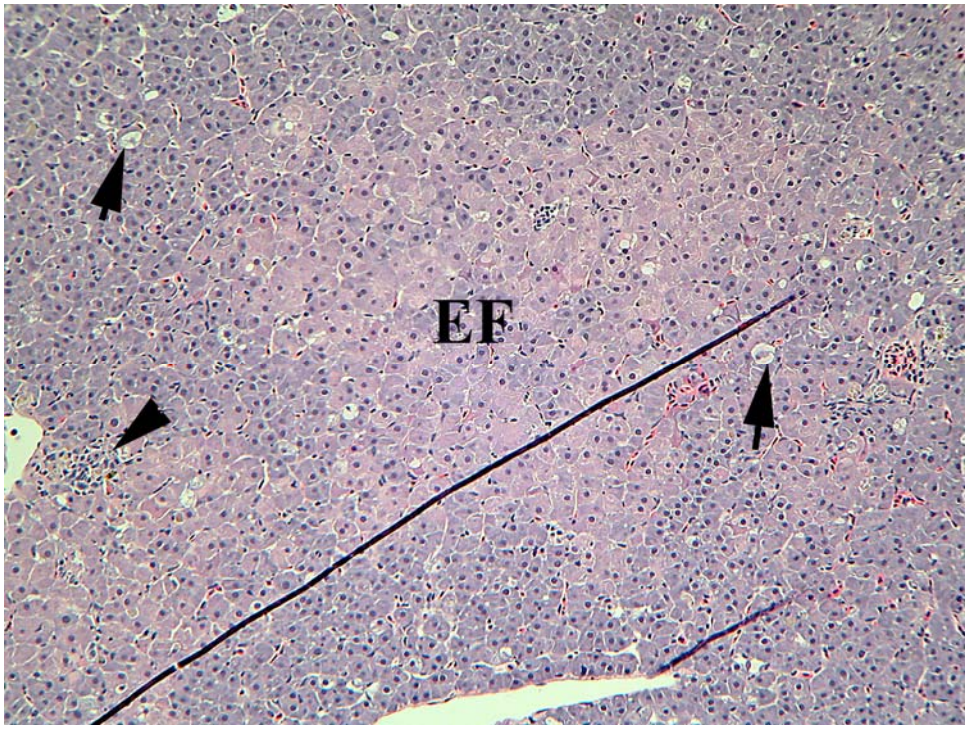

Figure 5 Large eosinophilic focus (EF) in trout liver (IGH-SH-A-2-L-2) collected from Iron Gate Hatchery. Arrows point to single cell necrosis and arrowhead points to macrophage aggregate. H&E stain

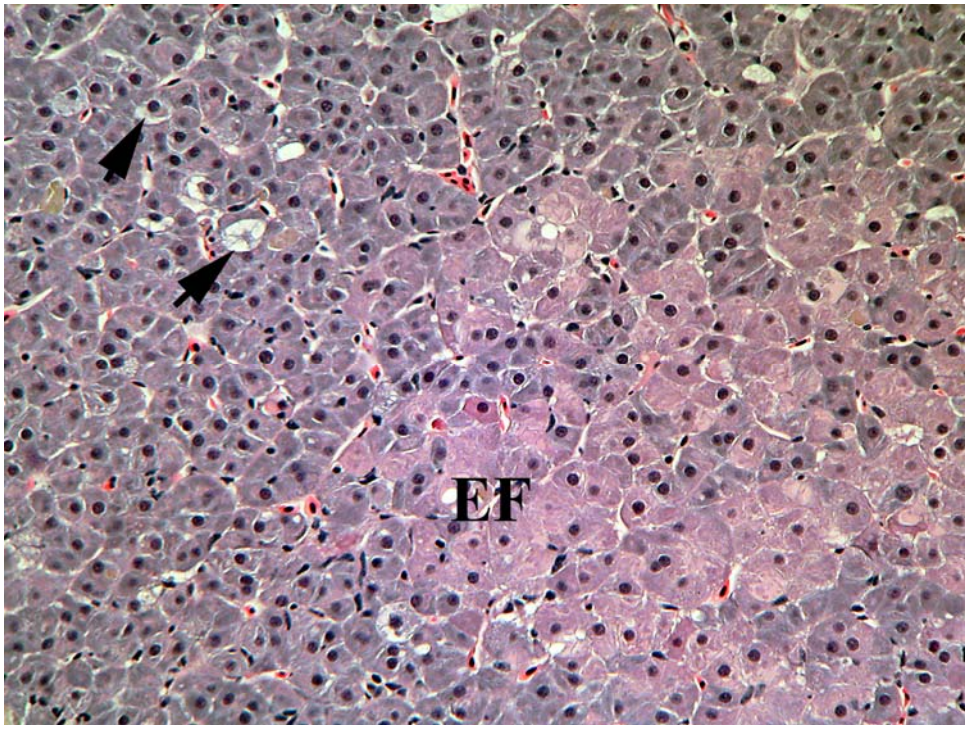

Figure 6 Higher magnification of the eosinophilic focus (EF) in Figure 7. Arrows point to single cell necrosis. H&E stain.

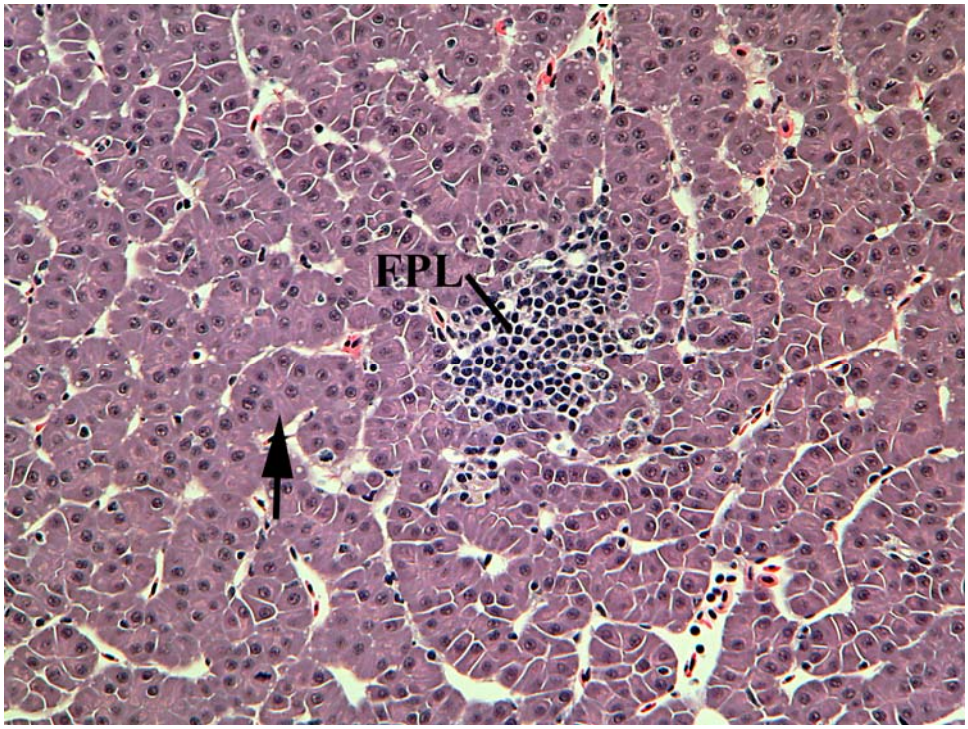

Figure 7 Mild focal parenchymal leukocytes or lymphocytes (FPL) and glycogen depletion (arrow) in liver of a salmon (LKR-CS-A-2) collected from Lower Klamath River. H&E stain

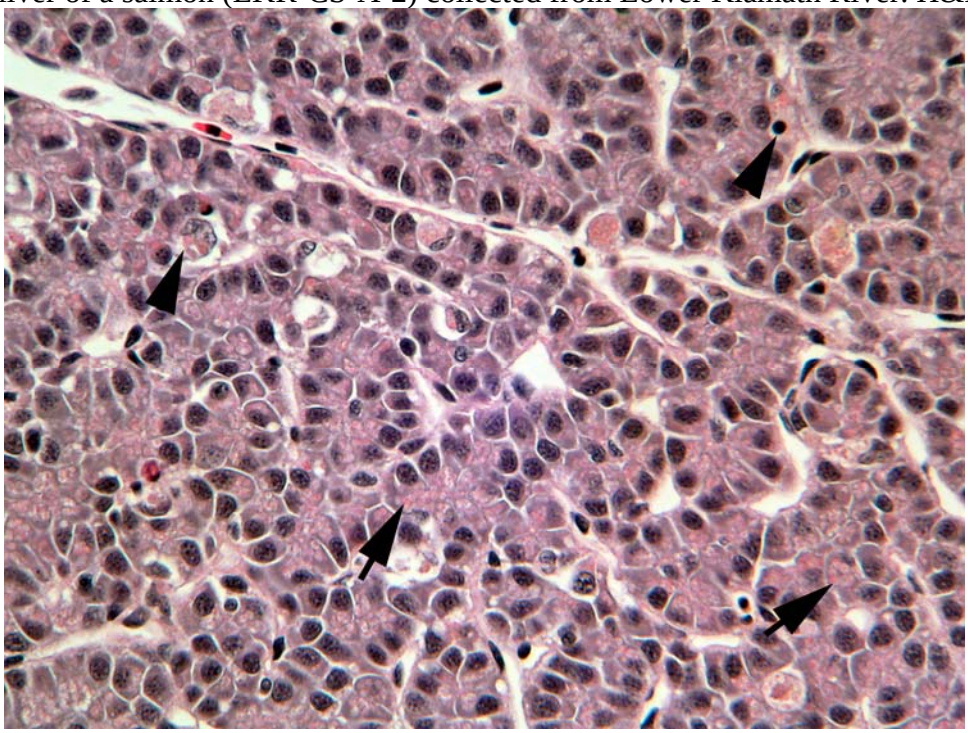

Figure 8 Severe single cell necrosis (arrowheads), glycogen depletion, and cytoplasmic inclusions (arrows) in liver of a salmon (IGH-CS-A-1-L-1) collected from Iron Gate hatchery. H&E stain.

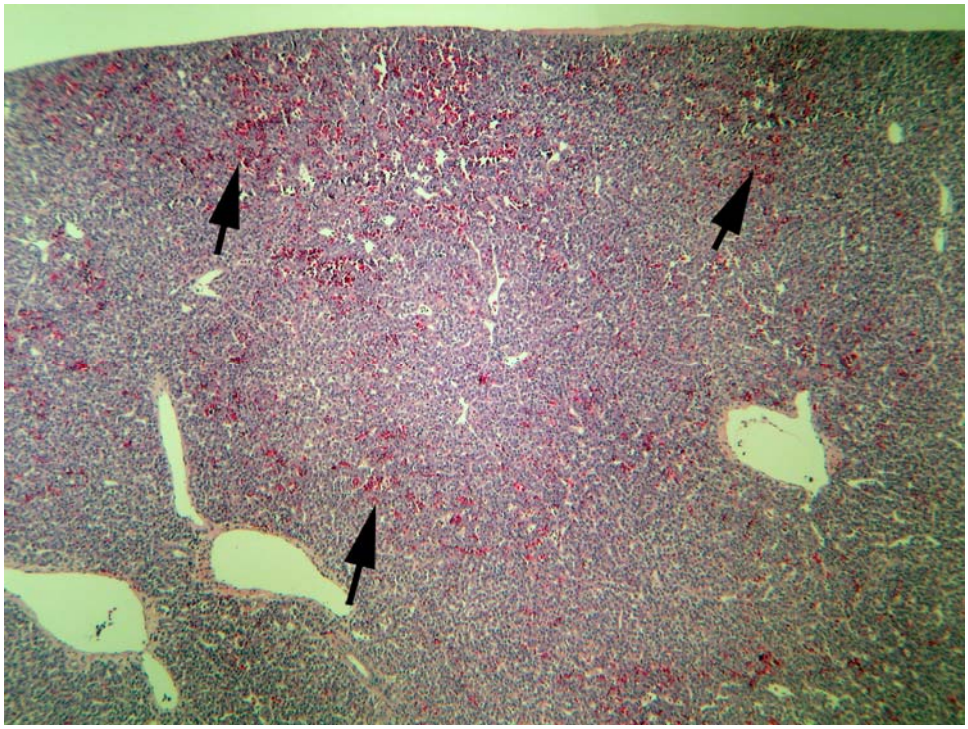

Figure 9 Hemorrhagic lesions (arrows) in a salmon liver (IGH-CS-A-3-L-3) collected from Iron Gate hatchery. H&E stain.

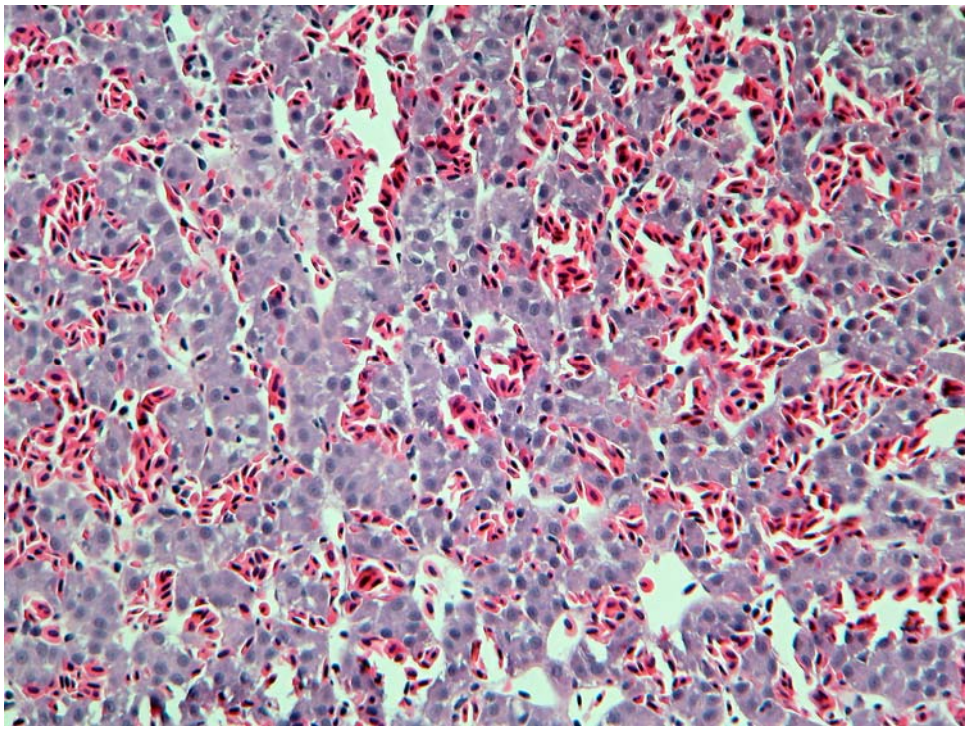

Figure 10 Higher magnification of Figure 5 showing severe sinusoidal dilation and congestion. H&E stain.

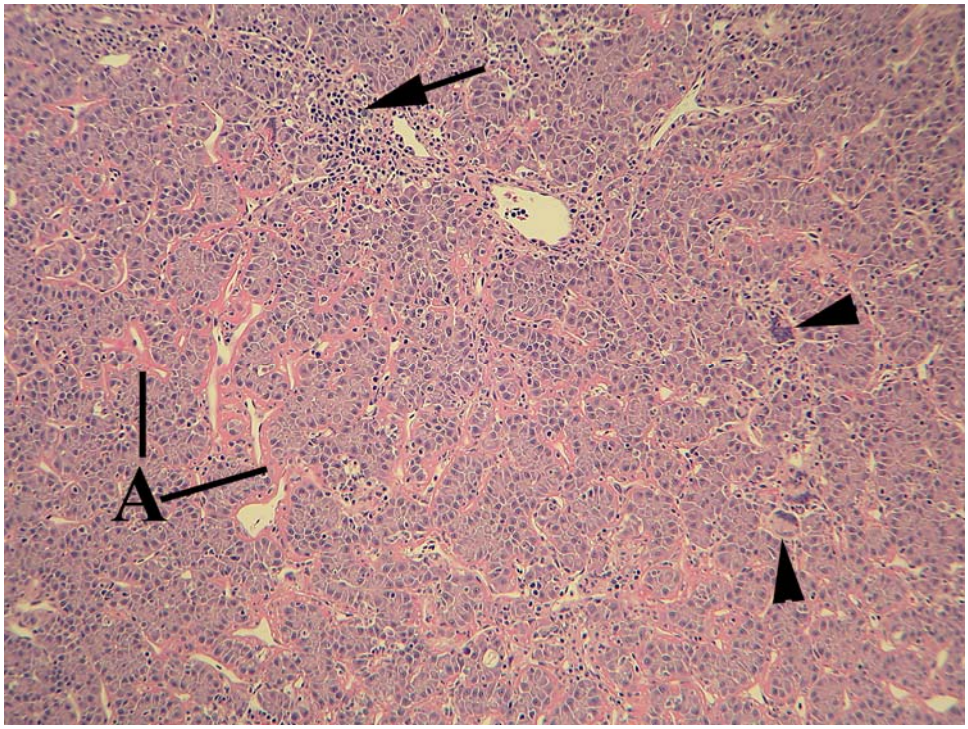

Figure 11 Amyloidosis (A) in liver of a salmon (IGH-CS-A-1-L-1) collected from Iron Gate hatchery. Arrow points to perivascular leukocytes and arrowheads point to giant cells. H&E stain

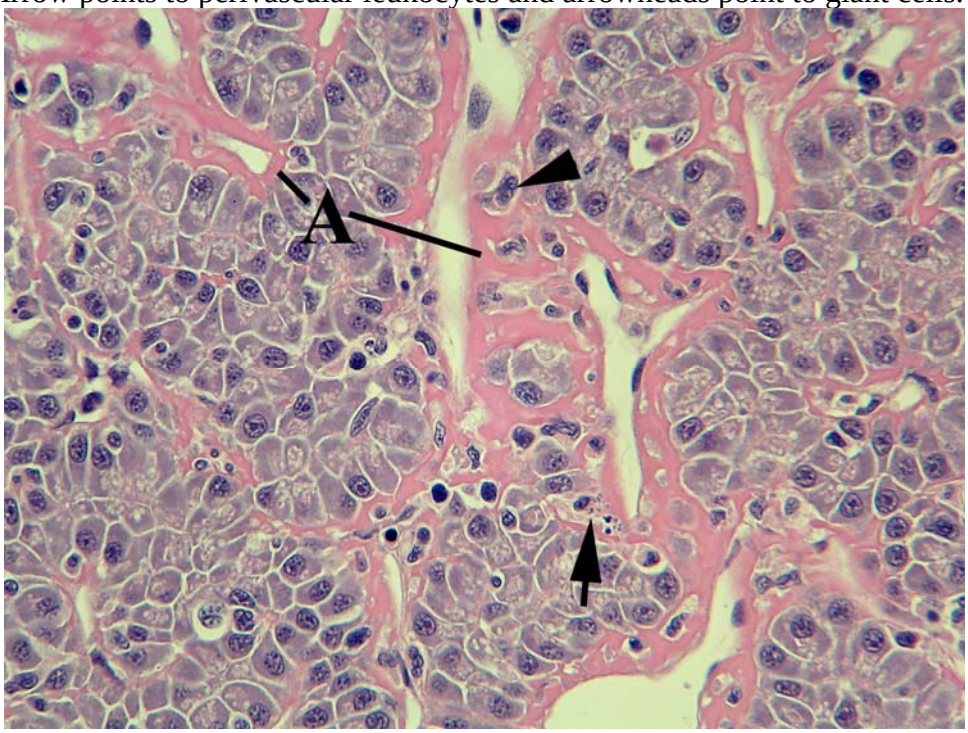

Figure 12 Higher magnification of Figure 11 showing single cell necrosis (arrow). Note amyloid (A) is deposited and occupied spaces between sinusoidal lining cells and hepatocytes. Arrowhead is pointing at hepatocytes undergo atrophy. H&E stain.

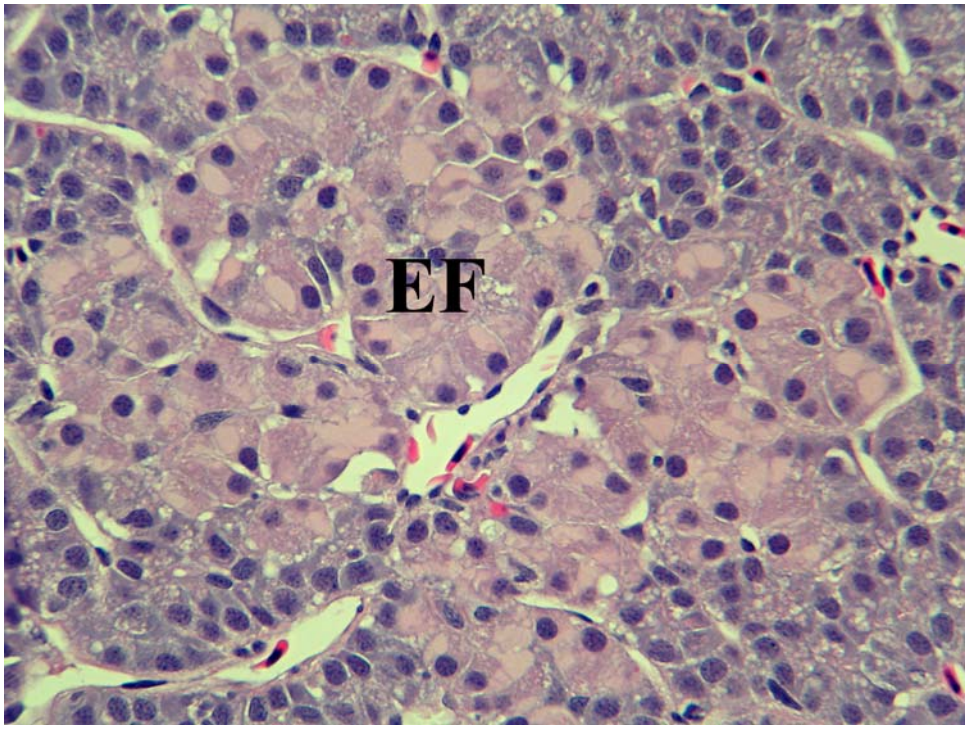

Figure 13 Eosinophilic focus in liver of a salmon (IGH-CS-A-1-L-1) collected from Iron Gate hatchery. H&E stain

Table 1. Histologic Lesion criteria

1. **GD = glycogen depletion**. Glycogen depletion was characterized by decreased size of hepatocytes, loss of the 'lacy', irregular, and poorly demarcated cytoplasmic vacuolation typical of glycogen, and increased cytoplasmic basophilia (i.e., blue coloration)

2. **CI= Cytoplasmic inclusions** were characterized by accumulation of foreign and eosinophilic materials within the cytoplasm of hepatocytes,

3. **LIP = Lipid or fatty vacuole**. This was characterized by lipid droplets appears as clear, round, well-demarcated, cytoplasmic vacuoles.

4. **FPCVL = focal/multifocal parenchymal leukocytes or lymphocytes and/or perivascular and/or pericholangial leukocytes**. This is an inflammatory lesion in response to infection or xenobiotic exposure. The lesions were characterized by focal to multifocal aggregates of lymphocytes, occasionally mixed with other inflammatory cells. Leukocytes, primarily lymphocytes, infiltrated the connective tissue around bile ducts or blood vessels or parenchyma.

5. **MA = macrophage aggregates**. This is a lesion of the hepatic parenchyma or capsule. Macrophage aggregates were usually pigmented yellow-brown to green-brown, and were occasionally mixed with lymphocytes.

6. **SCN = single cell necrosis**. This is a lesion of hepatocytes. This was characterized by cells having eosinophilic (i.e., pink coloration) cytoplasm with nuclear pyknosis and karyorrhexis.

7. **SC = Sinusoidal congestion or hemorrhagic lesions.** This lesion was characterized as the dilation of sinusoidal spaces due to vascular hemorrhage.

8. **AMY= amyloidosis.** This is a vascular disease. This lesion was characterized as the deposition of amyloid-like proteins in the spaces between sinusoidal lining cells and hepatocytes.

9. **PAR =** Helminthes and myxosporean parasites**.**

10. **FCA = foci of cellular alteration**. Foci of cellular alteration represent the earliest stage in the progression of fish hepatic neoplasia (tumor). Cells in the foci were composed of hepatocytes which are variable in size and were distinguished from the adjacent parenchyma primarily based on staining characteristics. Classes of FCA include amphophilic, basophilic, clear cell, eosinophilic, vacuolated or mixed. Because of the importance of foci in the progression of fish hepatocarcinogenesis, lesions were enumerated rather than scored by severity.

Table 2 Average Histologic Score for Liver Lesions

Sample ID	Assign ID	# of Liver	Time Sample	Date Sample	Sex	FCA	Lesion Type								
							GD	CI	LIP	FPCVL	MA	SCN	SC	AMY	PAR
LKR-CS-A-1	07ST9-1	4	0853	10/10/07	M	0	0.00	0.25	2.25	1.00	0.25	1.33	0.00	0.00	0.00
LKR-CS-A-2	07ST9-2	4	0958	10/10/07	F	0	3.00	0.00	1.00	0.75	0.00	1.00	0.00	0.00	0.00
LKR-CS-A-4	07ST9-4	4	1340	10/10/07	F	0	2.75	0.00	1.00	1.00	0.25	0.75	0.00	0.00	0.00
LKR-CS-A-6	07ST9-6	4	1630	10/10/07	M	0	0.00	0.00	0.00	1.00	0.00	0.75	0.00	0.00	0.25
LKR-SH-A-1	07ST9-3	4	1100	10/10/07	F	0	2.00	0.00	0.00	0.75	0.75	1.00	0.00	0.00	0.00
LKR-SH-A-2	07ST9-5	4	1550	10/10/07	I	0	2.75	0.00	0.50	1.25	0.75	2.25	0.00	0.00	0.25
SB-SH-A-1	07ST9-7	4	1115	10/16/07	M	0	1.25	0.00	1.00	1.00	1.00	0.50	0.00	0.00	0.25
SV-CS-A-1	07ST9-8	4	1140	10/25/07	F	0	2.00	0.50	0.00	0.00	0.25	0.25	0.00	0.00	1.00
IGH-CS-A-1-L-1	07ST9-12	4	0991	10/31/07	F	EF	2.75	3.00	0.00	1.25	0.50	1.75	0.75	2.00	1.00
IGH-CS-A-2-L-2	07ST9-13	4	1037	10/31/07	F	0	2.00	1.50	0.00	1.50	1.00	1.00	1.25	0.50	0.50
IGH-CS-A-3-L-3	07ST9-14	4	1050	10/31/07	M	0	0.75	0.00	0.00	1.00	0.00	0.50	1.00	0.00	0.00
IGH-CS-A-4-L-4	07ST9-15	4	1104	10/31/07	F	0	3.00	1.25	0.00	1.25	0.50	1.50	0.00	0.00	0.50
IGH-CS-A-5-L-5	07ST9-18	4	1145	10/31/07	M	0	1.25	0.00	0.00	1.25	0.00	0.25	0.25	0.00	1.00
IGH-CS-A-6-L-6	07ST9-19	4	1257	10/31/07	M	0	1.50	0.00	0.00	0.25	0.00	0.00	0.25	0.00	1.00
IGH-SH-A-1-L-1	07ST9-9	4	0903	10/31/07	F	EF	3.00	0.00	0.00	1.00	0.00	0.75	0.00	0.00	0.25
IGH-SH-A-2-L-2	07ST9-10	4	0917	10/31/07	F	EF	3.00	0.00	0.00	0.75	1.25	2.75	0.00	0.00	0.25
IGH-SH-A-3-L-3	07ST9-11	4	0926	10/31/07	M	0	0.00	0.00	0.00	0.75	0.50	1.00	0.50	0.00	0.00
IGH-SH-A-4-L-4	07ST9-16	4	1116	10/31/06	M	EF	1.00	0.00	0.00	1.00	0.00	1.00	0.50	0.00	0.25
IGH-SH-A-5-L-5	07ST9-17	4	1132	10/31/07	F	0	0.00	0.00	0.00	1.00	0.00	1.00	1.25	0.00	0.00

Liver lesion severity scoring were based on a scale of 0 = not present, 1 = mild, 2 = moderate, and 3 = severe. LKR=Lower Klamath River; SB=Somes Bar located on Klamath River; SV= Selad Valley located on Klamath River; IGH= Iron Gate Hatchery; F= female, M =male, I= possible intersex fish or female with ovarian atrophy (Observation described by CH2MHILL staff); GD= glycogen depletion; CI = cytoplasmic inclusions; LIP= lipidosis or fatty vacuolation; FPCVL= focal/multifocal parenchymal leukocytes or lymphocytes or perivascular and/or pericholangial leukocytes; MA= macrophage aggregate; SCN = single cell necrosis; SC= sinusoidal congestion or hemorrhage; AMY= amyloidosis; PAR= parasitic infections. FCA = foci of cellular alteration also called preneoplastic foci; EF = eosinophilic focus, a type of the foci of cellular alteration. Because of the importance of foci in the progression of fish hepatocarcinogenesis, lesions were enumerated rather than scored by severity.

Appendix F:
Microcystin Data Obtained in Water Samples below Iron Gate Dam, June through October 2007

Table 1. Microcystis and microcystin data obtained at various river sites in the Klamath River below Iron Gate dam, 2007 (source: various reports and memos).

Date	Approx RM	Name	Microcystin (µg/L)	Lab	Source
June 12, 2007	189.5	Klamath River Below Iron Gate			Kann 2007 memos
June 26, 2007	189.5	Klamath River Below Iron Gate	1.60	EPA	Kann 2007 memos
July 10, 2007	189.5	Klamath River Below Iron Gate	3.00	EPA	Kann 2007 memos
July 23, 2007	189.5	Klamath River Below Iron Gate	0.98	EPA	Kann 2007 memos
August 7, 2007	189.5	Klamath River Below Iron Gate	3.10	EPA	Kann 2007 memos
August 21, 2007	189.5	Klamath River Below Iron Gate	5.40	EPA	Kann 2007 memos
September 5, 2007	189.5	Klamath River Below Iron Gate	1.00	EPA	Kann 2007 memos
September 18, 2007	189.5	Klamath River Below Iron Gate	1.00	EPA	Kann 2007 memos
September 24, 2007	189.5	Klamath River Below Iron Gate	1.70	EPA	EPA lab report
September 27, 2007	189.5	Klamath River Below Iron Gate	0.16	CH	PacifiCorp
October 3, 2007	189.5	Klamath River Below Iron Gate	1.40	EPA	Kann 2007 memos
October 4, 2007	189.5	Klamath River Below Iron Gate	0.22	CH	PacifiCorp
October 9, 2007	189.5	Klamath River Below Iron Gate	0.08	CH	PacifiCorp
October 16, 2007	189.5	Klamath River Below Iron Gate	0.17	EPA	Kann 2007 memos
October 18, 2007	189.5	Klamath River Below Iron Gate	0.18	CH	PacifiCorp
October 29, 2007	189.5	Klamath River Below Iron Gate	0.19	EPA	Kann 2007 memos
June 26, 2007	128.5	Klamath River at Seiad Valley	1.40	EPA	Kann 2007 memos
July 10, 2007	128.5	Klamath River at Seiad Valley	6.60	EPA	Kann 2007 memos
July 23, 2007	128.5	Klamath River at Seiad Valley	0.08	EPA	Kann 2007 memos
August 7, 2007	128.5	Klamath River at Seiad Valley	5.10	EPA	Kann 2007 memos
August 21, 2007	128.5	Klamath River at Seiad Valley	2.70	EPA	Kann 2007 memos
September 5, 2007	128.5	Klamath River at Seiad Valley	0.08	EPA	Kann 2007 memos
September 12, 2007	128.5	Klamath River at Seiad Valley	1.20	EPA	EPA lab report
September 18, 2007	128.5	Klamath River at Seiad Valley	1.10	EPA	Kann 2007 memos
September 27, 2007	128.5	Klamath River at Seiad Valley	0.16	CH	PacifiCorp
October 3, 2007	128.5	Klamath River at Seiad Valley	1.10	EPA	Kann 2007 memos
October 4, 2007	128.5	Klamath River at Seiad Valley	0.24	CH	PacifiCorp
October 16, 2007	128.5	Klamath River at Seiad Valley	0.14	EPA	Kann 2007 memos
October 18, 2007	128.5	Klamath River at Seiad Valley	0.08	CH	PacifiCorp
October 29, 2007	128.5	Klamath River at Seiad Valley	0.11	EPA	Kann 2007 memos
July 10, 2007	59.0	Klamath River at Orleans	3.70	EPA	Kann 2007 memos
July 23, 2007	59.0	Klamath River at Orleans	0.99	EPA	Kann 2007 memos
August 7, 2007	59.0	Klamath River at Orleans	2.60	EPA	Kann 2007 memos
August 21, 2007	59.0	Klamath River at Orleans	1.60	EPA	Kann 2007 memos
September 5, 2007	59.0	Klamath River at Orleans	1.10	EPA	Kann 2007 memos
September 12, 2007	59.0	Klamath River at Orleans	1.20	EPA	EPA lab report
September 18, 2007	59.0	Klamath River at Orleans	1.30	EPA	Kann 2007 memos
September 29, 2007	59.0	Klamath River at Orleans	0.08	CH	PacifiCorp
October 2, 2007	59.0	Klamath River at Orleans	0.08	CH	PacifiCorp
October 3, 2007	59.0	Klamath River at Orleans	0.08	EPA	Kann 2007 memos
October 11, 2007	59.0	Klamath River at Orleans	0.08	CH	PacifiCorp
October 16, 2007	59.0	Klamath River at Orleans	0.13	EPA	Kann 2007 memos
October 17, 2007	59.0	Klamath River at Orleans	0.08	CH	PacifiCorp
October 29, 2007	59.0	Klamath River at Orleans	0.19	EPA	Kann 2007 memos
July 24, 2007	43.5	Klamath R. at Weitchpec	1.30		Fetcho 2007 memos
August 7, 2007	43.5	Klamath R. at Weitchpec	1.30	EPA	Fetcho 2007 memos
August 21, 2007	43.5	Klamath R. at Weitchpec	1.80	EPA	Fetcho 2007 memos
September 5, 2007	43.5	Klamath R. at Weitchpec	1.60	EPA	Fetcho 2007 memos
September 18, 2007	43.5	Klamath R. at Weitchpec	1.10	EPA	Fetcho 2007 memos
October 2, 2007	43.5	Klamath R. at Weitchpec	0.08	EPA	EPA lab report
August 7, 2007	42.5	Klamath R. below Weitchpec	1.20	EPA	Fetcho 2007 memos
August 21, 2007	42.5	Klamath R. below Weitchpec	2.00	EPA	Fetcho 2007 memos
September 5, 2007	42.5	Klamath R. below Weitchpec	1.30	EPA	Fetcho 2007 memos
September 18, 2007	42.5	Klamath R. abv Tully Cr.	1.10	EPA	Fetcho 2007 memos
October 2, 2007	42.5	Klamath R. abv Tully Cr.	1.40	EPA	EPA lab report

Table 1. Microcystis and microcystin data obtained at various river sites in the Klamath River below Iron Gate dam, 2007 (source: various reports and memos).

Date	Approx RM	Name	Microcystin (µg/L)	Lab	Source
July 24, 2007	6.0	Klamath River at Turwar	1.20	EPA	Fetcho 2007 memos
August 7, 2007	6.0	Klamath River at Turwar	0.08	EPA	Fetcho 2007 memos
August 21, 2007	6.0	Klamath River at Turwar	1.00	EPA	Fetcho 2007 memos
September 5, 2007	6.0	Klamath River at Turwar	1.50	EPA	Fetcho 2007 memos
September 18, 2007	6.0	Klamath River at Turwar	0.98	EPA	Fetcho 2007 memos
September 29, 2007	6.0	Klamath River at Turwar	0.08	CH	PacifiCorp
October 2, 2007	6.0	Klamath River at Turwar	1.20	EPA	EPA lab report
October 2, 2007	6.0	Klamath River at Turwar	0.08	CH	PacifiCorp
October 17, 2007	6.0	Klamath River at Turwar	0.08	CH	PacifiCorp