Monitoring of Mountain Whitefish Prosopium williamsoni, from the Columbia River System near Castlegar, British Columbia: **Health Parameters & Contaminants in 1992**

Nener, J., D. Kieser, J.A.J. Thompson, W.L. Lockhart, D.A. Metner, and R. Roome

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Canadian Manuscript Report of Fisheries and Aquatic Sciences 2036



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Correct citation for this publication:

Nener, J., D. Kieser, J.A.J. Thompson, W.L. Lockhart, D.A. Metner, and R. Roome. 1995. Monitoring Mountain Whitefish Proposium williamsoni, from the Columbia River System near Castlegar, British Columbia: Health Parameters & Contaminants in 1992. Can. Manuscr. Rep. Fish. Aquat. Sci. 2036: 89p.

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ABSTRACT

Nener, J., D. Kieser, J.A.J. Thompson, W.L. Lockhart, D.A. Metner, and R. Roome. 1995. Monitoring Mountain Whitefish Proposium williamsoni, from the Columbia River System near Castlegar, British Columbia: Health Parameters & Contaminants in 1992. Can. Manuscr. Rep. Fish. Aquat. Sci. 2036: 89p.

In July 1992, adult mountain whitefish (*Prosopium williamsoni*) were collected from two reaches of the Columbia River (Genelle and Beaver Creek) and one reference site on the Slocan River, for assessment of fish health and contaminant levels. This was the first of three sampling events scheduled to take place over five years, in a program designed to assess improvements which are anticipated to follow changes in manufacturing processes and upgrading of effluent treatment systems at a bleached kraft pulp mill near Castlegar, and at a smelter-fertilizer complex located at Trail, B.C.

Results of the health assessment indicate that fish from both Genelle and Beaver Creek had a higher incidence of stress-related abnormalities than fish from the reference site. Abnormalities commonly associated with bleached kraft pulp mill effluent were not found. Genelle fish were significantly older than fish from the other two sites, which had a similar mean age, compromising the ability to make comparisons between this site and the Slocan reference site.

Concentrations of polychlorinated dibenzodioxins (PCDD) and dibenzofurans (PCDF) were significantly higher in fish from the Columbia River compared with fish from the reference reach. Concentrations appear to have declined since 1991 (Boyle et al., 1992), however the small sample size of the earlier study did not allow for statistical comparisons between the two years.

Measurement of polychlorinated biphenyl (PCB) congeners showed higher levels of Arochlor 1254/1260 and coplanar (dioxin-like) PCBs in fish from the Columbia River compared with fish from the reference site, but total PCB concentrations were below the Health Canada criterion of 2 ppm for limiting consumption by humans. Body burdens of Arochlors do not appear to have declined in mountain whitefish between the 1991 and 1992 sampling periods. No source of PCBs has been identified in the area of the Columbia River under study.

PCBs contributed slightly less than half the Toxic Equivalents (TEQs) (calculated relative to toxicity of T4CDD) contributed by dioxins and furans in Columbia River fish. The sum of TEQs from dioxins, furans, and PCBs was not related to the Cumulative Disease Severity (CDS) for these fish, however a relationship could have been obscured by relatively small sample sizes with high variability of contaminant concentrations and ages.

Mixed function oxidases (MFOs) were measured in all fish which were assessed for organic contaminant concentrations. Results were difficult to interpret due to high variability in MFO data, and because the pulp mill was on strike for approximately 10 days prior to sampling. Activities of both EROD and AHH showed a decline in Genelle fish from the 1991 levels, possibly as a result of both reduced body burdens of dioxins and furans, and because pulp mill effluent discharges to the river ceased during a strike.

Whitefish muscle contained measurable levels of copper, mercury, strontium and zinc, and with the exception of mercury, concentrations were significantly higher at the reference site. Mercury concentrations were highest at the Genelle site and may relate to the greater fish ages at this reach, or exposure to compounds of that element which had historical uses in the pulp and paper industry.

Levels of metal-binding proteins and the associated metals, copper, cadmium and zinc were determined in 45 whitefish livers. Metal-binding proteins correlated strongly with tissue-bound copper and cytosolic cadmium only in fish from the Beaver Creek site. Comparison of liver and muscle Cu and Zn data indicated that Cu (and to a lesser extent, Zn) was being stored preferentially in fish livers from Beaver Creek. It was considered that smelter slag particles could be a vector for these metals, as slag was identified in the gut contents of fish from Beaver Creek.

RESUMÉ

Nener, J., D. Kieser, J.A.J. Thompson, W.L. Lockhart, D.A. Metner, and R. Roome. 1995. Monitoring Mountain Whitefish Proposium williamsoni, from the Columbia River System near Castlegar, British Columbia: Health Parameters & Contaminants in 1992. Can. Manuscr. Rep. Fish. Aquat. Sci. 2036: 89p.

En juillet 1992, des ménominis des montagnes adultes (*Prosopium williamsoni*) ont été capturés dans deux segments du fleuve Columbia (Genelle et Beaver Creek) ainsi qu'en un site témoin (rivière Slocan) pour l'évaluation de l'état de santé et du niveau de contamination des poissons. Il s'agissait du premier de trois échantillonnages devant être réalisés au cours d'une période de cinq ans dans le cadre d'un programme d'évaluation des améliorations prévues suite à la modification des processus de fabrication et de l'amélioration des systèmes de traitement des effluents d'une usine de pâte kraft blanchie située à proximité de Castlegar et d'un complexe regroupant une fonderie et une usine d'engrais situé à Trail (C.-B.).

L'évaluation de l'état de santé a montré que les poissons provenant de Genelle et de Beaver Creek présentaient une incidence élevée d'anomalies liées au stress, comparativement aux poissons du site témoin. Les anomalies généralement associées aux effluents d'une usine de pâte kraft blanchie n'ont pas été décelées. Les poissons de Genelle étaient significativement plus âgés que ceux des deux autres sites, d'un âge moyen semblable, et cela a nuit de beaucoup à l'établissement de comparaisons entre ce site et le site témoin de la Slocan.

Les concentrations de polychlorodibenzodioxines (PCDD) et de polychlorodibenzofuranes (PCDF) étaient significativement plus élevées chez les poissons du Columbia, comparativement à ceux du site témoin. Les concentrations semblent avoir diminué depuis 1991 (Boyle et coll., 1992), mais le faible échantillon de l'étude antérieure ne permet pas d'établir de comparaisons statistiquement valables entre les deux années.

L'analyse des congénères des biphényles polychlorés (BPC) a permis de déceler une teneur plus élevée en Arochlor 1254/1260 et en BPC coplanaire (type dioxine) chez les poissons du Columbia que chez ceux du site témoin, mais les concentrations en BPC totaux étaient inférieures à la teneur maximale limite de 2 ppm fixée par Santé Canada pour la consommation humaine. La charge corporelle en Arochlor ne semble pas avoir diminué chez les ménominis des montagnes entre les périodes d'échantillonnage de 1991 et 1992. Aucune source de BPC n'a été décelée dans la région du Columbia faisant l'objet de l'étude.

Les BPC représentaient légèrement moins de la moitié des équivalents toxiques (EQT, calculés en fonction de la toxicité du TCDD) attribués aux dioxines et aux furanes présents dans les poissons du Columbia. La somme des EQT des dioxines, furanes et BPC ne présentait pas de corrélation avec l'indice cumulatif de gravité de maladie de ces poissons, mais il est possible qu'une telle relation ait été masquée par la petitesse relative de l'effectif de l'échantillon et la forte variabilité des concentrations des contaminants et des âges.

Les oxydases à fonction mixte (OFM) ont été caractérisées chez tous les poissons ayant fait l'objet d'un dosage des contaminants organiques. Les résultats étaient difficiles à interpréter à cause de la forte variabilité des données OFM et du fait que l'usine de pâte à été inactive pendant 10 jours, suite à une grève, avant le prélèvement des échantillons.

Les muscles des poissons contenaient des quantités mesurables de cuivre, de mercure, de strontium et de zinc et, à l'exception du mercure, les concentrations étaient significativement supérieures au site témoin. Les concentrations de mercure les plus élevées ont été notées au site de Genelle et peuvent s'expliquer par l'âge plus avancé des poissons à cet endroit ou par une exposition à des composés de cet élément qui étaient utilisés dans l'industrie des pâtes et papiers.

Les protéines se liant aux métaux et les métaux qui leur sont associés, à savoir le cuivre, le cadmium et le zinc, ont été recherchés dans le foie de 45 ménominis. Ces protéines ne présentaient une forte corrélation avec le cuivre lié aux tissus et le cadmium cytosolique que chez les poissons de Beaver Creek. La comparaison des teneurs de Cu et de Zn du foie et des muscles a montré que le Cu et, dans une moindre mesure le Zn, étaient surtout emmagasinés dans le foie des poissons de Beaver Creek. Des particules du laitier produit par les fours pourrait être le vecteur de ces métaux car on en a trouvées dans les viscères des poissons de Beaver Creek.

INTRODUCTION

In October 1990 the Department of Fisheries and Oceans (DFO) was contacted with regard to apparent health problems in mountain whitefish caught in the Columbia River between Hugh Keenleyside Dam and the Canada/U.S. border. Emaciated whitefish and fish with external lesions were noted by field biologists (R.L. & L. Environmental Services Ltd., Edmonton, Alberta). A preliminary study was undertaken by DFO in January 1991 to document the condition of fish prior to the planned expansion and environmental upgrading of the bleached kraft pulp mill located at Castlegar, B.C. (Boyle et al., 1992). Results of this study indicated that the health of mountain whitefish collected from two reaches of the Columbia River may have been impaired.

DFO's Green Plan Toxic Contaminants Program provided additional funds to monitor fish health in this portion of the Columbia River over a subsequent five year period, with three sampling seasons (July 1992, 1994, and 1996). The purpose of these studies is to monitor the reduction in body burdens of dioxins and furans, and changes in fish health which are anticipated to follow scheduled improvements in effluent quality from the bleached kraft pulp mill. The Columbia River Integrated Environmental Monitoring Program (CRIEMP) Committee provided funds to expand the program for inclusion of studies on tissue metal concentrations and metallothioneins in whitefish, associated with the lead/zinc smelter and fertilizer complex located at Trail. The smelter and fertilizer plant are currently implementing a number of programs to improve the quality of its effluents. This report presents results of the July 1992 sampling.

Changes to bleaching sequence and installation of an air activated sludge secondary treatment system at the pulp mill between the January 1991 study and the July 1992 sampling resulted in a 42% reduction in the release of dioxins and furans in mill effluents. Between the January 1991 fish sampling program and the July 1992 sampling the pulp mill switched to 40% chlorine dioxide substitution for chlorine in the bleaching process, and began using hydrogen peroxide in the delignification process. Modernization was completed in June 1993 with the startup of the new mill, which has the capacity to produce 1200 ADtpd of kraft pulp. Although production capacity of the mill was more than doubled, loadings of resin/fatty acids, BOD, and AOX have been substantially reduced since start-up of the new facility in June 1993. The effluent has also been non acutely toxic to rainbow trout (96-hour LC50) during this time period.

The 1991 mountain whitefish study did not involve sampling downstream from Trail. The smelter/fertilizer plant located at Trail is undergoing a major modernization, and effluent quality has been improving since the 1992 mountain whitefish sampling was completed. Results presented here will serve as important baseline information for monitoring improvements in fish health and reductions in levels of some metals in response to these ongoing changes.

METHODS

FISH COLLECTION

Sampling for mountain whitefish was conducted at three river reaches between July 6th and July 17th 1992. The upper Slocan River, approximately 2 km downstream from Slocan Lake near the Passmore Bridge, served as the reference site. Mountain whitefish were also collected from the Genelle and Beaver Creek areas on the Columbia River (Figure 1). Efforts were made to angle whitefish, in order to avoid the possibility of damage to fish gills that can result from electroshocking (Boyle et al., 1992). Angling was reasonably successful on the Slocan River but not on the Columbia River, where electroshocking proved to be a viable alternative. Thus, one group of fish on the Slocan were angled and another group were electroshocked, so that possible effects of capture methods (bias for healthy/unhealthy fish respectively, and interference of electroshocking with histology results) could be identified to aid in comparisons between the three sites. Electroshocking was done at 2.5 to 4 A using a boom-mounted unit on a jet boat.

On the Slocan River forty-eight fish were angled, and an additional twenty-two whitefish were captured by electroshocking. Sixty-six fish were captured from Genelle (1 seined, 4 angled, and 61 electroshocked), and 60 fish from Beaver Creek were captured by electroshocking. All Slocan fish were captured during daylight. Most Genelle fish were captured at approximately 18:30 h local time. All Beaver Creek fish were captured at night (2100 - 2300 h).

Captured fish were held in a live tank on board the boat and delivered to pens immersed near the river banks. Fish between 26 and 37 cm in fork length were targeted for sampling, in an attempt to ensure similar age distributions among sampling reaches. At Genelle several larger fish were used due to a lack of smaller fish. Approximately equal numbers of males and females were used in each component of the study. Fish were killed by pithing. Fork length (+ 0.1 cm) was recorded and fish were weighed on a Mettler electronic balance. Fish were examined and sampled as described below for disease survey and analysis of contaminants. Stomachs were removed, weighed, and preserved in 10% formalin for gut content analysis. Liver, gonad, and gutted carcass weights were obtained for each fish. Otoliths were collected from all fish, and sent to the Fish Aging Lab at Pacific Biological Station (P.B.S.) in Nanaimo, B.C. for age determination. Although ages were determined with a high degree of confidence, no validation has been done using mountain whitefish of known ages.

GUT CONTENT ANALYSES

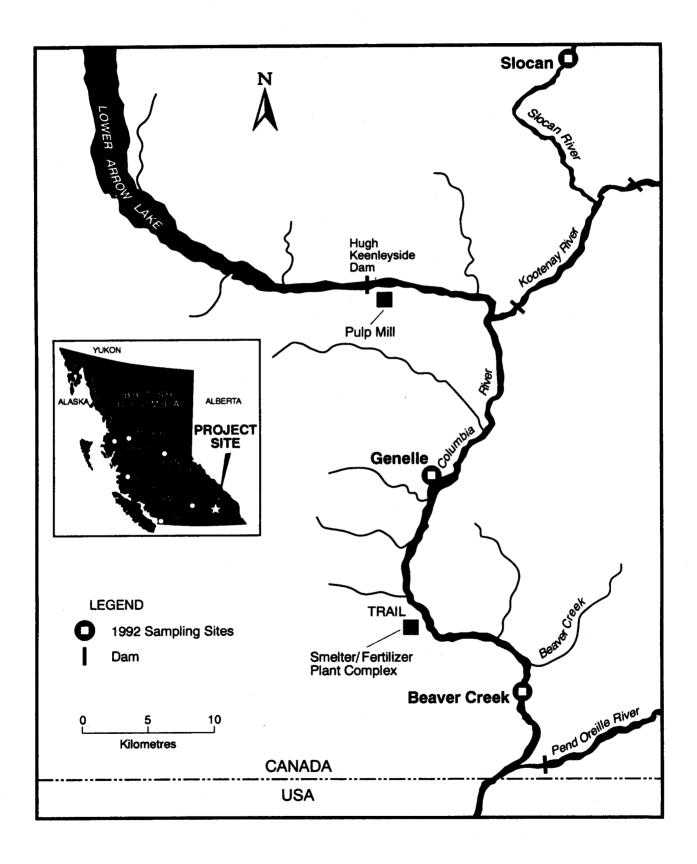
Preserved fish stomachs were shipped to Applied Technical Services (Saanichton, B.C.), where contents were identified, measured, counted, and weighed. Percent digestion was also estimated, as it has some bearing on the level of identification that can be reached, and on the lengths and weights of individual organisms.

In preparation for analyses, stomachs were removed from jars and the contents rinsed into a clean jar, containing 5% formalin. Contents were rinsed in a small-mesh Nitex sieve (150 μ m), blotted on filter paper, and weighed to the nearest milligram on an electric balance (OHaus).

Identifications were performed using a Wild M5A binocular dissecting microscope, and if necessary for smaller organisms or fragments, wet mounts were examined on a Wild M12 binocular compound microscope. Where a species new to the analyst was encountered, it was preserved in a reference collection.

Each organism was measured to the nearest millimeter using the eyepiece micrometer of the microscope. Measurements were from the front of the head to the end of the body and did not include antennae, setae, hooks, or other projections. When only part of an animal was present body length was extrapolated from measured organisms present in the sample.

FIGURE 1. Fish Collection Sites on the Columbia and Slocan Rivers.



Whole animals present in each sample were counted. Counting criteria are presented in Table 1. The body part used depended upon the degree of digestion, and in some samples separate counts of each part were made and the highest count used. If numbers of a single organism were greater than 100, a subsample of about 100 organisms was measured and counted, and remaining individuals of that species were counted, and length categories were assigned by extrapolation.

Each organism or length group was weighed to the nearest milligram. Where weight was less than 1 milligram estimates were obtained from known weights of a large number of a particular size category. Total weight of stomach contents recorded at the start of the procedure usually equalled the sum of the contents. When contents were heavily digested, estimated weights exceeded to original weight.

Each organism or group was categorized as benthic (B), surface or drift (D), or water column (C), or not at all (e.g. mucus). Benthic organisms include nymphs, larva, and pupae of aquatic insects that are buried or closely associated with the bottom. Those present in the water column include fish, corixids, etc., and those found on the surface include terrestrial organisms that fall in, or adults of aquatic insects that live on the surface film or are laying eggs or mating at the surface.

DISEASE SURVEY

The numbers of fish sampled and the disease testing methodology followed protocols outlined in the Fish Health Protection Regulations: Manual of Compliance (DFO, 1984).

Gross Examination

Fish were examined externally and internally. Any abnormalities were noted, and abnormal tissues were preserved in Davidson's solution for histological examination.

Bacteriology and Virology

Kidney tissue from each fish was cultured on tryptic soy agar plates for a minimum of five days. Selected bacterial colonies were identified using API 20E strips (API, St. Laurent, P.Q.), or using slide agglutination tests with commercially prepared antisera (Micrologix Inc., Victoria, B.C.) together with standard cytochrome oxidase tests, motility tests, and Gram stains. In addition, two smears of kidney were prepared from every fish. One smear was examined microscopically for bacterial and protozoal agents following Gram staining. The other smear was tested for the presence of mycobacteria by acid-fast staining.

Kidney tissue from 57 fish (# 31, 41-57, 91-110, 118, and 121-140) was tested for mycobacteria by culture on commercially prepared Lowenstein-Jensen (PML Microbiologicals, Richmond, B.C.) medium at 20°C for a minimum of six weeks. Positive acid-fast stain of bacterial colonies was used for confirmation of mycobacterial growth.

From each fish, tissue from gills, kidney, spleen, and pyloric caeca were collected and pooled. Pooled tissue from each fish was homogenized and tested for the presence of filtrable viral agents using the epithelioma papillosum cyprini (EPC) cell line and standard virus assay methods (DFO, 1984).

In addition to the above routine tests, material from any lesions observed was tested for the presence of infectious agents by culture and stained smears.

Parasitology

For each of the three sampling reaches, internal organs from 10 fish were collected, frozen, and submitted to the Parasitology Laboratory, P.B.S. for evaluation. Wet-mounts of liver, intestine, kidney, gall bladder, and urinary bladder were prepared and 50 fields were examined at 350x magnification to detect protozoan parasites. Helminths were identified after removal from tissues by the bicarbonate separation technique.

Histology

For each fish, tissue from the gills, liver, kidney, spleen, posterior intestine, and pyloric caeca/pancreas was collected and preserved in Davidson's solution. In addition, material from gross abnormalities was preserved. Tissues were stained with haematoxylin-eosin solution, and evaluated histologically. Special stains, such as acid-fast staining, were used where necessary.

Data Analyses

All meristic and fish health data were analyzed by Aquametrix Research Ltd. Analytical tests used included a combination of univariate descriptive statistics, distribution-based comparisons (ANOVA/regression, Chi-Square), and multivariate descriptive techniques. Statistical summaries and tests were completed using SIGMA-STAT. SIGTREE and CLUSTER (inhouse program package) were employed for the gut content analyses.

Prior to each ANOVA, tests for homogeneity of variance (Levene Median Test) and sample normality (Kolmogorov-Smirnov Test) were performed on the data. The ANOVA is considered robust with non-normal data, but requires that sample variances be equal between groups which are being compared. The parametric ANOVA approach was employed where both of the above tests resulted in a nonsignificant outcome, or when the Levene Median test demonstrated that variances were equal among groups. Where data failed both normality and heteroscedasticity tests a log₁₀ transformation was tried, followed by a square-root and arc-sin transformation. Failure to achieve homogeneity of variance resulted in use of ANOVA procedures using ranked data (Kruskal-Wallis).

ORGANIC CONTAMINANTS

Approximately 18 fish per reach were designated in the field for determination of organic contaminants. These fish were sampled for histopathological parameters as described above, and following removal of internal organs, selected fish were sealed individually inside a contaminant-free plastic bag, and frozen on dry ice. Livers from approximately 18 mountain whitefish were frozen in individual clean containers on dry ice within approximately 2 minutes of death, for subsequent analysis of Mixed Function Oxidases (MFOs). Each liver was weighed and a small piece of tissue was removed for histology, prior to freezing.

Upon completion of field work, a subsample of 12 to 14 fish from each reach were selected for analyses. Fish were selected on the basis of age and sex, with the objectives of obtaining a similar age distribution in the subsamples from the three reaches, and a 1:1 sex ratio for each reach.

Fish muscle was homogenized at the Institute of Ocean Sciences (I.O.S.) ultra-trace contaminants laboratory in Sidney B.C. using ultra clean protocols which are standard in preparing samples for analysis of organic contaminants. Homogenized tissues, including four blind duplicates, were frozen and delivered to AXYS Analytical Laboratories. Tissues were analyzed using High Resolution GCMS for dioxins and furans. In addition, full analyses of polychlorinated biphenyls (PCBs) were performed, including monoortho substituted and non-ortho substituted (coplanar) PCBs (congeners 77, 126, and 169). As an additional Quality Control/Quality Assurance (QA/QC) measure, five samples were also analyzed at I.O.S. for comparison of the dioxin, furan, and PCB results between the two laboratories

Toxic equivalence factors (TEFs) for dioxin and furan congeners were used to calculate Toxic Equivalents (TEQs) in order to obtain an estimate of toxic body burden of all the dioxin and furan congeners which exert similar effects upon organisms (Table 2). The TEFs used in this study were obtained from NATO, 1988, and relate toxicity to the most toxic of the dioxin and furan congeners, 2,3,7,8-T4CDD.

TEFs were also used to calculate toxic equivalents (TEQs) for some PCB congeners as a means of standardizing their toxicity relative to that of 2,3,7,8-T4CDD, as these congeners affect biota through similar mechanisms (Ahlborg et al., 1994; Walker et al., 1991). There is currently no internationally accepted standard for PCB TEFs. The TEFs used (Table 3) were obtained from Ahlborg et al. (1994). They were developed by an international panel and were based on data contained in 57 articles or manuscripts which involved study of PCB congeners with a T4CDD or PCB reference. Detection limits available at the analytical laboratory were in the same order of magnitude for Non-Ortho Substituted PCBs as for dioxins and furans (pg/g), however detection limits for all other PCBs were three orders of magnitude less sensitive (ng/g).

Data Analyses

Data for organic contaminant concentrations were analyzed with SYSTAT (Windows version) using univariate descriptive statistics. Relationships between contaminant concentrations and other parameters such as age and CDS were explored using ANOVA following the procedure outlined above for meristic and fish health parameters.

MIXED FUNCTION OXIDASES (MFOs)

Livers were frozen on dry ice and shipped to the Freshwater Institute in Winnipeg for analysis of ethoxyresorufin O-deethylase (EROD), benzpyrene hydroxlase (also called aryl hydrocarbon hydroxylase (AHH)), and cytochrome P-450 using procedures described previously (Boyle et al., 1992). MFO data were transformed to logarithms and means were compared among locations using covariance analysis of the General Linear Models procedure of SAS.

METALS IN MUSCLE TISSUE

Muscle tissue from a total of 51 mountain whitefish from the three sampling reaches was analyzed for trace metals. Following sampling for histology, and removal of internal organs, fish carcasses were placed individually in contaminant-free plastic bags. Tissues from these 51 mountain whitefish were analyzed for trace metals. Fish carcasses were shipped to the contracting laboratory (Quanta Trace Laboratories, Burnaby, B.C.) where samples of muscle were removed from the right side equidistant from the anterior and posterior borders. Portions were trimmed so that no exposed flesh was included in the analysis. The entire sample was oven dried (55 °C) and percent moisture determined. Dried samples were ground and portions (approx. 0.5 g) were weighed and transferred to teflon vessels for microwave-enhanced digestion in nitric acid. Thirty elements were determined by inductively coupled plasma spectrometry. Mercury was determined by cold-vapor atomic absorption spectrometry.

Livers were excised from 45 (15 from each site) of the original, freshly killed fish and frozen to -80 °C for return to the Ocean Chemistry Division Laboratories at the I.O.S. Metals (cadmium, copper and zinc) in liver tissue were determined by flameless atomic absorption spectrometry after microwave-enhanced nitric acid digestion of tissue homogenates and cytosols. Metal-binding proteins (MBP, metallothioneins) were determined in heat-denatured cytosols by the method of Thompson and Cosson (1984) with the following modifications: 1. cell temperature was maintained at 5.0 + 0.5 °C; and, 2. the concentration of the hexamminecobalt catalyst in the electrolytic buffer was increased to 80 mg·L⁻¹. Total protein content of the cytosols was determined according to Bradford (1976) prior to heat denaturation.

For QA/QC of the liver analysis the following measures were taken: Three of the liver samples were taken for triplicate analysis. Each sample was carried through the entire procedure. In addition, one sample (#155) was triplicated for whole-tissue analysis only. During each day's sample processing, blank aliquots of the homogenization buffer (50 mM TRIS, pH 7.5 with one mM dithiothreitol as an antioxidant) was treated identically to the liver samples, including the homogenization and all subsequent sample division and treatment steps. In total, eight heat-denatured cytosol blanks and seven tissue metal blanks were run. Metal detection limits were determined for each matrix as 3x the standard deviation of the blank signals. Metal recoveries for the method were determined using the dogfish liver reference material, DOLT-1 (NRC Canada).

Statistical analysis of the muscle and liver data was accomplished by use of the PC-based software, Statistical for Windows (Statsoft, Tulsa, OK). All analysis was performed on \log_{10} - transformed values of the dry-weight data. Details of the analysis are provided in the text or in tables.

FISH HEALTH PARAMETERS - RESULTS, INTERPRETATION, AND DISCUSSION

Biometric Data

Of the 70 fish sampled from the Slocan River site, fish #408 was not used for health summaries as histology was not done. Fish numbers 501 - 505 inclusive were not used for health summaries of the Genelle reach as histology was not done on these samples. All 60 fish sampled from the Beaver Creek reach were used in health summaries.

Biometric data are summarized in Table 4 which provides mean age, fork length, wet weight, condition index, gutted weight, stomach weight, gonad weight, gonadosomatic index, liver weight, and hepatosomatic index, from each sampling reach. Appendix 1 provides data for these parameters for individual fish, and also identifies the sex where known.

The average age of fish sampled from the Slocan River was 6.3 years with a range of 2 to 18 years. Genelle fish had an average age of 13.6 years, and ranged between 3 and 23 years. Fish from Beaver Creek averaged 7.5 years, with a range of 2 to 20 years. Analysis of Variance and Multiple Range tests revealed that the mean ages of fish sampled from the Genelle site were significantly greater than the mean ages of fish sampled at Beaver Creek or the Slocan River reference site (P<0.001, G>B=S) (Cross, 1994). Age distributions for fish from the three reaches are shown in Figure 3.

Fish sampled at the Slocan River had a mean fork length of 273.7 mm, with a range of 194-370 mm. Fish from the Genelle sampling reach were larger on average, with a mean fork length of 353.3 mm, and a range from 260 mm to 380 mm. A range in fork length of 260 mm to 380 mm, with of mean of 324.8 mm, was obtained for fish sampled at Beaver Creek. Mean fork length differed significantly among fish sampled from the three sites. Genelle and Beaver Creek fish were found to have similar mean fork lengths. Fish sampled from the reference site had significantly lower mean fork lengths than the Columbia River fish (2-way ANOVA, with sex as Factor 2) (Cross, 1994). Fork length was determined to be a poor predictor of age, confirming findings of Boyle et al. (1992).

Fish collected from the Slocan River had a mean weight of 237.5 g, with a range of 76 g to 606 g. The mean weight of Genelle fish was 466.0 g with a range of 212 g - 878 g. Fish from the Beaver Creek area had a mean weight of 449.2 g and ranged from 250 g - 689 g. Mean weight was significantly greater at the Genelle and the Beaver Creek reaches compared with fish sampled from the reference site (2-way ANOVA with sex as Factor 2) (Cross, 1994).

Condition Index and Hepatosomatic Index

Condition index was calculated as (Weight x Length³) x 10⁵ (Boyle et al., 1992). The mean condition index of fish sampled at the Slocan River was 1.09. The mean condition index of fish sampled at Genelle and Beaver Creek was 1.05 and 1.33, respectively. Analysis of Variance and Multiple Range tests demonstrate that the mean condition index differs significantly between sites. Mean condition index values from the Slocan River fish and the Genelle fish are similar, while the mean condition index of the Beaver Creek fish is significantly higher (P<0.001, ANOVA, Tukey Multiple Comparisons).

A hepatosomatic index (HSI) was calculated as a ratio of liver weight to total body weight (Goede and Barton, 1990). Increases or declines in the HSI may indicate exposure to certain types of stressors such as pollutants (Goede and Barton, 1990). The mean HSI of fish collected from the Slocan River was 0.6. The mean HSI of fish collected from Genelle and Beaver Creek were 0.7 and 0.9 respectively. No significant difference in mean hepatosomatic index was found between any of the sites sampled (P=0.057, ANOVA).

The condition index measures the overall "plumpness" of a fish, and interpretation assumes that healthy fish of a given species will have a greater body weight per body length than unhealthy fish. Although it was noted that Genelle mountain whitefish generally had less body fat than fish sampled at the Slocan River, and a greater proportion of fish sampled from the Genelle site were considered thin compared with Slocan fish, mean condition index values from fish sampled at the Genelle and Slocan River sites were not significantly different. The mean condition index value from fish sampled at the Beaver Creek site was significantly higher than Genelle and Slocan. The significant difference between condition indices for Genelle and Beaver Creek fish may reflect the fact that fish from the two sites did not differ significantly in fork length, but that more fish from Genelle were considered to be "thin".

There is some question as to whether "thin" should be classified as an abnormality, or a variation in body form. Mountain whitefish with a "snake-like" body form have been observed elsewhere such as in the Fraser River system and not necessarily in areas impacted by pollution (D. McPhail, U.B.C., personal communication). Plots of length vs weight data for Columbia River fish did not fall into two groups which would have suggested two distinct morphologies. When thinness is eliminated as an abnormality there is no significant difference (P=0.05) in mean severity of external abnormalities (see below) among sites.

Condition and hepatosomatic indices can be used as indicators of general physiological health. The condition index and HSI may not accurately reflect differences in health between sites in this study because of differences in mean age, weight, and length found between sites. These indices may be more appropriate to measure changes over time at one site, or between populations of similar age and fork length.

Gonadosomatic Index

A gonadosomatic index (GSI) was calculated as a ratio of gonad weight to total body weight (Poels et al., 1980). It provides a measure of gonad development or ripeness. The mean GSI of Slocan River fish was 1.1. The mean GSIs of fish collected from the Genelle and Beaver Creek reaches were 1.7 and 2.7, respectively. The GSI of Beaver Creek fish was significantly higher than that of Genelle or Slocan River fish, which were similar to one another (P<0.001, ANOVA, Tukey Multiple Comparisons). This difference may be a reflection of the 6.7% of Beaver Creek fish which were ripe, as compared with no ripe fish noted at the other two sampling reaches.

Gut contents

Fish collected from the Beaver Creek area had slightly greater stomach weights and were slightly fuller than those from either Genelle or Slocan. The level of digestion of stomach contents was not significantly different (P=0.56) between the three reaches even though fish were collected at different times of the day (Cross, 1994).

Results of cluster analysis of the three survey reaches, based on individual prey taxa abundances, are presented in Figure 2. This figure illustrates that the Slocan and Beaver Creek fish have similar composition of gut contents (81.5%), and that the degree of similarity is 39.3% for both of these reaches in comparison with fish from Genelle reach. No further detailed analyses of gut content information was performed.

Results indicate that fish from all sampling locations were feeding. The consultant performing the gut content analyses reported finding shiny, metalic-looking material in the guts of fish from the Beaver Creek reach. This material is likely slag which is currently discharged from the lead-zinc smelter at Trail into the Columbia River. Slag contains metals, including copper and zinc, which vary in concentration in each batch of slag (Nener, 1992). Whitefish are bottom feeders, and slag particles were likely ingested either incidentally with food items, or as part of invertebrate cases. In future field programs it may be of value to collect some benthic invertebrates from the Beaver Creek area so that cases can be examined. No intact cases were observed in gut contents, however they may have been broken down by the digestive process.

TABLE 1. Identification and Enumeration Crtiteria for each Taxon and Life Stage obtained from Gut Contents.

Taxa	Life Stage	Identification Criteria	Enumeration Criteria	Level of Identification
Nematoda	n/a	n/a	n/a	usually parasitic and not counted
Trematoda	n/a	n/a	n/a	parasitic and not counted
Hydracarina	n/a	general	bodies	suborder
Ephemeroptera	nymph	head and gills	heads, eyes, cerci	genus (species)
	adult	head and wings	heads, wings	genus (usually too badly damaged)
Plecoptera	nymph	head, thorax, cerci	heads, cerci	genus
	adult	head and wings	heads, wings	genus
Homoptera	adult	general	heads	family (usually terrestrial)
Heteroptera	adult	head, wing cases	heads	genus
Chironomidae	larva	head capsules	heads	subfamily
	pupa	exuviae	head + thorax	genus
	adult	wings, legs	head + thorax	subfamily
Simulidae	larva	head capsule	head	genus
	pupa	case	case	genus
Other Diptera	larva	head capsule	head	family (genus if possible
	pupa	case	case	and if aquatic)
	adult	wings	head, wings	
Hymenoptera	adult	head, wings	head	family
Coleoptera	larva	head, legs	head	genus
	adult	head and elytra	head, elytra	genus if aquatic, family if terrestrial
Lepidoptera	larva	head	head	order
Trichoptera	larva	head, thorax, hooks	head	genus (species)
-	pupa	exuviae	head	genus (species)
	adult	head, thorax, wings	head	genus (species)
Mollusca	n/a	complete shell	shell	genus
Fish	n/a	complete or partial skeleton	bones	family

TABLE 2. International Toxicity Equivalency Factors (TEFs) for Dioxins and Furans.

	Dioxin/Furan	Toxic Equivalency Factor
	2,3,7,8-tetrachlorodibenzodioxin	1
	1,2,3,7,8-pentachlorodibenzodioxin	0.5
	1,2,3,4,7,8-hexachlorodibenzodioxin	0.1
	1,2,3,7,8,9-hexachlorodibenzodioxin	
	1,2,3,6,7,8-hexachlorodibenzodioxin	
	1,2,3,4,6,7,8-heptachlorodibenzodioxin	0.01
	octachlorodibenzodioxin	0.001
	2,3,7,8-tetrachlorodibenzofuran	0.1
	2,3,4,7,8-pentachlorodibenzofuran	0.5
v	1,2,3,7,8-pentachlorodibenzofuran	0.05
	1,2,3,4,7,8-hexachlorodibenzofuran	0.1
	1,2,3,7,8,9-hexachlorodibenzofuran	
	1,2,3,6,7,8-hexachlorodibenzofuran	
	2,3,4,6,7,8-hexachlorodibenzofuran	
	1,2,3,4,6,7,8-heptachlorodibenzofuran	0.01
	1,2,3,4,7,8,9-heptachlorodibenzofuran	
	octachlorodibenzofuran	0.001

After NATO, 1988.

TABLE 3. Interim Toxic Equivalent Factors (TEFs) relating PCB Toxicity to 2,3,7,8-T4CDD.

Туре	Cong	gener	
	IUPAC No.	Structure	TEF
Non-ortho (Coplanar)	77	3,3',4,4'-TCB	0.0005
	126	3,3',4,4',5-PeCB	0.1
	169	3,3',4,4',5,5'-HxCB	0.01
Mono-ortho	105	2,3,3',4,4'-PeCB	0.0001
	114	2,3,4,4',5-PeCB	0.0005
	118	2,3',4,4',5-PeCB	0.0001
	123	2',3,4,4',5-PeCB	0.0001
	156	2,3,3',4,4',5-HxCB	0.0005
	157	2,3,3',4,4',5'-HxCB	0.0005
	167	2,3',4,4',5,5'-HxCB	0.00001
	189	2,3,3',4,4',5,5'-HpCB	0.0001
Di-ortho	170	2,2',3,3',4,4', 5-HpCB	0.0001
	180	2,2',3,4,4',5,5'-HpCB	0.00001

After Ahlborg et al. (1994).

DISEASE SURVEY: RESULTS AND INTERPRETATION

In order to interpret the fish health significance of any of the abnormalities found (external, internal, bacterial, histological, and occurrence of parasites), a coding system was developed which subjectively rated the abnormalities as to their potential impact on fish health (Appendix 2). This rating system is very loosely based on quantitative, autopsy-based systems of fish health assessment, developed to provide a simple and inexpensive means of rapidly assessing general fish health in field situations (Goede and Barton, 1990; Adams et al., 1993). An abnormality such as a parasite cyst was rated light (1), reflecting the small impact it is likely to have on fish health. The impact of causative organisms such as myxobacteria was considered to be moderate (2), while the finding of mycobacteria is considered more damaging, and was rated as severe (3). The normal condition was given a rating of (0). The total numerical value of all abnormalities found in one fish at a given location was used to indicate the health status of that fish, and is referred to as the Cumulative Disease Severity (CDS).

Gross Examination

Results of the gross examination of each fish are provided in Appendix 1. A summary of the total number of abnormalities found and the cumulative disease severity is provided in Appendix 2, and the severity rating codes used are also indicated here.

A sample of mountain whitefish received by the Department of Fisheries and Oceans in October, 1990, from consultants working on the Columbia River, were unusually thin and had external lesions and discolouration. In a preliminary study of fish health parameters conducted by DFO in January, 1991, a few mountain whitefish sampled from the Columbia River at Genelle and Trail also had these abnormalities (Boyle et al., 1992).

Gross External Abnormalities

Results of the gross examination of each fish are provided in Appendix 1. Two fish each from the Slocan River and Genelle had hemorrhagic spots on the belly, and one each of Genelle and Beaver Creek fish had signs of mechanical damage.

Of the Genelle (G) and Beaver Creek (B) fish, 34.4% and 20.0% respectively, appeared to be thin (27% of the combined Columbia River fish), compared with 4.4% at the reference site. External examination revealed that 9.8% and 15.0%, of the Genelle and Beaver Creek fish, respectively, had dark gills in comparison with only 2.9% of Slocan fish. The total number of external abnormalities at each site was: 12 at the Slocan River (S) (17.4%), 33 at Genelle (54.1%), and 28 at Beaver Creek (46.7%). Significant differences in mean severity of external abnormalities were found among the three sites (S<B<G) (P< 0.001, ANOVA) (Cross, 1994).

In a study carried out in March 1993 on mountain whitefish collected from Genelle, Trail, and Beaver Creek, external abnormalities including emaciation, fin erosion, hemorrhaging at the base of the fins, and external lesions were found (Roome, 1994). The above abnormalities were also observed in low frequencies in mountain whitefish, rainbow trout (Oncorhynchus mykiss), and three species of sucker (Catostomus sp.) sampled from a similar section of the Columbia River in 1990 - 1992 (R.L. & L. Environmental Services Ltd., 1992). Exposure to lethal and sublethal concentrations of bleached kraft pulp mill effluent (BKME) has been shown to affect various tissues in a number of species, and fin erosion has been one of the most consistently described effects. Fin erosion has been found in rainbow trout, perch (Perca fluviatilis), pike (Esox lucius), winter flounder (Pseudopleuronectes americanus) and three-spined sticklebacks (Gasterosteus aculeatus) (Lindesjöö and Thulin, 1990; Lehtinen, 1989; Couillard et al., 1988; Sindermann, 1979) following exposure to BKME. Chronic exposure of minnows (Phoxinus phoxinus) to zinc and cadmium also resulted in fin damage similar to that produced by pulp mill effluents (Sindermann, 1979).

Hemorrhaging at the base of the fins can be due to a number of causes. It has been described as an associated abnormality in some species of fish exposed to pulp mill effluent or metal contaminants, however in our July 1992 study it was found with the greatest frequency in fish sampled at the reference site. No other external abnormalities described in the literature as possible effects of contaminant exposure were noted in any of the fish examined in the July 1992 study.

Gross Internal Abnormalities

The total number of gross internal abnormalities noted at each site was: 18 at the Slocan River (26.1 %), 24 at Genelle (39.3 %), and 18 at Beaver Creek (30.0 %). Of the Genelle and Beaver Creek fish, 16.4% and 11.7%, respectively, had an enlarged spleen, compared with 5.8% at the Slocan River. Three fish at the reference site had lumpy spleens. Three fish sampled at the Slocan River, and one fish at Beaver Creek, had pale livers. Two Genelle fish were noted as having dark brown livers. Of the 61 fish sampled at Genelle, four had liver lesions, and two had internal hemorrhagic areas. A few Genelle fish had cysts in the liver, kidney, spleen, and ovary. In Beaver Creek fish, internal adhesions, hemorrhagic areas, and cysts in the kidney and ovary were noted. At the Slocan River a few fish had cysts in the spleen, pyloric caeca, and ovary.

Statistical evaluation of gross internal abnormalities revealed no significant differences in mean severity among the fish sampled from the Columbia River sites and the reference site (Table 5) (Cross, 1994). This is similar to results of the baseline monitoring study in January, 1991, in which no apparent differences in gross abnormalities were found among the four collection sites. Enlarged spleens were found to be more prevalent in fish sampled at the Genelle and Beaver sites compared with the Slocan River.

Bacteriology

Table 6 summarizes the bacteriological results for the three sites and a statistical evaluation of data is present in Table 5. Myxobacteria were detected in 5 of 69 fish from the Slocan River (7.3 %), and in 6 of 61 (9.8 %) fish sampled from Genelle. No myxobacteria were detected in any of the Beaver Creek fish.

Mycobacteria were found in fish at all three sites: 5 of 69 (7.3 %) fish at the Slocan River, 9 of 61 (14.8 %) at Genelle, and 4 of 60 (6.7 %) at Beaver. Only one other bacterium known to be pathogenic to fish was isolated. *Yersinia ruckeri*, the causative agent of enteric redmouth disease (ERM), was isolated in one fish from the Slocan River.

A lower prevalence of myxobacteria was found in fish sampled from Genelle (9.8 %) and Beaver Creek (0 %) than was found in January of the previous year, in which 17.7 % of fish sampled at the Columbia River sites were infected. Myxobacterial infections tend to be seasonal with the highest frequency of outbreaks occurring during the colder months. For the 1992 sample, there was a significant difference in frequency of myxobacteria and other bacteria between the sites, which could be related to a difference in water quality. Mycobacteria were isolated from fish sampled at all three sites, and all three sites showed an increase in the number of mycobacteria-positive fish in comparison with the January 1991 samples. This indicates that as speculated in 1991, fish heavily infected with mycobacteria may have been eliminated from the population prior to the January sampling, and the summer infection level may be closer to the July, 1992 rate. The 1992 finding is also consistent with the January, 1991 conclusion that more fish downstream of Castlegar were infected with mycobacteria, than at the reference site (Boyle et al., 1992). Both myxo- and myco- bacteria are opportunistic rather than primary pathogens, and like other infectious agents may cause disease if the environmental conditions are right (Snieszko, 1974).

Virology

All pools of tissue remained free of cytopathic effects which would indicate the presence of a virus infection in the fish.

Parasitology

Table 7 summarizes the results of the examination for selected parasites. No parasites of any significance including myxosporeans, microsporideans, or *Diphyllobothrium* cestodes were found in fish from any of the three sampling reaches. At the two sampling sites on the Columbia River, the following trematodes were recovered: *Plagioporus shawi* from the intestine in all ten fish examined from both Genelle and Beaver Creek, and *Crepidostomum farionis* was found in the gall bladder in 1 of 10 fish examined at the Beaver site. The following nematodes were recovered from Genelle and Beaver Creek: encapsulated *Philonema agubernaculum* in 1 of 10 fish at both sites, *Rhabdochona sp.* in the intestine in 1 of 10 fish at both sites, and *Truttaedacnitus truttae* from the intestine in 8 of 10 fish from both sites.

In the Slocan River sample, the trematode *Crepidostomum farionis* was recovered from the intestine in 1 of 10 fish examined. *Eustrongylides sp.* larvae, (a nemarode) encapsulated on the stomach, were found in 3 of 10 fish, and *Truttaedacnitis truttae* was recovered from the intestine in 5 of 10 fish examined.

Except for the detection of Sanquinicola in the gills of a single fish from Beaver Creek, no parasites of any fish health significance including myxosporean and microsporean parasites, were found in fish from any site in the July, 1992 study. The findings from Genelle and Beaver Creek were essentially the same, but the Slocan River fish differed slightly. The sample sizes (n=10 fish for parasitology at each of the sampling reaches) were too small to be confident that there is a real difference between sites. The Slocan River was the only location in which fish carried Eustrongylides sp., and the only reach where fish did not carry Plagioporus shawi, a parasite found in the other two groups of fish examined. The finding of Plagioporus at the Columbia River sites is of interest because this parasite has usually been found in Oncorhynchus species and not in Prosopium species. None of the trematodes and nematodes noted were present in unusual numbers.

Fewer protozoan parasites were found in the gills of fish sampled from Genelle and Beaver Creek than were previously found in fish sampled from the Columbia River in January, 1991. A correlation between environmental stress, gill damage, and parasite frequency at low concentrations of pulp mill effluent has been described in the literature for a number of species. European flounder exposed to BKME were found to be infested by a ciliate (*Trichodina sp.*). This parasite is found in many wild fish in an ectocommensal state in which it does not harm the host. In its parasitic state it feeds on the gill epithelial cells of the host, stimulates mucus production, and damages the epithelium (Lehtinen et al., 1984). An increase in parasite frequency was also noted in perch exposed to sublethal concentrations of pulp mill effluents. Parasite cysts, identified as *Oodinium sp.*, were found between the secondary lamellae in all exposed fish, however, the lamellae appeared normal, even in the most heavily afflicted (Lehtinen and Oikari, 1980). Adams et al. (1989) indicated that high parasite infestations in fish exposed to effluents may indicate immune system dysfunction.

Histology

A summary of the total number of abnormalities and the cumulative disease severity found in each organ for fish from the three sampling reaches is provided in Appendix 2. A statistical evaluation of the mean disease severities determined from histological analyses of the organs and tissues examined are presented in Table 5. Frequencies of abnormalities for each organ are also provided.

Liver

The frequency of liver abnormalities identified through histology was 70.5% for Genelle fish, 45% for Beaver Creek fish, and 8.7% for fish from the Slocan reference population. Genelle fish had more severe liver abnormalities than fish sampled from Beaver Creek, which in turn had more severe abnormalities

than fish from the reference site. As well, the Genelle site had many more fish with multiple abnormalities than the Beaver Creek site or the Slocan River site. The types of severe liver abnormalities found at Genelle included inflammatory foci, foamy areas, cirrhosis, and tumours.

Changes in liver tissue of fish exposed to BKME have been described in the literature. In European flounder hepatocytes showed nuclear condensation and cytoplasmic vacuolization to varying degrees, from focal to massive distribution, and an increase in the number of macrophages was thought to indicate an increase in dying hepatocytes (Lehtinen et al., 1984). In sticklebacks, hepatocytes stained poorly and showed nuclear pyknosis (Lehtinen, 1989). An increase in liver size is another abnormality noted in a variety of species exposed to toxic compounds and is attributed to hyperplasia and hypertrophy (Adams et al., 1989). None of the abnormalities noted above were detected in the present study. Another study also found no histological lesions in the liver, spleen, kidney, stomach, intestine, and pancreas in rainbow trout exposed to untreated BKME (Couillard et al., 1988).

There was a marked increase in prevalence of liver abnormalities detected by histology in fish sampled from the Columbia River in July, 1992. In January, 1991, 5.9% of fish collected on the Columbia River downstream from Castlegar showed areas of inflammatory foci in the liver. In July, 1992, the prevalence of inflammatory foci in the liver was 42.6% and 18.3% of fish sampled from the Genelle and Beaver Creek sites respectively. The prevalence at the Slocan site was only 7.2%, and is similar to the rate of 6% observed in fish from the reference sites of January, 1991. This difference in frequency of liver inflammation between the two years may be due to seasonal differences in levels of irritants in the Columbia River causing inflammatory response. Inflammation is the vascular and cellular response of living tissue to injury caused by living or non-living agents. Bacteria, viruses, fungi and parasites, as well as physical and chemical injuries, including toxins and mechanical damage, can cause the fish to respond to the damage by mounting an inflammatory response (Thomson, 1978). The large number of fish sampled at Genelle and Beaver Creek showing an inflammatory response in the liver may indicate that more fish at these sites are responding to injury or irritants than those at the reference site.

Fish sampled at Genelle and Beaver Creek had melanosis/hemosiderin deposits in the liver at a rate of 13.1% and 15.0%, respectively. This is an increase in occurrence over the January, 1991 study in which 5.88% of the Genelle fish had melanosis in the liver. Melanosis is the presence of melanin in an abnormal location, and macrophages that pick up granules of melanin may be numerous in melanosis (Thomson, 1978). A pronounced accumulation of hemosiderin in one area of a tissue may be an indication of a previous hemorrhage (Thomson, 1978).

Other liver abnormalities found in Genelle fish, which were not noted in the 1991 study, were the presence of foamy areas (13.1%), and cirrhosis (4.9%). Cirrhosis is the outcome of prolonged hepato-cellular injury, and may be seen occasionally in older fish (Roberts, 1978). The Genelle fish had the highest number and most severe liver abnormalities. This site also had more fish with multiple liver abnormalities than the Beaver Creek or the Slocan River sites.

Kidney

The frequency of kidney abnormalities identified through histology was 19.7% for Genelle fish, 18.3% for Beaver Creek fish, and 1.5% for fish from the Slocan reference population. Statistical evaluation of the mean severity of kidney abnormalities showed Genelle and Beaver Creek sites to be similar in severity, and both sites to be greater than the reference site in mean severity (Table 5) (Cross, 1994). The types of severe abnormalities found at higher rates in fish sampled from Genelle and Beaver were: granulomas, interstitial hypercellularity, tumour, and lymphocystosis.

In the mountain whitefish sampled in July, 1992, unusual melano-macrophage activity in the kidney was found in only one fish sampled from the Beaver Creek site. This is not consistent with the finding in January, 1991, where most fish from the Genelle site exhibited this abnormality. This indicates a less severe inflammatory response in the kidneys of fish sampled in the later study.

Abnormalities such as lymphocyte infiltration, granulomas, and melano-macrophage activity are part of the inflammation and repair mechanism. Their underlying cause is considered to be capable of causing a severe impact on fish health, at least while lesions are present. Once the causative factor has been overcome, the lesion can be repaired unless damage is too massive and the fish succumbs to further problems (Thomson, 1984).

Hind Gut and Pyloric Caeca

Several abnormalities (granulomas, foamy lesions in the pancreas, hypercellular submucosa, lymphoid infiltration, and tumor) of the pyloric ceaca and hind gut were found with greater frequency at Genelle than at Beaver Creek or the Slocan site (Table 5).

Gill

Gill tissue has been described as the primary target of untreated bleached mill effluent at lethal concentrations, and exposure of rainbow trout to this effluent has caused a fusion of the gill lamellae. Cellular hyperplasia was found after the trout were exposed to sublethal levels of effluent for forty days (Couillard et al., 1988). In European flounder (*Platichys flesus*) the effects of BKME on gill tissue included a diffuse epithelium with detachment from the basal membrane occurring at higher effluent concentrations (Lehtinen et al., 1984). Gross changes in the gill similar to those produced by exposure to BKME were found in copper poisoning of winter flounder, and rainbow trout exposed to zinc sulphate showed damage to the gill epithelium (Baker, 1969; Skidmore, 1970). In the July, 1992 study, lamellar clumping was only found in one fish from the Beaver Creek site. No focal hyperplasia was noted in fish from any site, and gill aneurysms were found in similar prevalences at Genelle and the reference site.

Overview of Cumulative Disease Severity (CDS)

Table 5 summarizes the Analysis of Variance results of the mean CDS of each organ examined. It shows that among the three sites there were significant differences in mean severity of abnormalities in the liver, kidney, pyloric caeca, and hind gut (P<0.05, ANOVA). Multiple Range tests revealed that fish sampled from the Genelle site had a greater mean severity of abnormalities than fish sampled from the other sites. Fish sampled from the Slocan River had equal or less mean severity of abnormalities than fish from the Beaver Creek reach. Overall, the CDS was found to be greatest in fish collected from Genelle, and Beaver Creek fish had a greater CDS than Slocan River reference area fish.

During the 1992 study, the severe abnormalities found in the kidneys, pyloric caeca, and hindgut of fish sampled from Genelle and Beaver Creek were interstitial hypercellularity, tumour, and lymphocytosis. Hyperplasia occurs in response to a loss of tissue, increased functional demands, or disturbed hormonal activity. Hypercellularity is defined as an abnormal increase in the number of cells present, and can occur in response to damage at other sites.

For the most part specific types of abnormalities found in these organs from fish sampled in July, 1992, were similar to those found in January, 1991. Increased numbers of granulomas in the liver, kidney, and pyloric caeca were found in fish sampled at the Columbia River sites compared with the Slocan River, where no granulomas were found in any organ examined. Granulomas represent a prolonged struggle between the fish and a material which cannot be easily eliminated (Thomson, 1978). Examples of granuloma-inducing agents are parasites or chronic bacterial infections (Boyle et al., 1992).

FISH AGE AND DISEASE SEVERITY

The average age and age range of the fish populations sampled differed between the sampling reaches. Genelle fish were significantly older than fish from Beaver Creek and the Slocan River. Fish from the latter two reaches were similar in age and had similar age distributions (Figure 3). Genelle fish had an average age of 13.6 years and ranged between 3 and 23 years. The average age of fish sampled from the Slocan River was 6.3 years with a range of 2 to 18 years. Fish from Beaver Creek averaged 7.5 years, with a range of 2 to 20 years.

With increasing age, the number of abnormalities, such as parasitism, generally increase in fish as in other animals. This was shown in the preliminary fish health investigation of Columbia River mountain white-fish in January, 1991 (Boyle et al., 1992). In the present study a significant linear relationship (P<0.01) was found between fish age and cumulative disease severity at both the Genelle and Beaver Creek sites (Figure 4). The lack of an age-CDS relationship at the Slocan River site is likely related to the higher proportion of younger fish sampled at this site (Cross, 1994), and the small number of older fish obtained here.

An among-station comparison of CDS and age was done on the subset of fish 0-12 years old to determine if there were differences in this relationship between sites for this age group. ANOVA results indicate that the age-CDS relationship was not significant at any of the three sites (Cross, 1994) for this young subset.

The lack of representation of older fish groups at the reference site does not allow for a statistical comparison of mean severity of abnormalities with Genelle fish, because of the relationship between fish age and CDS. The differences in CDS between Beaver Creek and Slocan fish can be meaningfully compared, however, as there was no significant difference in age for fish from these two sites.

Within all age categories, including the youngest (1-5 years), the proportion of fish with abnormalities is consistently higher in the Columbia River fish in comparison with Slocan River fish (Table 8). Of the 12 Genelle fish in the youngest age group (1-5 years), 83% were classified as having at least one abnormality, in comparison with only 50% of Slocan fish and 68% of Beaver Creek fish in the same age group. Of the 6-10 year old age group, 14 of 23 (61%) Slocan River fish showed some kind of abnormality, while 80% of Columbia River fish had some kind of problem (12 of 15 fish). Although sample sizes within age groups are too small to permit reliable use of statistics, the data suggest that fish in the Columbia River do have a greater prevalence of abnormalities in all age groups.

In summary, fish sampled at Genelle were significantly older, had consistently greater frequencies of abnormalities, and significantly more severe abnormalities in several tissues than fish sampled from the Beaver Creek area and the Slocan River. Because of the significant relationship between age and disease severity, however, it is difficult to separate differences in occurrence and severity of abnormalities due to different environmental conditions from the natural effects of aging. The overall increased inflammatory response found in Genelle fish may be due to the length of time that older fish were exposed to irritants, but a direct comparison with older fish from a reference site is necessary. Beaver Creek fish were found to have more severe liver and kidney abnormalities, and a higher overall CDS than similarly aged Slocan fish. A major function of liver is detoxification of chemicals, therefore the high prevalence of liver abnormalities in Beaver Creek fish relative to fish of the same mean age from the Slocan reference site may reflect exposure to higher contaminant levels.

Gas Bubble Disease

This study also investigated the previous findings of gas bubble disease in rainbow trout, mountain white-fish, and sucker species noted by consultants sampling in the Columbia River in 1990 to 1992 (R.L. & L. Environmental Services Ltd., 1992). The external signs of gas supersaturation include bubbles or blisters

located under the skin, between fin rays, on the head, and in the lining of the mouth. In chronic gas bubble disease, hemorrhages may occur at the base of the paired fins and exophthalmia may be observed. Gas bubble disease has been described in mountain whitefish in the upper reaches of the Kootenay River system below the Libby dam. Fish held in live cages were dead within four days, at total dissolved gas supersaturation above 130 % (cited in Weitkamp and Katz, 1980). Mountain whitefish downstream of Castlegar may be exposed to a sudden or chronic increase in gas supersaturation due to the Hugh Keenleyside dam which leads to total dissolved gas levels of up to 144% (B.C. Hydro, 1990). Histopathological signs of gas supersaturation in different tissues were described in chinook fingerlings (*Oncorhyncus tshawytscha*) (Pauley and Nakatani, 1967). Tissue changes observed in these fish were comparable to those associated with chemical toxicity, and included congested and edematous gill filaments, and degenerative liver changes involving enlarged hepatocytes with vesicular cytoplasm and pyknotic nuclei.

No signs of gas bubble trauma were found during the external and internal gross examination, or during histological evaluation at either of the Columbia River sites. This is consistent with the January, 1991 findings. Electrofishing was thought to perhaps cause gill damage which could mask subtle changes in the gill tissue indicating gas bubble disease. As noted previously, the total number of gill abnormalities was not significantly increased in an electrofished group versus an angled group of the Slocan population during the 1992 study. Thus it seems unlikely that gill damage due to electrofishing would be so widespread as to obscure any gill changes resulting from gas bubble damage.

Effects of Electroshocking

The 1992 mountain whitefish study (Boyle et al., 1992) suggested that electroshocking could have caused some of the gill abnormalities observed. The present study compared angled and electroshocked fish to evaluate the effects of electroshocking so that results of histology work could be interpreted with greater confidence.

Abnormalities of interest for this component of the study were as described in Boyle et al. (1992): dark gills, hemorrhaging at the base of the fins, bleeding in the kidney, aneurysms in the gills, and hemorrhagic areas in the spleen and liver. In 1992, comparisons were made between 48 angled and 11 electroshocked fish from the Slocan Reach. It was found that the frequency of gill aneurysms is probably not associated with electroshocking under the conditions used in this study. Hemorrhaging at the base of the fins and dark gills were present at slightly higher frequencies in the electro-shocked fish, and may be related to sampling method. Interestingly, hemorrhaging at the base of the fins was noted at the reference site at a higher frequency (5.9 %), than at either Genelle (0 %) or Beaver Creek (1.7 %). No other abnormalities thought likely to be caused by electroshocking were found in fish sampled from the Slocan River.

In general, the present results indicate that electroshocking did not have a pronounced impact on fish, which would affect interpretation of data pertaining to abnormalities. This is in contrast with Boyle et al. (1992) where perhaps higher currents were used to capture fish.

CONCLUSIONS

Although there were some differences in occurrence of abnormalities between the July, 1992 study and the January, 1991 study, similar conclusions can be drawn. In general the abnormalities found suggest inflammatory response in several tissues. Dramatic tissue changes characteristic of fish exposed to pulp mill effluents, metal contaminants, and gas bubble trauma were not consistently seen. The type of abnormalities found seemed to indicate a deterioration in fish health, likely related to the stress imposed by impaired water quality. Stress has been defined as "a state produced by any environmental or other factor which extends the adaptive responses of an animal beyond the normal range or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced" (Brett, 1958).

Genelle fish were significantly older than both Beaver Creek and Slocan fish, therefore abnormalities and CDS could not be compared with the other two reaches without age acting as a confounding factor. Overall, fish from Beaver Creek had a higher CDS than similarly aged fish from the reference site, suggesting some degradation of fish health in the Columbia River fish. Impacts to fish health may occur from a combination of factors including stress from pollution, and the rapidly changing flows which result from altering water releases from the Hugh Keenleyside Dam.

Mountain whitefish from Genelle were significantly older than fish sampled at Beaver Creek and the Slocan River, despite efforts to obtain fish within a specified size class. Scatter plots of fork length versus age indicate that there is not a strong relationship between these two parameters in fish exceeding 30cm in length, meaning that age cannot be predicted by fork length. This should be a consideration in selecting size classes, and in determining sample size for future studies of mountain whitefish where fish age is of importance to the integrity of the study design.

The relatively high incidence of abnormalities in fish from the Slocan River reference site is worthy of comment. Although the level and severity of abnormalities in Slocan fish was always similar to or less than levels observed in Columbia River fish, approximately 55% of individuals were noted as having some type of abnormality. Further studies of mountain whitefish in pristine areas must be done in order to ascertain what a normal background rate of abnormalities is for a range of ages. Given that the purpose of this study was to compare the health and body burdens of contaminants of fish exposed to industrial discharges with unexposed fish, the Slocan fish did serve as a suitable reference population. The Slocan River does not receive any notable industrial discharges.

TABLE 4. Mean Age, Fork Length, Wet Weight, Condition Index, Gutted Weight, Stomach Weight, Gonad Weight, and Liver Weight, of Mountain Whitefish (*Prosopium williamsoni*) sampled from two sites within the Columbia River and a reference site within the Slocan River.

		Slocan	Genelle	Beaver
···		(n=69)	(n= 61)	(n=60)
Age	Mean	6.32	13.62	7.48
(yr)	Standard Deviation	± 3.25	± 5.90	± 5.56
Fork Length	Меап	273.65	353.25	324.83
(mm)	Standard Deviation	± 37.78	± 40.03	± 32.70
Wet Weight	Mean	237.45	465.97	449.20
(g)	Standard Deviation	± 107.58	± 142.01	± 106.55 (n=59)
Condition Index ^a	Mean	1.09	1.05	
	Standard Deviation	± 0.10	± 0.20	1.33 ± 0.25 (n=59)
Gutted Weight	Mean	216.90	423.98	396.14
(g)	Standard Deviation	± 97.21 (n=66)	± 125.50	± 93.64
Stomach Weight	Mean	7.87	9.29	9,59
(g)	Standard Deviation	± 5.52 (n=60)	± 4.13 (n=56)	± 3.25
		(11-00)	(11=30)	(n=50)
Gonad Weight (g)	Mean Standard Deviation	3.55	8.68	12.50
(B)	Standard Deviation	± 4.47 (n=37)	± 7.35 (n=58)	± 9.66 (n=50)
Hepatosomatic Index	Mean	0.6	0.7	0.9
	Standard Deviation	± 0.1 (n=68)	± 0.2 (n=59)	± 1.4 (n=49)
Liver Weight	Mean	1.48	3.31	4.43
(g)	Standard Deviation	± 0.88 (n=68)	± 1.96 (n=59)	± 9.26
Gonadosomatic Index	Mean	1.1	1.7	2.7
	Standard Deviation	± 1.1 (n=58)	± 1.2 (n=59)	± 1.7 (n=49)

a: Condition Index = (Weight x Length⁻³) x 10^s

Note: Values for n are provided only where the number is less than the total number of fish sampled from the site.

TABLE 5. Statistical Evaluation of Frequency and Severity of Disease, Gross Internal and External Abnormalities, Tissue Histopathology and Bacteriology.

		Slocan			Genelle			Beaver		Analysis of Variand	e of CDS
Parameter	Frequency (%)	Mean Severity	Std. Dev.	Frequency (%)	Mean Severity	Std. Dev.	Frequency (%)	Mean Severity	Std. Dev.	F, Prob., Sig.	MRT
Fish Age (covariable)	n/a	6.32	3.25	n/a	13.62	5.90	n/a	7.48	5.56	39.09, P<0.001, *	S=B <g< td=""></g<>
Gross Examination											
External Problems	17.39	0.29	0.69	49.18	0.98	1.18	40.0	0.65	0.86	9.20, P<0.001, *	S <b<g< td=""></b<g<>
Internal Problems	23.19	0.30	0.60	31.15	0.56	0.87	28.33	0.35	0.71	1.94, P=0.147, ns	-
Histological Evaluation											
Gill	11.59	0.13	0.38	13.11	0.16	0.49	6.67	0.13	0.54	0.10, P=0.907, ns	_
Liver	8.69	0.25	0.81	70.49	1.87	1.34	45.00	1.08	1.25	32.70, P<0.001, *	S <b<g< td=""></b<g<>
Kidney	1.45	0.01	0.12	19.67	0.34	0.77	18.33	0.35	0.80	6.11, P=0.003, *	S <b=g< td=""></b=g<>
Pyloric Caeca	0.00	0.00	0.00	11.48	0.26	0.75	6.67	0.13	0.50	4.28, P=0.015, *	S=B <g< td=""></g<>
Gut/Hind Gut	2.90	0.07	0.43	11.48	0.25	0.72	0.00	0.00	0.00	4.14, P=0.017, *	S=B <g< td=""></g<>
Other Organs	0.00	0.00	0.00	6.56	0.11	0.49	1.67	0.02	0.13	2.98, P=0.053, ns	-
Bacteria		= 	·								
Myxobacteria	7.25	0.14	0.52	9.84	0.20	0.60	0.00	0.00	0.00	2.94, P=0.055, ns	_
Other Bacteria	1.45	0.03	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.88, P=0.418, ns	_
Acid Fast Bacteria	7.25	0.22	0.78	14.75	0.44	1.07	8.33	0.20	0.75	1.45, P=0.233, ns	
Cumulative Severity	n/a	1.52	1.9	n/a	5.85	3.86	n/a	3.18	3.14	33.28, P<0.001, *	S <b<g< td=""></b<g<>

MRT = SNK Multiple Range Test Comparing Sample Means

TABLE 6. Numbers of Infected Fish and Frequency (%) of Myxobacteria, Mycobacteria, and other bacteria found in Mountain Whitefish (*Prosopium williamsoni*) sampled from two sites within the Columbia River and a reference site within the Slocan River, July 6 - 15, 1992.

	Slocan (n=69)	Genelle (n=61)	Beaver (n=60)
Myxobacteria	5	6	0
(Gram Stain)	7.25 %	9.84 %	
Mycobacteria	5	9	4
(Cultured or Acid Fast Stain)	7.25 %	14.75 %	6.67 %
Other Bacteria	1 ^a	0	0
(Culture or Gram Stain)	1.45 %		

a: identified as Yersinia ruckeri

TABLE 7. Selected Parasites found in Mountain Whitefish (*Prosopium williamsoni*) sampled from the Slocan River, and the Genelle and Beaver Creek Sites on the Columbia River near Castlegar, B.C.

Parasite Species/Tissue		Prevalence	
	Slocan	Genelle	Beaver Creek
Myxidium in urinary bladder	0/10	0/10	0/10
Myxidium in kidney	0/10	0/10	0/10
Chloromyxum in gall bladder	0/10	0/10	0/10
Ceratomyxa in gall bladder	0/10	0/10	0/10
Myxidium in liver	0/10	0/10	0/10
Ceratomyxa in liver	0/10	0/10	0/10
Ceratomyxa in intestine	0/10	0/10	0/10
Crespidostomum farionis in intestine	1/10	0/10	0/10
Eimeria in intestine	0/10	0/10	0/10
Truttaedacnitis truttae in intestine	5/10	8/10	8/10
Philonema agubernaculum (encapsulated)	0/10	1/10	1/10
Rhabdochona sp. in intestine	0/10	1/10	1/10
Plagioporus shawi in intestine	0/10	10/10	10/10
Eustrongylides sp. (larva) encapsulated on stomach	3/10	0/10	0/10

Note: No parasites of any significance were found in fish from any of the three sites. No myxosporeans, microsporidans, or *Diphyllobothrium* cestodes were detected.

TABLE 8. Proportion of Mountain Whitefish (Prosopium williamsoni) with any Abnormality.

			Age			_
	1 to 5	6 to 10	11 to 15	16 to 20	21+	Total
Slocan						
Total Number	36	23	9	1	0	69
# with abnormality	18		· 6	0	0	38
% with abnormality	50.0	60.9	66.7	0	0	55.1
Genelle		<u></u>	· · · · · · · · · · · · · · · · · · ·			
Total Number	12	5	7	36	1	61
# with abnormality	10	5	6	34	1	56
% with abnormality	83.3	100	85.7	94.4	100	91.8
Beaver Creek	· · · · ·			······································	· · · · · · · · · · · · · · · · · · ·	·
Total Number	34	10	6	10	0	60
# with abnormality	23	7	5	10	0	45
% with abnormality	67.6	70.0	83.3	100.0	0	75.0

FIGURE 2. Fish Stomach Contents Clustered by Sampling Site.

(Variable = Numbers)

No. of stations = 3 No. of prey taxa = 86

Cluster Analysis Options

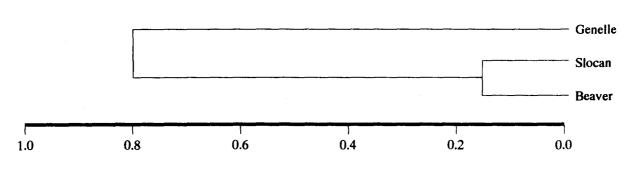
1. Coefficient = Euclidean Distance

2. Linkage = UPGMA
3. Linkage scale = Distance

Distance Matrix

	Genelle	Slocan	Beaver	
Genelle	0.00000			
Slocan	0.65238	0.00000		
Other Bacteria	0.56192	0.18521	0.00000	

Linkage	Clusters	Linked	Distance	
1.	Slocan	Beaver	0.18521	
2.	Genelle	Slocan	0.60715	



DISTANCE

From Cross, 1994.

FIGURE 3. Age Distributions of Fish Sampled from the Columbia River and Slocan Reference Site.

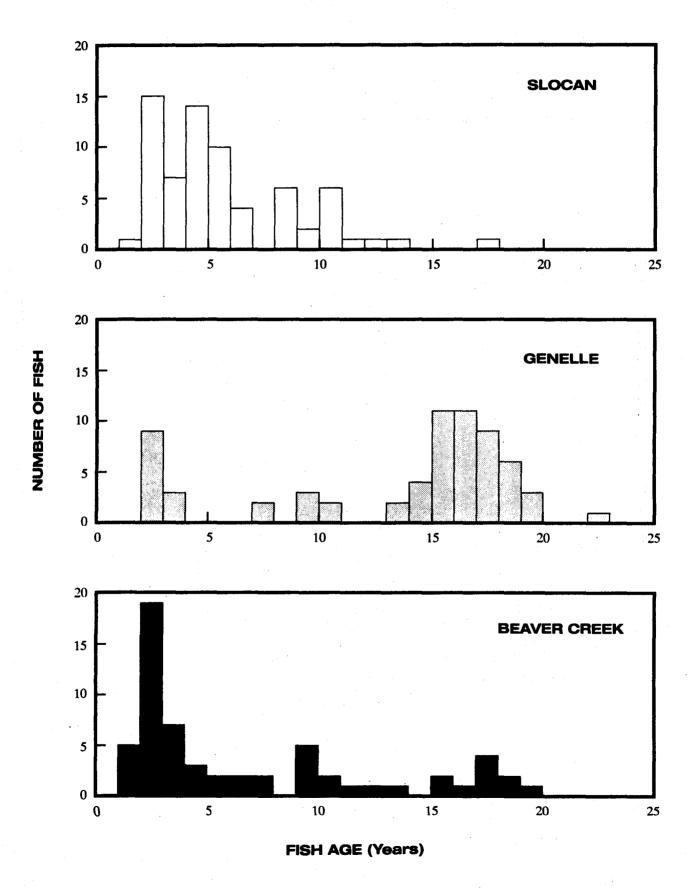
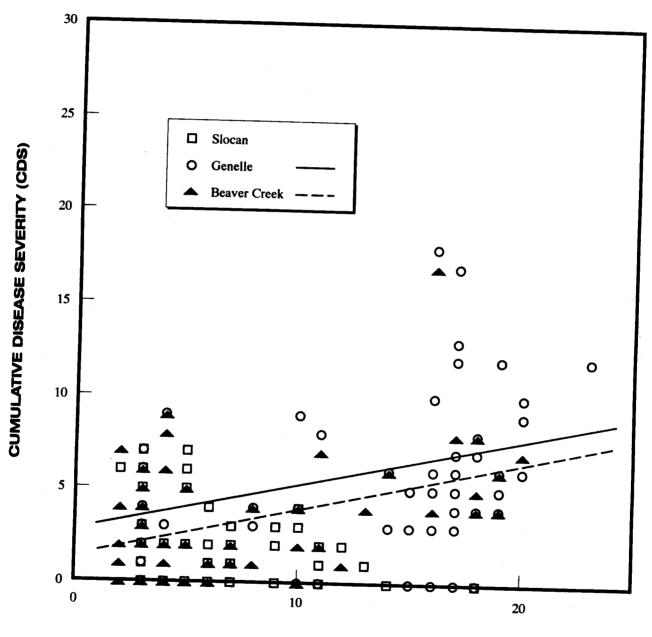


FIGURE 4. Cumulative Disease Severity in Mountain Whitefish as a Function of Fish Age.



FISH AGE (Years)

Slocan: No apparent relationship r = 0.0170 ns Genelle: CDS = 2.76+0.23 (age) r = 0.347* (p<0.01) Beaver Creek: CDS = 1.41+0.24 (age) r = 0.420* (p<0.01)

From Cross, 1994.

ORGANIC CONTAMINANTS - RESULTS AND DISCUSSION

DIOXINS AND FURANS

Data obtained from individual fish are provided in Appendix 3, including results obtained for blind duplicates and replicates analysed at the DFO Institute of Ocean Sciences (I.O.S.). In general results were similar for both blind duplicates submitted to AXYS and replicate samples analysed at I.O.S., indicating good repeatability both within and between analytical laboratories. Analysis of Variance was done to compare surrogate recovery of injected standards at the three sites and results indicate that recoveries were not significantly different between sampling stations.

For the purpose of statistical analysis, minimum detectable concentrations were considered to be the absolute level (i.e. where data were below detection limit, the detection limit was assumed to be the concentration). Where all congeners in a family (i.e. P5CDDs, etc.) were non-detectable, the detection limit was treated as the total concentration of the congener family. This provides a worst case estimate of tissue contaminant levels. Table 9 presents means and ANOVA results for muscle dioxin and furan concentrations from the three reaches. The upper table compares dioxin and furan subgroups using uncorrected data, and the lower table presents data corrected for muscle lipid content. Results indicate that none of the subgroups except for T4CDD and T4CDF were significantly different between sites, however these are the compounds which contribute the greatest toxicity (Table 10). Concentrations of T4CDD and T4CDF were both significantly higher (P=0.002 and P=0.012, respectively) in fish muscle from the Genelle and Beaver Creek reaches in comparison with the Slocan reach but did not differ significantly between the two Columbia River reaches. There is a difference of two orders of magnitude between the average T4CDD TEQ of Genelle (TEQ = 10.75 pg/g) and Beaver Creek fish (TEQ = 8.90 pg/g), compared with that determined for fish from the Slocan River (TEQ = 0.035 pg/g) (Figure 5).

Table 10 displays the T4CDD TEQs of individual dioxin/furan congeners as a percentage of the total toxic equivalents. At each site 2,3,7,8-T4CDF contributes the greatest percentage of toxicity of all dioxin and furan congeners to the fish samples. In the Columbia River fish, which have much higher TEQs than Slocan fish, 88-97% of the calculated toxicity results from the combined effects of 2,3,7,8-T4CDD and 2,3,7,8-T4CDF. For the Slocan samples, the latter compound contributes the majority of the low toxicity calculated.

Regressions were performed to determine whether a relationship between percent lipid (wet wt.) and dioxin/furan levels was present in Columbia River fish, because dioxins and furans are highly lipophylic (Safe, 1990). No significant relationship (p < 0.05) was found at either of the sites, nor in data combined from the two sites (Table 11).

No statistically significant relationship between fish age and tissue concentrations of total dioxins and furans was found using regression analyses (Table 13). Fish from the Slocan River had extremely low concentrations of dioxins and furans regardless of age, as they are presumably not exposed to these chemicals in high concentrations through effluent sources. Among Columbia River fish some of the young individuals from the Genelle and Beaver Creek reaches had relatively high dioxin and furan concentrations, while some older fish had very low levels of these contaminants (Figure 6). This is an interesting finding as dioxins and furans are known to bioaccumulate (Safe, 1990) thus body burdens are typically correlated with duration and level of exposure.

It is possible that individuals were exposed to widely different levels of these chemicals, resulting in the observed range of values. Older, uncontaminated fish may have moved to the sampling area from upstream of the Hugh Keenleyside Dam where there are no known sources of contamination, shortly before they were captured. Also, contaminant concentrations in individuals may be related to their diet preferences, or there may be localized "hot spots" in which individuals were feeding or otherwise spending their time, resulting in non-homogenous exposure of individuals to contaminants. Tagging studies have shown that individual mountain whitefish on the Columbia River downstream of the Hugh Keenleyside dam tend to remain within a relatively small radius of approximately 5 km (R.L. & L., 1992), thus if localized contaminated areas exist, they could have a pronounced effect on individuals.

One fish captured at Genelle had a very different contaminant pattern in comparison with all other fish. The T4CDD and T4CDF congeners were the commonly observed contaminant in Columbia River fish, as would be expected due to their association with pulp mill effluents. Fish number G71 had low levels of these congeners, but had extremely high levels of H6CDD, H7CDD, H6CDF, and H7CDF congeners (320, 600, 300, and 160 pg/g respectively). All other fish captured had either extremely low or non detectable levels of these H6 and H7 compounds. Contamination of the sample during processing or analyses is unlikely to explain results obtained for this fish. Samples were handled and processed in batches and no other fish showed this pattern of contamination. This fish might have been exposed to contamination elsewhere in the river prior to moving into the study area. The congeners which were elevated in this individual are associated with pentachlorophenol which was formerly commonly used in sawmills (R. Macdonald, I.O.S. personal communication).

Comparison of dioxin and furan data for fish analyzed in the present study with levels found in fish captured in January 1991 (Boyle et al., 1992) suggests that levels in fish tissues are declining in the lower Columbia River. The maximum concentration of T4CDF in whitefish muscle in the present study is 190 pg/g. In the 1991 study only one of six fish captured at Genelle had a muscle T4CDF concentration as low or lower than this level, while five of the six had concentrations between 350 and 580 pg/g. In the 1991 study, five of the six fish analyzed had T4CDD concentrations between 12 and 24 pg/g, while in the present study only 2 of 15 fish analyzed from at Genelle had T4CDD concentrations of 12 pg/g or greater. The highest concentration of T4CDD measured in fish from Beaver Creek in the present study was 7.2 pg/g. This reach was not sampled in the 1991 work. The apparent decline in body burdens of dioxins and furans is likely linked with improvements made to the pulping process and effluent treatment system at the pulp mill in Castlegar, which result in the release of much smaller amounts of dioxins and furans to receiving waters. Also, it was found during a survey of the river bottom that a fiber mat, believed to harbour high levels of contaminants, had disappeared from the Columbia River downstream of the pulp mill sometime before the present fish samples were collected (J. McLaren, Celgar Pulp Mill, personal communication). Thus, sediment levels of contaminants would be expected to be deminished. As mountain whitefish feed heavily on benthic organisms, a decrease in sediment contaminant levels should eventually translate into a reduction in contaminant levels in fish tissue.

PCBs

A variety of PCB congeners were detected in the muscle samples analysed. For the purpose of data management and interpretation PCB data were treated in three groups: Arochlors, Non-Ortho Substituted (NOS) PCBs, and all others. Summary data for fish from the three sampling reaches are provided in Table 12. Appendix 4 contains summarized PCB data for individual fish, as well as QA/QC data (blind duplicates, and replicate samples analyzed at IOS). In general results were similar for both blind duplicates submitted to AXYS and replicate samples analysed at IOS, indicating good repeatability both within and between analytical laboratories.

The analytical laboratory reported concentrations of Arochlor 1254/1260 which is a brand name for a mixture of PCBs. Concentrations of the specified commercial mixture are calculated by the analytical laboratory based upon levels of specific individual congeners measured.

Total PCB concentrations were calculated by adding measured concentrations of Di-, Tetra-, Penta-, Hexa-, Hepta-, and Octa families of PCBs for each individual. Where non-detectable concentrations were found for an entire congener family, the detection limit was considered to be the total concentration of that PCB family. NOS PCBs were not included in the calculated concentration of Total PCBs because they were measured using different detection limits (pg/g vs ng/g). This calculated value of Total PCBs provides a more accurate measure of all PCB congeners present than concentrations indicated for Arochlors, however for most fish the numbers were very similar. Arochlor concentrations are provided in addition to Total PCB concentrations so that data can be compared with results obtained by Boyle et al. (1992). For the purpose for this discussion Total PCBs excludes Arochlors and NOS, for which data are analyzed and interpreted separately.

ANOVA results indicate that Arochlors, NOS PCBs, and the Di-, Tetra-, Penta-, Hexa-, Hepta-, and Octa- families of PCBs were all present at significantly higher levels (P<0.05) in fish from the two Columbia River reaches in comparison with fish from the Slocan reference site. Paired comparisons indicate no difference (P<0.05) between Genelle and Beaver Creek fish for any of the PCB groups. The total concentrations of all congeners analyzed did not exceed the Health Canada criterion of 2 ppm in any of the samples.

Results of regression analyses (Table 13, Figure 7) indicate that for Columbia River fish there was a significant relationship between age and Total PCB concentration ($r^2 = 0.51$, P=0.000). The relationship was also significant in the Genelle and Beaver Creek fish when data for the two sites were analyzed independently (P=0.02 and P=0.00 respectively). There was no significant relationship between age and Total PCBs in Slocan fish ($r^2 = 0.007$, P=0.77), as most congeners were below detection limits. There was no statistically significant relationship between NOS PCBs and age in fish from any of the three sampling reaches.

No strong relationships were found between levels of PCBs and lipid content (Table 13) using regression analyses, where Arochlors, non-ortho substituted PCBs, and Total PCBs were considered separately. Very weak relationships between both Total PCB and Arochlors, and lipid content (r² values of 0.15 and 0.16 respectively), for combined Columbia River sites were significant (P<0.05). PCBs are lipophylic, therefore higher levels of PCBs would be expected in fish with higher lipid levels given a similar duration and level of exposure. The fact that only weak relationships were found here could be a result of the relatively small sample sizes, and also because other factors such as non-uniform exposure of individuals to PCBs may have had a large role in determining body burdens of PCBs.

Comparison of Arochlor 1254/1260 concentrations between the present study and results obtained from the 1991 fish of similar age show that levels are alike. Due to the small sample size obtained in 1991 (six fish from Genelle), this was not examined statistically. Possible trends can be identified only with further sampling. No individual congeners were measured in the 1991 study therefore conclusions with regard to trends in levels of specific PCB congeners will await results of future investigations.

Regressions were performed to investigate the relationships between the total dioxin and furan concentrations found in Columbia River mountain whitefish, and levels of PCBs (Table 13). There was no relationship between dioxins and furans, and Arochlors, NOS PCBs, or Total PCBs. PCBs have chemical properties similar to dioxins and furans and therefore bioaccumulate in a similar manner, so one would expect to find similar patterns of contamination in organisms exposed to the two families of chemicals if they were similarly dispersed in the environment.

Dioxins and furans are commonly associated with pulp mill effluent, while PCBs are associated with different sources such as dielectric fluids for power transformers and capacitors, hydraulic fluids, and a variety of industrial oils, plasticizers, and wood sealants (Safe, 1990). PCBs were also widely distributed in the environment through pesticide use, being added to pesticides as volatilization suppressants. The use of PCBs has been legally restricted since the early 1980s in Canada. PCBs are hydrophobic and therefore tend to bind to sediment particles as do dioxins and furans. In the Columbia River, sediments are thought to be scoured from the river bottom during high flows as evidenced by the disappearance of the fiber mat from downstream of the pulp mill. Unless there is a continuing source of PCBs to the Canadian portion of the Columbia River, exposure of fish to this family of chemicals should therefore be minimal, and body burdens should decrease over time, although depuration rate is slow.

The very low levels of PCBs detected in Slocan fish may be the result of atmospheric deposition, or from unknown sources. The higher PCB levels found in Columbia River fish may have originated from discharges or seepage from the nearby pulp mill, sawmill, or other industries. One significant spill occurred in 1976, approximately 700 m from the Columbia River near Trail, when 1200 kg of PCBs were spilled on the ground (Garrett, 1983). Soil contaminated with up to 4% PCB was removed, but the remainder was left as it was believed that leaching/surface runoff would not be a problem in the area due to low levels of precipitation. This is not likely a large source of PCBs found in Columbia River fish. If it was a significant source one would expect to find higher PCB concentrations in fish caught near Beaver Creek than in fish from the Genelle area, and this was not the case.

DIOXIN AND FURAN, AND PCB TEQS, AND IMPLICATIONS FOR FISH HEALTH

PCBs vary in their chemical properties, however like dioxins most congeners are chemically very stable, and are very hydrophobic and therefore lipid soluble. Like dioxins, they bioaccumulate, and highest concentrations are generally found in organisms at the top of the food chain. Some of the congeners are structurally very similar to dioxins and furans and therefore exert effects on biota through the same biochemical mechanisms (Sonzogni et al., 1991; De Voogt et al., 1990). These congeners, which include non-ortho, mono-ortho- and di-ortho-substituted PCBs, bind to the Ah receptors and elicit dioxin-like biochemical and toxic responses in biota (Ahlborg et al., 1994). Because of these similarities, considerable effort has gone into developing toxic equivalency factors for dioxin-like PCBs (PCB TEQ_{diox}), so that the cumulative effects of both types of chemicals can be considered (Walker and Peterson, 1991; Ahlborg et al., 1994). These dioxin-like PCBs include the three non-ortho-, eight mono-ortho-, and two di-ortho-substituted PCBs (Ahlborg et al., 1994).

Toxic equivalents were calculated for dioxins and furans, and PCB congeners that were measured and for which TEFs have been determined (see Tables 2 and 3). All TEQs are expressed relative to the most toxic dioxin congener, 2,3,7,8-T4CDD. The most important limitation of the TEFs which are used to calculate TEQs is that the combined toxic effects of component compounds are addressed as though they are additive, and possible synergistic or antagonistic effects are not accounted for (Ahlborg et al., 1994). The relative toxicities of compounds were determined through in vitro and in vivo studies which investigate the effects of exposure to individual substances.

Results of the present work show that fish from the reference site have higher TEQs resulting from PCBs compared with dioxins and furans, while in Columbia River fish the reverse is true (Figure 5). For Columbia River fish, TEQs from PCBs were approximately half the value of TEQs from dioxins and furans, accounting for about one third of the total T4CDD TEQs calculated. With regard to Slocan fish the PCB TEQs diox largely reflect achievable detection limits rather than measurable concentrations. Fish from both the Genelle and Beaver Creek reaches had significantly greater PCB TEQdiox values (P<0.05) in comparison with fish from the Slocan River (Table 12), a reflection of the overall higher PCB concentrations in Columbia River fish.

Detection limits for all PCB congeners except NOS compounds were much less sensitive than for dioxins and furans (i.e. ng/g vs pg/g). Detection limits for PCBs were in the range of 0.1 ng/g for most congeners (except NOS PCBs), while detection limits for dioxins and furans were typically in the range of 0.1 pg/g. Detection limits for NOS PCBs were similar to those attained for dioxins and furans. Because detection limits are taken to be the absolute concentration of PCBs, concentrations at detection limits are inflated by a thousand-fold compared with dioxins. This is also true for calculated TEQs where measured levels did not exceed detection limits.

Regression analyses were done to investigate relationships between dioxin and furan TEQs, and CDS in the same individuals. Values of r^2 were close to zero at all reaches (Table 14). A weak relationship ($r^2 = 0.20$) between CDS and PCB TEQ_{diox}s for Total PCBs was statistically significant (P=0.03) when data from the two Columbia River sites were pooled, but no significant relationship was found in fish from individual reaches. A similar relationship was found between non-ortho substituted PCBs and CDS ($r^2 = 0.31$ for pooled Columbia River data). Total TEQs from dioxins, furans, and PCBs were calculated, and regression analyses indicated that there was no significant relationship with CDS (Table 14), reflecting results obtained for dioxins and furans.

The significant relationship between PCB TEQ_{diox}s and CDS at first appears to be an anomaly considering that the dioxin and furan TEQs were approximately twice as high as TEQs from PCBs, yet there was no relationship between dioxin and furan TEQs and CDS (Figure 8). The statistically significant relationship between PCB TEQ_{diox}s and CDS may reflect the fact that both PCB concentrations in fish muscle, and CDS, were age-dependant while body burdens of dioxins and furans were not age dependant. Older whitefish from the Columbia River in general had higher concentrations of PCBs and a greater CDS than younger fish, but not necessarily higher concentrations of dioxins and furans. It is not possible to determine the relative implications of TEQs and aging on CDS, an indicator of fish health, from the present data due to a lack of older fish (> 12 years) sampled from the reference population.

This lack of a relationship between Total TEQs and CDS may result from a variety of interacting factors including interfering effects from aging, and varying exposures to contaminants in fish of similar ages. Effects of dioxins, furans, and PCBs on biota are well established in the literature. Small samples sizes (n=13) for the two Columbia River sites also make it difficult to identify relationships between contaminant levels and CDS. Some of the documented effects of PCBs on fish such as impaired cortisol stress response (Hontela et al., 1992) were not examined in the study. Also, the present study has no way of accounting for duration of exposure of individuals to chemicals, and duration of exposure may be related to actual health impacts of chemicals on biota. Exposure to other chemicals such as resin acids may also affect fish health, causing a high CDS in individuals which do not necessarily have high levels of PCB, dioxin, and furan TEQs. Other factors such as age may have had an over-riding affect on CDS and masked relationships between CDS and toxic equivalents.

Data available for individual PCB congeners were examined by De Voogt et al. (1990), who concluded that biochemically active PCBs are present in the environment at background concentrations several orders of magnitude greater than T4CDD. The authors concluded that the relatively low potency of PCBs, compared with dioxins, could still exert significant toxic impacts on biota. Although PCB concentrations in the present study were high compared with dioxins and furans, mean PCB TEQ_{diox}s were lower at Genelle and Beaver Creek (4.35 and 3.65 pg/g 2,3,7,8-T4CDD, respectively) than TEQs for dioxins and furans (10.75 and 8.89 pg/g for the two sites, respectively), presumably because the pulp mill located upstream of the Columbia River sampling reaches was a source of the most toxic T4CDD and T4CDF congeners. With continued reduction of dioxin and furan loadings from the pulp mill, however, it is expected that PCBs will soon become the largest source of T4CDD TEQs in mountain whitefish in the Columbia River.

At the reference site PCB TEQ_{diox}s (0.81 pg/g) were higher than TEQs associated with dioxins and furans (0.035 pg/g), however this difference may be largely an artifact of less sensitive analytical detection limits obtained for PCBs relative to dioxins and furans (where detection limits were not exceeded they were treated as the actual values).

Dioxins, furans, non-ortho, and mono-ortho substituted PCBs are known to exert a range of toxic effects including weight loss, thymic atrophy, dermal disorders, liver damage, teratogenicity, reproductive toxicity, immunotoxicity, and high binding affinity for Ah receptors in liver (Kannan et al., 1989; Safe, 1984). The present study focussed on gross abnormalities, histological abnormalities for key organs, and some indicators of disease. Diseased fish may be quickly removed from the population through predation and other mechanisms, therefore highly impacted individuals may not be found in high numbers. The fish health analyses showed that abnormalities found were those usually associated with stress, and exposure to contaminants is one form of stress. Other significant forms of stress, such as rapidly fluctuating water levels and gas saturation levels resulting from operational changes at the Hugh Keenleyside Dam, could also cause stress, leading to some of the abnormalities observed in the present study.

CONCLUSIONS

- 1) Body burdens of polychlorinated dibenzo- dioxins and furans in mountain whitefish from the Columbia River downstream of Castlegar appear to have declined since January 1991. This is likely a result of process changes and improved effluent treatment at the pulp mill in Castlegar.
- 2) Body burdens of Arochlors in mountain whitefish downstream from Castlegar on the Columbia River do not appear to have declined since 1991, when only 6 fish were sampled, and exceed levels measured at the Slocan reference site. Further sampling undertaken in July 1994 will indicate whether levels are stable or changing.
- 3) Toxic equivalents from body burdens of PCBs are slightly less than half of the TEQs resulting from body burdens of dioxins and furans in mountain whitefish. When data from Genelle and Beaver Creek fish were combined, a significant relationship (P = 0.03, r² = 0.20) was found between the total TEQs from PCBs, and cumulative disease severity, suggesting a possible link between PCB levels and fish health. Both CDS and PCB concentrations were statistically linked with fish age, however. No specific source for these PCBs has been identified.
- 4) Body burdens of dioxins and furans (as 2,3,7,8-T4CDD) did not appear to be linked with fish health, based on the cumulative disease severity index used to quantify health impacts. This could be due to a variety of confounding factors including fish ages, which were not similar between all sampling reaches, duration of exposure, and small sample sizes.

TABLE 9. Dioxin and Furan Concentrations by Congener Family in Mountain Whitefish Muscle (pg/g). ANOVA Results for Site Totals; Raw (Top) and Lipid Corrected (Below) Data.

Congener	Sloc	can (n=15)	Genel	le (n=13)	Beaver (Creek (n=13)		ANOV	'A Results	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Normality	F ratio (2df)	Significance	Paired Comp
T4CDD	0.13	0.04	4.11	4.51	2.46	2.07	P<0.001	7.23	P=0.002	B=G>S
P5CDD	0.18	0.07	0.43	0.96	0.33	0.45	P<0.001	0.6	P=0.56	
H6CDD	0.25	0.11	24.84*	88.68	0.34	0.24	P<0.001	1.08	P=0.35	
H7CDD	0.41	0.17	46.46*	166.32	0.81	0.88	P<0.001	1.07	P=0.35	
O8CDD	1.07	0.8	5.72	17.81	1.7	1.59	P<0.001	0.84	P=0.44	
T4CDF	0.37	0.37	56.1	84.49	63.45	59.75	P<0.001	5.02	P<0.001	B=G>S
P5CDF	0.14	0.04	3.34	7.67	1.26	1.94	P<0.001	1.83	P=0.17	
H6CDF	0.24	0.11	23.26*	83.15	1.71	5.2	P<0.001	1.01	P=0.37	
H7CDF	0.3	0.14	12.54*	44.31	1.7	4.6	P<0.001	0.96	P=0.39	
O8CDF_	0.47	0.24	1.08	2.43	0.65	0.49	P<0.001	0.69	P=0.51	
TEQ (TOT)	0.04	0.04	10.75	12.72	8.9	7.8	P<0.001	6.64	P=0.003	B=G>S
T4CDD	5.12	2.92	263.9	437.21	354.26	1096.68	P<0.001	1.06	P=0.36	
P5CDD	7.28	5.04	29.19	65.91	54.33	171.78	P<0.001	0.72	P=0.49	
H6CDD	9.96	7.44	437.26*	1333.21	69.56	227.06	P<0.001	1.25	P=0.30	
H7CDD	15.78	10.26	767.39*	2507.2	112.86	349.77	P<0.001	1.11	P=0.34	
O8CDD	51.54	49.5	187.72	356.89	152.81	428.73	P<0.001	0.72	P=0.50	
T4CDF	10.45	5.85	1324.21	1537.2	1157.64	834.29	P<0.001	7.55	P=0.002	B=G>S
P5CDF	5.58	3.14	79.65	122.54	64.75	144.88	P<0.001	1.92	P=0.16	
H6CDF	9.32	6.42	385.06*	1253.36	121.65	316.8	P<0.001	0.96	P=0.39	
H7CDF	11.72	8.49	237.96*	670.7	136.14	365.06	P<0.001	0.98	P=0.39	
O8CDF	18.32	12.3	78.06	174.75	100.59	315.53	P<0.001	0.62	P=0.54	
LIPID (g/g wet	wt.) 3.18	1.44	4.2	2.66	5.8	2.7		4.57	P=0.02	B=G>S

^{*}Note: Genelle results for H6 and H7 congeners are largely skewed by results for fish # G71. Exclusion of outlier results in mean concentrations of H6 and H7 congeners which are extremely similar to the Slocan and Beaver Creek results.

TABLE 10. Individual Dioxin and Furan Toxic Equivalents (as pg/g T4CDD) as a Percentage of Totals.

	Pe	ercent of Total Toxic Equiv	valents
Congener	Slocan	Genelle	Beaver Cree
2,3,7,8-T4CDD	0.0	37.9	25.7
1,2,3,7,8-P5CDD	0.0	0.2	0.0
1,2,3,4,7,8-H6CDD	0.0	0.0	0.0
1,2,3,6,7,8-H6CDD	0.0	2.4	0.0
1,2,3,7,8,9-H6CDD	0.0	0.3	0.0
1,2,3,4,6,7,8-H7CDD	0.4	1.5	0.0
O8CDD	2.0	0.0	0.0
2,3,7,8-TCDF	95.9	52.1	71.0
1,2,3,7,8-P5CDD	0.0	0.2	0.1
2,3,4,7,8-P5CDD	0.0	4.5	2.9
1,2,3,4,7,8-H6CDD	0.0	0.2	0.1
1,2,3,6,7,8-H6CDD	0.0	0.1	0.1
2,3,4,6,7,8-H6CDF	1.8	0.2	0.1
1,2,3,4,6,7,8-H7CDF	0.0	0.3	0.1
O8CDF	0.0	0.0	0.0
Total %	100.1	100.0	100.1
TEQ (pg/g as T4CDD)	0.0	11.0	8.7

Note: Bold values cumulatively represent >95% of Total Toxic Equivalents.

TABLE 11. Relationship of Dioxin/Furan Concentrations with Lipid Levels in Columbia River Mountain Whitefish.

Site	Mean	Mean Total Dioxin	Regression of Total D & F conc. vs. Lipid						
% Lipid	% Lipid	& Furan Conc. (pg/g)	R-squared	Std. Error	F-Ratio	P Value			
Genelle	4.2	171.85	0.10	408.8	1.22	0.29			
	4.2	*60.23	0.06	96.16	0.64	0.44			
Beaver Creek	5.8	74.42	0.3	52.19	4.6	0.06			
Genelle* & Beaver Creek	5.0	67.3	0.13	73.13	3.57	0.07			

^{*} Outlier removed

TABLE 12. Summary of PCB Concentrations (ng/g) by Congener Family and Site.

Congener	Slocan I	River (n=15)	Genel	le (n=13)	Beaver (Creek (n=13)		ANOVA Resu	lts
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	F Ratio	P Value	Pairwise Comp.
Di CB	0.10	0	0.12	0.04	0.15	0.05	8.11	0.001	B=G>S
Tri CB	0.11	0.05	0.21	0.25	0.16	0.1	1.29	0.288	
Tetra CB	0.22	0.24	2.37	2.54	1.93	1.4	6.87	0.003	B=G>S
Penta CB	1.63	1.06	16.23	15.69	14.71	14.79	6.24	0.005	B=G>S
Hexa CB	1.88	1.55	34.29	29.49	25.72	26.34	8.09	0.001	B=G>S
Hepta CB	0.95	0.84	28.74	27.18	19.96	25.6	6.48	0.004	B=G>S
Octa CB	0.21	0.15	7.61	6.86	6.42	8.8	5.73	0.007	B=G>S
Nona CB	0.15	0.05	0.39	0.33	0.97	1.65	2.7	0.08	
Deca CB	0.1	0	0.1	0	0.11	0.03	1.08	0.349	
Arochlors	6.1	4.76	118.31	104.05	94.27	102.39	7.37	0.002	B=G>S
*NOS	15.61	12.42	50.16	30.62	52.17	18.07	13.21	0	B=G>S
TEQs (pg/g)	0.81	0.55	4.35	0.59	3.65	0.59	11.04	0	B=G>S

Note: *Non-Ortho Substituted PCB concentrations are in pg/g.

TABLE 13. Results of Regression Analyses for Organic Contaminants with Age and % Lipid.

			Regre	ession	ANC	OVA
Site	Dependant	Independant	R-squared	Std. Error	F-Ratio	P value
	DF TOT	Age				
Slocan		-	0.08	1.44	1.13	0.31
Genelle			0.10	400.04	1.26	0.285
Beaver Creek	٠		0.03	61.37	0.28	0.61
Genelle & Beaver			0.04	293.64	0.89	0.36
	DF TOT	% Fat				
Slocan			0.05	1.46	0.66	0.43
Genelle			0.10	408.79	1.22	0.29
Beaver Creek			0.30	52.19	4.60	0.06
Genelle & Beaver			0.04	292.46	1.09	0.31
	TOTAL PCB	Age				
Slocan			0.01	3.80	0.09	0.77
Genelle			0.40	61.76	7.45	*0.02
Beaver Creek			0.60	50.31	16.60	*0.002
Genelle & Beaver			0.51	54.04	24.91	*0.00
	TOTAL PCB	% Fat				
Slocan			0.14	3.53	2.16	0.17
Genelle			0.07	77.16	0.82	0.39
Beaver Creek			0.23	70.03	3.25	0.10
Genelle & Beaver			0.15	71.00	4.34	*0.05
	Arochlor	Age				<u> </u>
Slocan		•	0.01	4.92	0.12	0.73
Genelle			0.40	84.02	7.40	*0.02
Beaver Creek			0.76	52.00	35.51	*0.00
Genelle & Beaver			0.58	67.63	32.73	*0.00
	Arochlor	% Fat				
Slocan			0.08	4.74	1.16	0.30
Genelle			0.09	103.67	1.09	0.32
Beaver Creek			0.23	93.94	3.26	0.10
Genelle & Beaver			0.16	95.10	4.69	*0.04
	NOS PCB	Age				
Slocan			0.12	12.06	1.84	0.20
Genelle			0.13	29.79	1.68	0.22
Beaver Creek			0.02	18.66	0.26	0.62
Genelle & Beaver			0.02	24.90	0.52	0.48
	NOS PCB	% Fat				
Slocan			0.01	12.85	0.08	0.78
Genelle			0.00	31.98	0.00	0.98
Beaver Creek			0.08	18.10	0.97	0.35
Genelle & Beaver			0.01	25.02	0.27	0.61
	TOTAL PCB	Total Dioxin and Furan				
Slocan			0.02	3.78	0.23	0.64
Geneile			0.20	71.94	2.47	0.15
Beaver Creek			0.00	79.53	0.05	0.84
Genelle & Beaver			0.05	75.90	1.07	0.31

N=15 at Slocan, and N=13 at both Genelle and Beaver Creek

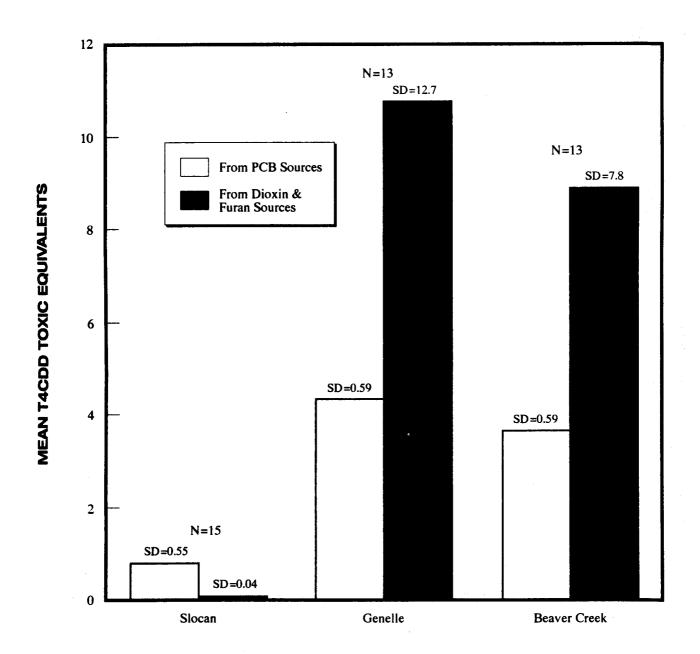
^{*} Statistically significant relationship

TABLE 14. Results of Regression Analyses of TEQs with Cumulative Disease Severity.

		_	Regr	ession	ANC	OVA
Site	Dependant	Independant	R-squared	Std. Error	F-Ratio	P value
	CDS	DFTEQ				
Slocan			0.02	0.97	0.29	0.60
Genelle			0.00	4.68	0.00	0.97
Beaver Creek			0.00	2.42	0.00	0.96
Genelle & Beaver			0.00	3.73	0.01	0.93
	CDS	PCBTEQ TOT				
Slocan			0.00	0.98	0.00	0.98
Genelle			0.29	3.95	3.99	0.07
Beaver Creek			0.05	2.36	0.52	0.49
Genelle & Beaver			0.20	3.34	5.70	*0.03
	CDS	NOS PCB				
Slocan			0.00	0.16	0.00	0.96
Genelle			0.45	1.98	8.21	0.17
Beaver Creek			0.01	1.12	0.05	0.83
Genelle & Beaver			0.31	1.61	10.14	*0.004
	CDS	Arochlor				
Slocan		* · · · · · · · · · · · · · · · · · · ·	0.12	0.91	2.08	0.17
Genelle			0.00	4.67	0.04	0.85
Beaver Creek		•	0.16	2.21	2.15	0.17
Genelle & Beaver			0.02	3.70	0.35	0.56
,	CDS	TEQ Total			-	
Slocan			0.00	0.98	0.00	0.96
Genelle			0.03	4.60	0.35	0.57
Beaver Creek			0.00	2.42	0.02	0.90
Genelle & Beaver			0.13	3.71	0.31	0.59
	PCBTEQ TOT	DFTEQ				
Slocan			0.07	0.36	0.91	0.36
Genelle			0.11	2.98	1.30	0.28
Beaver Creek			0.17	2.14	2.22	0.16
Genelle & Beaver			0.13	2.51	3.53	0.07

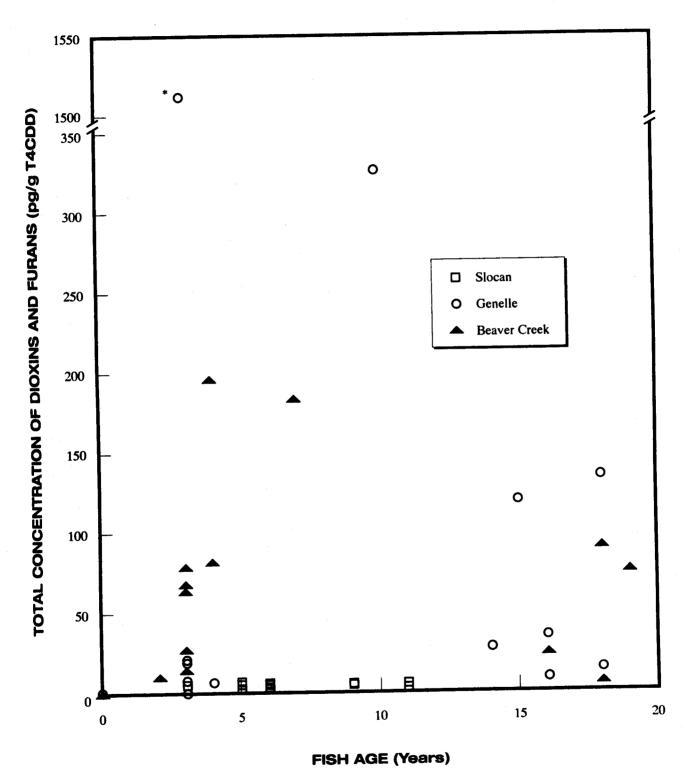
^{*}Statistically significant relationship.

FIGURE 5. T4CDD Toxic Equivalents from Dioxins, Furans, and PCBs for Mountain Whitefish from the Columbia River and Slocan River Reference Site.



SD=Standard Deviation

FIGURE 6. Relationship between Total Dioxin and Furan Concentration and Age of Mountain Whitefish.



Regression Equations:

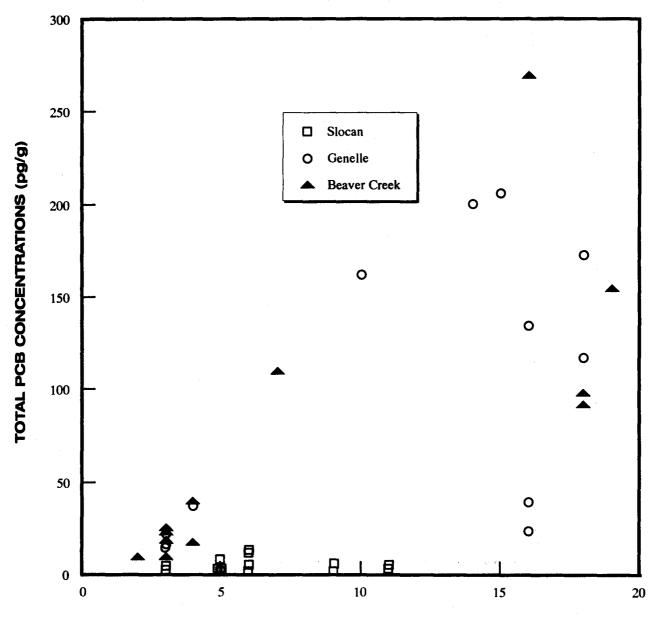
Slocan: Total D&F Concentration = 2.52 + (0.16) x Age

Genelle: Total D&F Concentration = 390.6 + (-20.46) x Age Beaver Creek: Total D&F Concentration = 85.1 + (-1.35) x Age

Genelle & Beaver Creek: Total D&F Concentration = 199.72 + (-8.23) x Age

^{*} Fish G71 with unusual contaminant profile

FIGURE 7. Relationship between Age and Total PCB Concentrations in Mountain Whitefish from the Columbia River and Slocan Reference Area.



FISH AGE (Years)

Regression Equations:

Slocan: $6.12 + (-.12) \times Age$

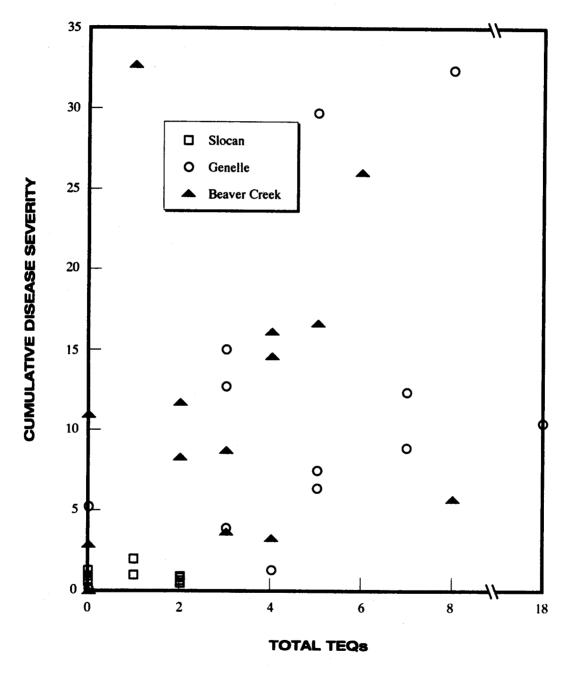
*Genelle: $9.67 + (7.52) \times Age$

*Beaver Creek: 2.47 + (8.52) x Age

*Genelle & Beaver Creek: 5.38 + (8.02) x Age

*Significant relationship at P<0.05.

FIGURE 8. Relationship between Cumulative Disease Severity and Total TEQs (as pg/g T4CDD) in Mountain Whitefish from the Columbia River and Slocan River Reference Site.



Regression Equations: Slocan CDS = $0.833 + (-0.039) \times \text{Total TEQs}$ Genelle CDS = $4.639 + (0.084) \times \text{Total TEQs}$

Beaver Creek CDS = 3.102 + (0.010) x Total TEQs

Genelle & Beaver Creek CDS = 3.833 + (0.046) x Total TEQs

MIXED FUNCTION OXIDASES (MFOs)

Results and Discussion

Mixed function oxidases (also known as monoxygenase enzymes) are among the cytochrome P-450 class of enzymes which is responsible for the metabolism of xenobiotic compounds in mammals and fish. Exposure to certain classes of compounds such as polycyclic aromatic hydrocarbons and several polychlorinated dibenzodioxins and furans causes the amount (and thus the catalytic activity) of one form of these enzymes, cytochrome P-450IA, to increase or be 'induced'. This induction response can be used as an indicator of exposure to these compounds or to mixtures and effluents containing these compounds (Hodson et al., 1991). For a further description of the cytochrome P-450 enzymes, the reactions that they catalyze, and their use as indicators of environmental contamination the reader is referred to several excellent books and articles on the subject (Jakoby, 1980; Jimenez et al., 1990; Stegeman et al., 1992).

Two liver microsomal monoxygenase catalytic enzyme activities were measured in liver from each fish, namely ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH). The cytochrome P-450 content was also measured, although it is generally less responsive than the catalytic activities. The same measurements were made on mountain whitefish taken at Genelle and Brilliant Reservoir in January, 1991 (Boyle et al., 1992). Seasonal cycles in the enzyme activities of some fish have been documented (e.g. Luxon et al., 1987; Boychuk, 1994), but these have not been described systematically for mountain whitefish although the species has been used in Alberta to study the effects of a mill there (Kloepper-Sams et al., 1994). Consequently, direct comparisons between the samples from January, 1991, and July, 1992, may reflect seasonal differences. However, any seasonal differences should also appear in the control or reference sites, and so comparisons of the relative differences between downstream sites and reference sites should be appropriate.

Results for individual fish are tabulated in Appendix 5, where the summary group statistics are also provided. EROD activities for each fish analyzed in 1992 are plotted in Figure 9 and group means from collections January, 1991, and 1992 are shown in Figure 10. For statistical comparisons the enzyme activities were transformed to logarithms and means were compared among locations using the covariance analysis of the General Linear Models procedure of SAS. EROD activities differed significantly among the sites in 1992 (p=0.004), with the highest values from the Genelle site, although these differences were less striking than they were in 1991 (Figure 10, top). Although the mean for the Genelle site was higher than controls in 1992, most of the EROD values for all three sites in 1992 were within the same range; the difference in mean values was caused by a few individuals with high activities in the Genelle group (Figure 9). In 1991, the EROD activities of fish from Genelle were consistently higher than controls from Brilliant Reservoir. This pattern suggests improvement over the interval from January, 1991, to July, 1992. A few fish in the Genelle area in 1992 still showed influence by inducing materials, but most had activities the same as controls. The pattern in mean AHH activities (Figure 10, middle) was the same as that for EROD, but AHH activities did not differ enough to meet the statistical criterion. Again this suggests an improvement between 1991 and 1992. Cytochrome P-450 levels were similar throughout all sites and sampling times (Figure 10, bottom); these are typically less responsive than EROD and AHH.

EROD, AHH and P-450 data correlated with each other (p<0.05). Correlations among these measurements is be expected since production of the enzymes responsible for the catalytic conversion of both of these substrates is mediated by binding to the same Ah receptor site.

The enzyme activities were examined for relationships to the body burdens of several of the dioxin and furan congeners (2,3,7,8-T4CDD, 2,3,7,8-T4CDF and 2,3,4,7,8-PCDF), the non-ortho substituted PCB's (PCBs 77, 126, 169 and their sum) and the individual and combined TEQs contributed by both of these sources. A significant overall correlation between EROD and AHH and the dioxin/furan congeners tested was observed.

This correlation held only at Genelle when broken down among the reaches. Overall the EROD correlated with PCB 126 and the total load of non ortho substituted PCBs but this disappeared when broken down into the various reaches. The TEQs correlated in identical manner as would be expected. No significant correlations were found for the P-450 content with the organochlorines tested.

Interpretation of the data is complicated by the wide variability seen in fish from the Columbia River sites, particularly those from Genelle. One Genelle fish had AHH and EROD values three to five times higher than any of the others. Results are not considered to be analytical artifacts since both enzyme activities were high, and are within the range expected from induced fish. No analytical quality control problem was observed when those assays were performed. Furthermore, sampling and storage artifacts would be expected to reduce enzyme activity rather than create it. The pattern of enzyme activities suggests that some feature of the life history of a few fish in 1992 has resulted in their exposure to inducing materials, but that most fish are no longer exposed.

Elevations in EROD and/or AHH activities have been linked to body burdens of contaminants such as dioxins and furans and polycyclic aromatic hydrocarbons (Rogers et al., 1989; Servos et al., 1994; Johnson et al., 1988). Fish G69 was at the high end of the range for some of the more potent inducers (2,3,7,8-TCDD, 2,3,7,8-TCDF, and PCB 126) but it was not the highest. The fish exhibiting the highest enzyme activities were all male. Males often exhibit higher monoxygenase activities than females (Spies et al., 1988; Lockhart and Metner, 1992; Boychuk, 1994). However, analysis of covariance incorporating both sex and location indicated that the differences among locations in 1992 was not an artifact of differences between males and females.

The reduction in EROD and AHH activity at Genelle, with respect to controls, in 1992 is a significant observation. The difference between Genelle fish and controls was much more pronounced in January, 1991, than in July, 1992. This may be partly attributable to a reduction in loadings of dioxins and furans to the Columbia River due to upgrades at the pulp mill as discussed earlier in the Introduction. The chemical residue data (see Dioxins and Furans) do indicate a decline in chlorinated dioxin and furan concentrations in the fish between the 1991 and 1992 sampling. Dioxin and furan body burdens in mountain whitefish are unlikely to have been affected by the pulp mill strike which began about two weeks before fish sampling. MFO induction by chlorinated dioxins and furans is not lost readily since these materials are cleared only very slowly by fish. Indeed, experiments by Delorme (Ph.D. thesis, University of Manitoba, spring, 1995) in which fish were given a single injection of a chlorinated dibenzofuran resulted in retention of the furan and retention of MFO induction for several years. A short-term reduction in stable chlorinated dioxins and furans would not be expected to result in loss of induction. If the observed induction in January, 1991, was in fact caused by chlorinated dioxins and furans, then the sources of these materials must have been reduced for a considerable time.

Unidentified components in pulp mill effluents have been implicated in causing a short term MFO induction response which decays rapidly after exposure to effluent ceases (Munkittrick et al., 1992). Induction has been observed at two non-chlorinating mills in Ontario (Carey et al., 1993; Munkittrick et al., 1994) and a sulfite pulp mill in Manitoba (Friesen et al., 1994) so the cause of induction in field populations is not exclusively chlorinated dioxins and furans in effluent (Servos et al., 1994). The mill at Castlegar had been shut down by a strike for approximately ten days prior to the sampling period in 1992 and this may have been partially responsible for the observed reduction in enzyme activity between 1991 and 1992.

CONCLUSIONS

Levels of MFO induction in the lower Canadian portion of the Columbia River declined between January 1991 and July 1992. Fish from Genelle might be expected to exhibit induction due to two types of exposure, a short-term component due to labile constituents, which fell because of the strike at the mill, and a long-term component due to persistent constituents released before the strike began. Presumably the fish taken in January, 1991, experienced both types of exposure while those taken in July, 1992, were not exposed to recent effluent. The enzyme activities as measured here would not discriminate between these two kinds of induction, so we cannot tell from the enzymes alone whether the improvement was due to better effluent quality or simply to the absence of effluent because of the strike. When considered along with the chemical residue data, however, the enzyme activities suggest that the long-term component has been reduced, although not eliminated.

FIGURE 9. Liver Microsomal EROD Activities in Mountain Whitefish from the Columbia River Area, July, 1992.

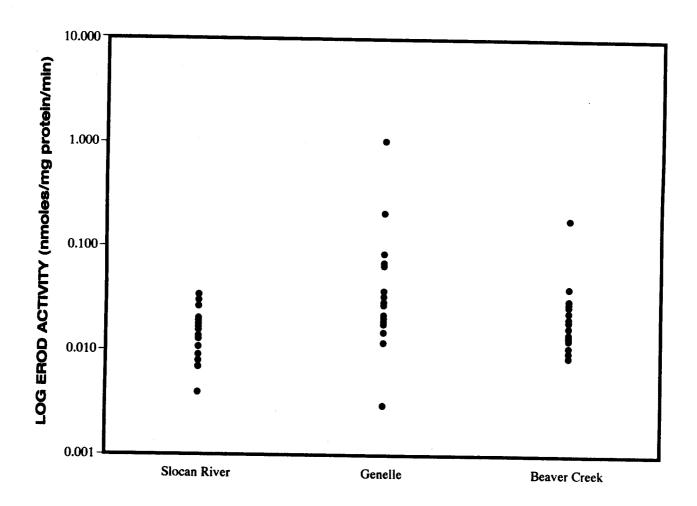
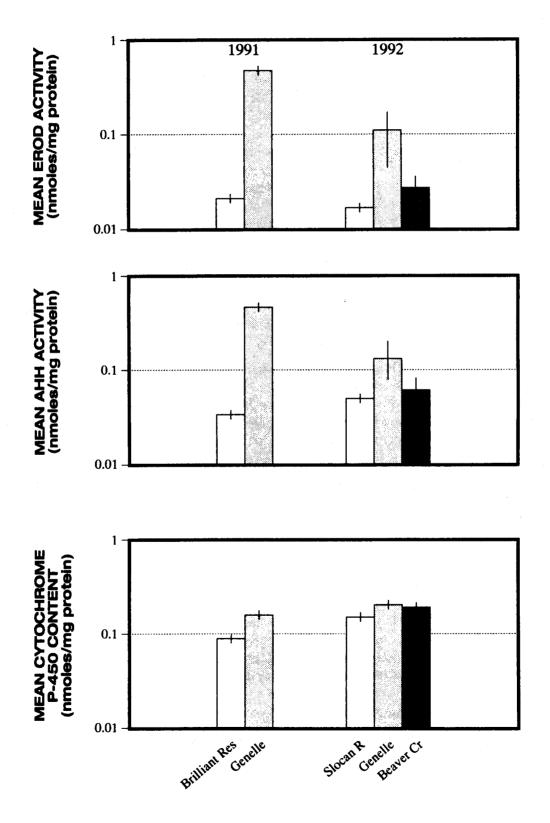


FIGURE 10. Log₁₀ of Mean EROD and AHH Activities and P-450 Content (+/- 1 standard error) from Mountain Whitefish in the Study Site in 1991 and 1992.



METALS AND METALLOTHIONEINS

RESULTS AND DISCUSSION

Table 15 presents the meristic data for the subset of 51 fish used for hepatic metal and metal-binding protein assays. Included are the results of the ANOVA which indicate that fish from the Genelle site were significantly older (P = 0.001) than those from Beaver Creek and fish from the Slocan were significantly smaller (P < 0.001) than those from either Beaver or Genelle.

Sufficient data above limits of detection were obtained for only 4 of 31 trace elements measured in white-fish muscle. Results are shown in Table 16 and in Figures 11-14 as categorized box and whisker plots. Analysis of variance (2-way, fixed effects model) and tests for differences (Tukey's Honest Significant Difference, HSD) indicate that significant differences (P < 0.05) exist between sites for Copper (Cu), Mercury (Hg), Strontium (Sr) and Zinc (Zn), with the higher values being found for Cu, Sr and Zn in muscle of fish from the Slocan River control site. Mercury was significantly higher (P = 0.007) in muscle from the Genelle site, but mean values were below the federal human health consumption guideline of $0.5 \mu g/g$ (wet weight). Female fish from Slocan had significantly higher (P < 0.05) copper levels than males and females from the Beaver Creek site. Copper in female muscle from Genelle was also greater than that in males from Beaver Creek.

Of all elements examined in muscle, only Cu, Hg, Sr and Zn provided data from which meaningful interlocation statistical inferences were possible. The highest concentrations were found for Zn, Sr and Cu in fish from the Slocan reference site, although no age-concentration relationship was established. Higher metal concentrations in Slocan fish may reflect higher levels of mineralization in the area, which has been historically a center for zinc, silver and lead extraction.

Muscle data for the 1992 sampling program were compromised by high limits of detection for a majority of the important trace metals. While such detection limits may suffice for evaluation of metal burdens for human consumption, they are not suitable for proper evaluation of ecologically relevant changes. With newer technology now in place it is expected that limits of detection will have been improved sufficiently to provide meaningful information for Cd and Pb, among others in the 1994 program.

Similarly to muscle data, taken at face value, concentrations of Cd, Cu and Zn in livers would also suggest that there is no apparent increased accumulation of metals at either of the sites which have been exposed to industrial wastes. Data from the previous study (Boyle et al., 1992) for the Genelle site only were somewhat higher for Cu $(2.72 \pm 0.59 \text{ vs } 1.59 \pm 0.21 \text{ mg.kg}^{-1})$ while means for Hg and Zn were comparable with data from the present study.

Hepatic metal and metal-binding protein (MBP) content are more specific indicators of exposure to these metals which are stored preferentially in the liver. Cadmium, copper and zinc were determined in whole tissue homogenates and in heat-denatured cytosols of 15 whitefish livers from each site. In addition, metal-binding proteins and total protein were determined in the cytosols only. Data for the metals and MBP are shown in Table 17. Concentrations of the metals are given in μ moles·kg¹. MBP values are also given in μ moles·kg¹ based upon a molecular weight of 6100 Da for metallothionein. Analysis of variance (1-way, random effects; one outlier removed) performed on the data shows that there were significant differences between sites (P < 0.05) for cytosolic and cell-bound Cd, Cu and Zn. Between-site differences for MBP were not significant. Total protein values for liver tissues were highest at the Slocan site.

Concentrations of the three metals were highest at the Slocan reference site in both the cytosolic and cellsolid compartments, except for cellular Cu which was highest in livers from the Beaver Creek site. Cytosolic to cellular metal ratios were also calculated. ANOVA followed by Tukey's test for differences showed that Slocan whitefish livers contained significantly higher proportions of each metal in the cytosol. Over 50% of the total Cd was present in the cytosols of Slocan fish, compared with under 20% at the two other sites. Copper in the cytosols accounted for between 21 an 43% of the total. However, between 95 and 98 % of the zinc was bound to the cellular fraction.

Significant correlations (Pearson Product Moment; P < 0.05; $\alpha = 0.05$) were found for Cd and Cu in Beaver Creek tissue and cytosolic compartments after removal of one outlier (i.e. Fish 155). No significant correlations were established for either of the other two locations. Multiple regression (ridge) analysis was performed on the Beaver Creek data to establish possible relationships between MBP and the three metals in cytosolic and cell-solid compartments. The final linear regression model equation was:

MBP =
$$57.48 + 0.115 \text{ Cu}_{tis} + 1.274 \text{ Cd}_{cvt}$$
 $R^2 = 0.805, P < 0.00007$

where Cutis and Cdcvt are the cell-bound copper and cytosolic cadmium, respectively.

Data for Cu and Zn mean concentrations in muscle were compared to those for livers at the respective locations (after first converting the muscle data to molar concentrations). Ratios (liver/muscle) for Slocan and Genelle were essentially equal at about 8.6 for copper, while that for the Beaver Creek samples was much higher at 29.7. This reflected somewhat lower values for the muscle and a 1.5 to 2-fold increase in liver concentrations at the latter site. Zinc data reflected the same trend but numerical differences were less striking (Slocan: 5.7; Genelle: 5.4; Beaver Creek: 7.1).

Correlations of MBP data with cytosolic- and tissue-bound metals in livers are important because only those for the Beaver Creek reach carry statistical significance. This suggests that although MBP concentrations are not any greater than at the other two sites, they may be driven by exposure to metals in the river below the discharges at Trail. It has been established previously that increased induction of MBP is a response to enhanced exposure to metals and serves as a detoxification process (Kägi and Nordberg, 1979). Particles of what appeared to be slag in stomach contents of fish (see p. 9) taken at Beaver Creek could be a direct source of metals through the chemical action of gastric fluids. The strong correlation between cell-bound copper, cytosolic cadmium and MBP as demonstrated by the linear regression model may serve as a useful indicator of temporal changes in bioavailable quantities of these metals. If slag particles are a major vector for metals it might be expected that the correlation significance will decrease following reduction in discharges (scheduled for December 1995) and subsequent long-term burial or dilution by riverine sediments, however resuspension of sedimented slag could lengthen the period of availability.

The comparison of liver to muscle data for Cu and Zn on a molar basis was also interesting. Ratios for Cu were identical for Slocan and Genelle, while the value for the Beaver Creek samples increased over three-fold. This is a strong indicator that this metal is being acquired in greater amounts by fish below the smelter and is being stored preferentially in the liver while Cu uptake by fish from Genelle and Slocan is through natural sources. This result gives further support to the strong relationship between Cu and MBP as shown by the above regression equation.

CONCLUSIONS AND RECOMMENDATIONS

Elemental analysis of mountain whitefish muscle was of limited utility due to high detection limits for many analytes. Copper, mercury, strontium and zinc data were obtained and were subjected to statistical analysis. In general, with the exception of mercury, concentrations were significantly higher at the reference site on the Slocan River. Mercury concentrations were highest at the Genelle site and may relate to compounds of the element which had historical uses in the pulp and paper industry.

Metal-binding proteins and the associated metals, copper, cadmium and zinc were determined in 45 white-fish livers. A multivariate linear regression analysis of the data provided a model which correlated MBT strongly with tissue-bound copper and cytosolic cadmium only in fish from the Beaver Creek site. Comparison of liver and muscle Cu and Zn data indicated that Cu (and to a lesser extent, Zn was being stored preferentially) in fish livers from Beaver Creek. It was concluded that smelter slag particles could be a vector for these metals

It is recommended that future sampling and analysis should focus on the liver metals, including mercury, and metal binding proteins. More sensitive analytical techniques will be required to provide useful, ecosytem-relevant information on metals in muscle tissues.

TABLE 15. Meristic Data for Mountain Whitefish Collected for Metal and Protein Determinations.

	Sa	ampling Locati	on	Analysis of Variance: 2-way					
Variable	Slocan	Genelle	Beaver	Location (p)	Sex (p)	Location x Sex (p)	MRT		
Age (y)	6.4 (1.3)	11.7 (1.2)	5.4 (1.1)	0.001*	0.055	0.349	B=S <g< td=""></g<>		
Weight (g)	233.3 (28.5)	418.5 (27.5)	418.5 (24.6)	< 0.001*	0.056	0.982	S <b=g< td=""></b=g<>		
Length (cm)	27.4 (0.9)	33.7 (0.9)	31.4 (0.8)	< 0.001*	0.064	0.694	S <b=g< td=""></b=g<>		

Probabilty ($\alpha = 0.01$). Asterisk indicates significance at that value of p.

Duncan's Multiple Range Test. Increasing values of the means. Underlining denotes no significant difference between sites. Mean (Std. Error) in mg.kg⁻¹.

TABLE 16. Trace Metal Data: Mountain Whitefish Muscle.

	Sa	ampling Location	on	Analysis of Variance Results					
Trace Metals	Slocan	Genelle	Beaver	Location (p)	Sex (p)	Location x Sex (p)	HSD (Location)		
Copper (Cu)	2.15 (0.39)	1.59 (0.21)	0.85 (0.12)	< 0.001*	0.015*	0.471	B <g=s< td=""></g=s<>		
Mercury (Hg)	0.11 (0.02)	0.29 (0.06)	0.15 (0.03)	0.007*	0.067	0.116	S=B <g< td=""></g<>		
Strontium (Sr)	3.56 (0.39)	1.70 (0.84)	0.84 (0.10)	<0.001*	0.571	0.97	B=G <s< td=""></s<>		
Zinc (Zn)	21.1 (1.0)	17.7 (1.4)	15.3 (1.2)	0.003*	0.259	0.669	B=G <s< td=""></s<>		

Slocan: N = 15; Genelle: N = 16; Beaver Creek: N = 20.

p = probability (a = 0.05)

HSD = Tukey's Honest Significant Difference Test for unequal sample sizes; p < 0.05.

Underlining denotes differences between locations are insignificant.

Mean (standard error of the mean). Units of mg.kg1 (dry weight).

^{*} denotes significance at the given \propto

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TABLE 17: Data and Statistics Summary for Metals and Proteins in Mountain Whitefish Liver (1992).

Site	MBP (μmol·kg ⁻¹)	Total Protein	Cytosolic Metals (μmol·kg¹)			Cellular Metals (µmol·kg¹)			Cytosol/Cellular (μmol·kg ')		
			Cd	Cu	Zn	Cd	Cu	Zn	Cd	Cu	Zn
Slocan	188 (27)	361 (21)	39.7 (15.2)	138 (32.8)	88.9 (11.3)	71.6 (20.3)	295 (45)	1872 (103)	0.504 (0.035)	0.429 (0.045)	0.047 (0.005)
Genelle	132 (18)	338 (17)	3.34 (1.2)	47.3 (8.6)	40.2 (3.1)	17.4 (3.7)	216 (21)	1495 (79)	0.161 (0.028)	0.213 (0.031)	0.027 (0.002)
Beaver Creek	142 (9)	303 (12)	11.1 (2.7)	130 (27)	38.6 (2.8)	63.4 (13.0)	400 (55)	1695 (64)	0.175 (0.024)	0.314 (0.028)	0.023 (0.024)
Tukey Test			G <b=s< td=""><td>G<b=s< td=""><td>B=G<s< td=""><td>G<b=s< td=""><td>G<s=b< td=""><td>G<b=s< td=""><td>G=B<s< td=""><td>G<b=s< td=""><td>G=B<s< td=""></s<></td></b=s<></td></s<></td></b=s<></td></s=b<></td></b=s<></td></s<></td></b=s<></td></b=s<>	G <b=s< td=""><td>B=G<s< td=""><td>G<b=s< td=""><td>G<s=b< td=""><td>G<b=s< td=""><td>G=B<s< td=""><td>G<b=s< td=""><td>G=B<s< td=""></s<></td></b=s<></td></s<></td></b=s<></td></s=b<></td></b=s<></td></s<></td></b=s<>	B=G <s< td=""><td>G<b=s< td=""><td>G<s=b< td=""><td>G<b=s< td=""><td>G=B<s< td=""><td>G<b=s< td=""><td>G=B<s< td=""></s<></td></b=s<></td></s<></td></b=s<></td></s=b<></td></b=s<></td></s<>	G <b=s< td=""><td>G<s=b< td=""><td>G<b=s< td=""><td>G=B<s< td=""><td>G<b=s< td=""><td>G=B<s< td=""></s<></td></b=s<></td></s<></td></b=s<></td></s=b<></td></b=s<>	G <s=b< td=""><td>G<b=s< td=""><td>G=B<s< td=""><td>G<b=s< td=""><td>G=B<s< td=""></s<></td></b=s<></td></s<></td></b=s<></td></s=b<>	G <b=s< td=""><td>G=B<s< td=""><td>G<b=s< td=""><td>G=B<s< td=""></s<></td></b=s<></td></s<></td></b=s<>	G=B <s< td=""><td>G<b=s< td=""><td>G=B<s< td=""></s<></td></b=s<></td></s<>	G <b=s< td=""><td>G=B<s< td=""></s<></td></b=s<>	G=B <s< td=""></s<>

Means based on N = 15 for Slocan and Genelle; N = 14 for Beaver Creek. Standard error of the mean in parentheses.

FIGURE 11. Concentration of Copper in Mountain Whitefish Muscle, sampled from the Columbia River and Slocan River Reference Site.

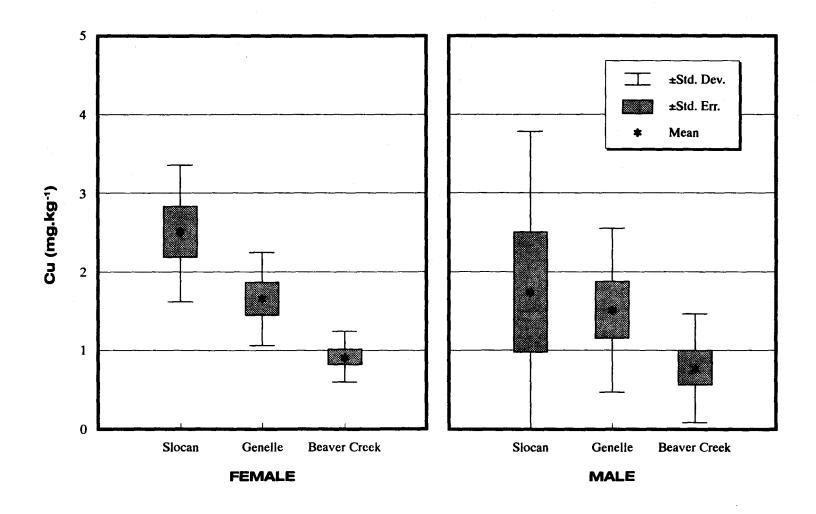


FIGURE 12. Concentration of Mercury in Mountain Whitefish Muscle, sampled from the Columbia River and Slocan River Reference Site.

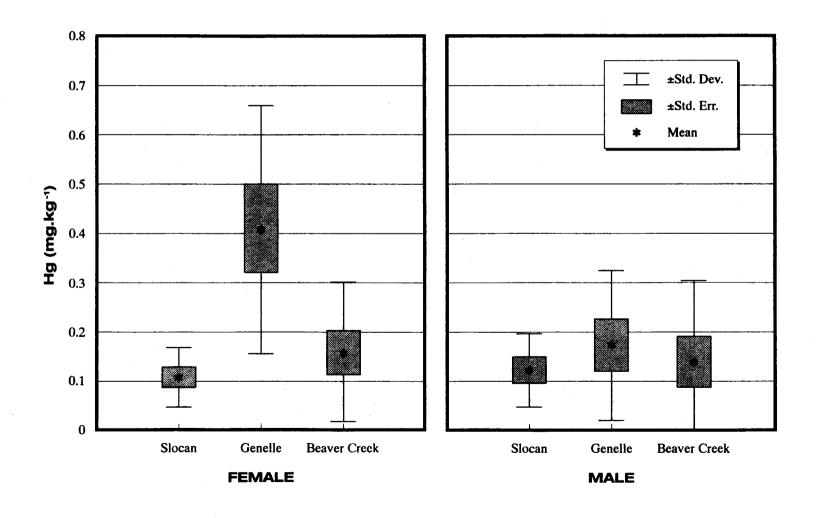


FIGURE 13. Concentration of Strontium in Mountain Whitefish Muscle, sampled from the Columbia River and Slocan River Reference Site.

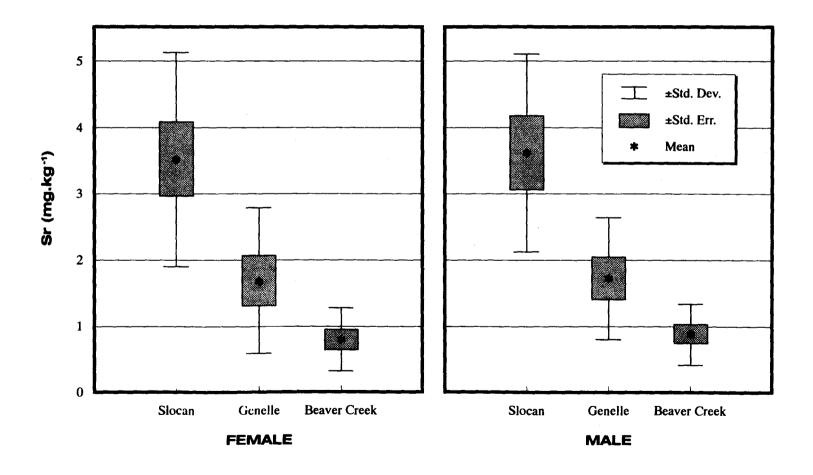
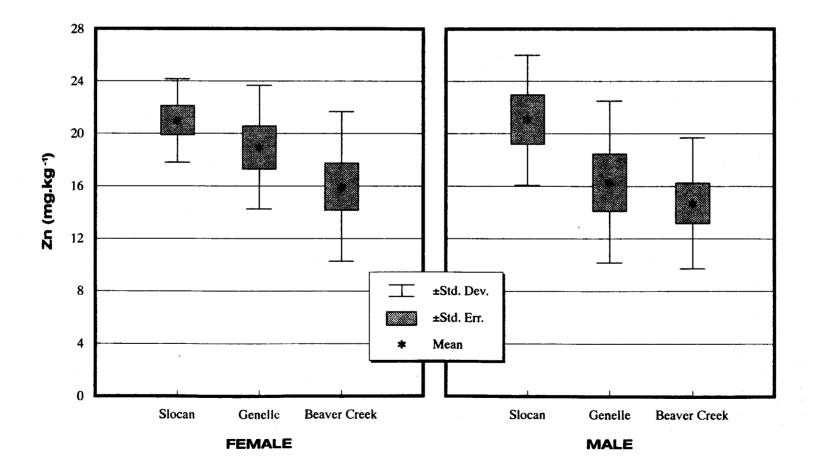


FIGURE 14. Concentration of Zinc in Mountain Whitefish Muscle, sampled from the Columbia River and Slocan River Reference Site.



OVERALL SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Results of the fish health assessment were similar to results obtained in a 1991 study (Boyle et al., 1992). Fish from the Genelle and Beaver Creek reaches of the Columbia River appeared to have compromised health in comparison with fish from the reference site. Fish from Beaver Creek and the Slocan reference site were similar in age, allowing legitimate comparison of the health data from these two reaches, while fish from Genelle were significantly older than fish from the other two sites. Abnormalities typically associated with bleached kraft pulp mill effluent were not found in Columbia River fish, however they did have stress-related health conditions.

Fork length was found to be a poor predictor of age, making it difficult to obtain similarly aged fish from different reaches even when restricted size classses were sampled. Where age is an important factor in future mountain whitefish studies, this weak relationship must be considered. Future study designs should address this difficulty by either further limiting size classes for sampling, or by increasing the sample size.

Levels of dioxins, furans, and PCBs were significantly higher in Columbia River whitefish muscle compared with fish from the Slocan reference site. Concentrations of dioxins and furans appear to be declining in Columbia River fish following improvements made to the upstream pulp mill. Previous sampling of PCBs was too limited in terms of sample sizes and congeners assayed to determine whether muscle concentrations of PCBs are declining in Columbia River mountain whitefish.

Body burdens of dioxins and furans and total T4CDD toxic equivalents did not appear to be correlated with cumulative disease severity at any of the reaches sampled, nor when data from the two Columbia River reaches were combined. A possible relationship could have been obscured by other factors, due to the complex environment in which these fish live.

Interestingly, muscle concentrations of non-ortho substituted (coplanar) PCBs were found to be related to CDS when data from the Genelle and Beaver Creek fish were combined. CDS was also related to the sum of TEQs calculated from the muscle concentrations of individual congeners as per Table 3. This may reflect the fact that two of the three non-ortho substituted PCBs have very high TEF values. As PCBs accounted for approximately one third of the T4CDD TEQs in Columbia River fish, they may become the greatest organic contaminant of concern here if dioxin and furan levels continue to decline. Levels of Arochlors do not appear to have declined since 1991, however few samples were obtained in the earlier study so comparisons are of limited value. No specific sources of PCBs in the portion of the Columbia River being studied have yet been identified. If data collected in 1994 do not show a decline in PCB concentrations in whitefish muscle, possible sources of these contaminants should be investigated.

Levels of mixed function oxidases (EROD and AHH) measured in livers of mountain whitefish in the present study have declined since the January 1991 sampling. The observed decrease in these enzyme activities is likely because of two factors: 1) reduced loadings of dioxins and furans to the Columbia River; and 2) the pulp mill strike, which would have eliminated the short-term MFO inductions observed in other studies on bleached kraft pulp mill effluent. Sampling completed in 1994 will confirm whether the declines are real, likely resulting from improvements at the pulp mill, or whether they were largely an artifact of the strike.

Results of metal analyses in muscle and liver proved interesting. Most muscle metal concentrations were highest in fish from the reference site, a surprise considering the relatively high metal loadings to the Columbia River at Trail. The levels in Slocan fish likely reflect naturally high levels in the environment. The Slocan area is known to be rich in some metals, as reflected by historic mining activity. Despite the higher muscle concentrations in Slocan fish, liver metals at this site were not correlated with metal-binding

proteins, suggesting that metals here were not a source of physiological stress. A multivariate linear regression analysis of the data provided a model which correlated MBT strongly with tissue-bound copper and cytosolic cadmium only in fish from the Beaver Creek site. Comparison of liver and muscle Cu and Zn data indicated that Cu (and to a lesser extent, Zn) was being stored preferentially in fish livers from Beaver Creek. It was concluded that smelter slag particles could be a vector for these metals.

It is recommended that future metals sampling and analysis should focus on the liver metals, including mercury, and metal binding proteins. Because most metals proved to be non-detectable in fish muscle it is concluded that more sensitive analytical techniques will be required to provide useful, ecosytem-relevant information on metals in mountain whitefish muscle tissues.

ACKNOWLEDGEMENTS

This project was a cooperative effort, and would not have been possible without the assistance of many people. Members of the CRIEMP Committee reviewed the program proposal and offered valuable input. The authors wish to thank Harry Connell and members of the Trail Wildlife Association, Brad Turner and friends from the Slocan area, and volunteers from the Trail and Castlegar area, for the time which they put into angling whitefish for this project. John Bell from the Ministry of Environment, Lands, and Parks (MELP) in Nelson also helped by organizing the fish angling. In addition we wish to thank B.C. Hydro, R.L. & L. Environmental Services Ltd. of Edmonton, Gerry Oliver (MELP - Cranbrook) and Les McDonald (MELP -Cranbrook) for carrying out the electroshocking. Les McDonald also generously provided the MELP mobile laboratory for the field program. The Fish Aging Laboratory at Pacific Biological Station (P.B.S.) in Nanaimo is gratefully acknowledged for aging all of the fish used in this study. K. Mullen and C. Westby of P.B.S. performed the viral and bacteriological analysis. J. Bagshaw of P.B.S. carried out the histological evaluation. T. MacDonald of P.B.S. performed the examination for selected parasites. Nes Sutherland performed the metal-binding protein and metal analyses of liver samples. Dave McKenna (Institute of Ocean Science, Sidney, B.C.) offered extensive advice, and is also thanked for preparing muscle samples for analyses of organic contaminants. Dave Sergeant provided guidance in writing up analytical contracts for this project. Erick Groot was invaluable as a project coordinator as well as in the field. Steve Sheehan and Gordon Ennis of the Eastern B.C. Unit of DFO supported the program in numerous ways, including the securing of Green Plan funds, and providing the program with its initial direction. Bonnie Antcliffe of the Eastern B.C. Unit coordinated the circulation of multiple drafts to authors and reviewers, and provided advice on statistics and valuable input in her review of the drafts. Al Colodey from DOE provided helpful input and offered many productive discussions. Many others from Environment Canada, MELP, DFO, Celgar, Cominco, and the public, are thanked for their generous assistance which was offered in many different ways.

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APPENDIX 1. Biological characteristics of Mountain Whitefish (*Prosopium williamsoni*) sampled from two sites within the Columbia River and a reference site within the Slocan River, July 6 - 15, 1992.

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index ^j
1	Slocan (July 6,1992)	F	5	183	256	169.5	6.4	2.8	1.0	•	-	1.09
2	S	M	6	223	281	209	10.5	0.2	1.4	-	-	1.01
3	S	F	5	194	261	175	7.2	3.7	1.2	-	1 a	1.09
4	S	F	6	215	277	204	6.5	0	0.8	-	7	1.01
5	S	F	9	231	283	215	6.2	3.5	1.5	-	-	1.02
6	S	M	5	232	282	214	8.1	0.4	1.7	-	-	1.03
7	S	F	9	289	303	263	9.7	6.1	2.2	-	•	1.04
8	S	F	11	570	370	516	28.4	10.8	4.5	-	2	1.13
9	S	M	3	134	232	125	5.2	•	0.7	•	-	1.07
10	S	F	9	535	315	471	29.6	13.3	3.5	-	-	1.71
11	S	M	6	212	274	195	8.1	1.1	1.0	•	-	1.03
12	S	M	9	231	281	214	10.2	0.4	1.4	-	1	1.04
13	S	F	6	231	269	207	9.5	4.0	1.6	-	-	1.19
14	S	F	6	303	292	269	13.0	7.7	2.2	•	-	1.22
15	S	F	5	252	283	227	7.1	4.9	1.6	-	-	1.11
16	S	M	5	251	278	-	6.9	2.9	1.2	-	-	1.17
17	S	F	6	269	290	246	7.2	5.2	2.1	-	-	1.10
18	S	M	6	216	268	201	8.3	0.4	0.8	•	-	1.12
19	S	F	5	239	283	212	9.6	3.6	1.1	4	-	1.05
20	S	M	3	125	239	118	3.4	0.1	0.6	_	•	0.92

APPENDIX 1. continued.

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
21	Slocan (July 6,1992)	M	5	173	264	163	5.1	0.1	1.0	-	9	0.94
22	S	M	5	187	264	176	4.1	0.3	0.7	-	-	1.02
23	S	M	4	217	273	197	9.5	0.7	1.4	-	1	1.07
24	(July 7,1992)	M	4	200	271	181	7.8	0.1	1.3	-	-	1.01
25	S	М	5	167	260	155	6.2	0.1	1.2	-	-	0.95
26	S	F	6	253	293	233	9.1	2.9	1.7	5	-	1.01
27	S	F	3	145	245	136	3.1	1.9	0.7	-	•	0.99
28	S	F	12	387	333	343	19.3	5.5	2.9	-	1	1.05
29	S	F	3	76	194	69	1.9	0.1	0.4	-	-	1.04
30	S	F	18	606	367	553	16.4	24.5	4.0	-	- :	1.23
31	S	M	4	134	232	123	2.9	0.1	0.9		8 ⁱ	1.07
32	S	F	3	125	229	114	3.4	1.8	1.0	-	-	1.04
33	S	F	3	109	213	101	2.2	0.3	0.6	-	-	1.13
34	(July 8,1992)	F	2	103	215	95.3	3.3	0.1	0.5	3	Ek	1.04
35	S	M	4	167	253	154	6.3	0.2	1.1	<u>-</u>	-	1.03
36	S	F	6	265	286	247.6	4.7	3.9	0.9	-	-	1.13
37	S	M	10	269	290	242.4	7.8	1.4	1.2	-	3,4	1.10
38	S	F	7	265	295	239.9	9.5	3.6	2.1	-	-	1.03
39	S	-	3	95	204	89.4	1.6	0.1	0.5	-	4,5	1.12
40	S	M	3	86	202	79.5	1.7	-	0.7	-	-	1.04

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
41	Slocan (July 8,1992)	F	11	367	324	317.5	14.9	9.3	3.1	•	_i	1.08
42	S	F	13	385.1	324	340.1	16.0	10.4	3.1	•	4 ⁱ	1.13
43	S	F	3	86.6	197	79.9	2.0	0.1	0.5	-	_i	1.13
44	S	M	3	158	241	145.1	1.9	0.1	1.2	-	_i	1.13
45	S	F	3	154	244	143.0	3.1	2.4	0.7	*	_i	1.06
46	S	M	3	104.1	216	98	1.7	0.1	0.6	5,6	_i	1.03
47	S	F	6	198	265	-	4.3	4.1	1.4	-	_i	1.06
48	S	F	7	403.7	335	356.2	12.0	6.8	3.2	-	_i	1.07
49	(July 14,1992)	M	5	210	266	192.2	6.4	0.5	1.2	2	_i	1.12
50	S	M	9	295	296	271.0	7.5	0.9	1.9	-	_i	1.14
51	S	M	7	264.6	290	242.5	7.2	1.6	1.9	1	_i	1.09
52	S	F	11	384	334	348.3	8.6	8.9	2.0	2	_i	1.03
53	S	M	14	351	320	314.8	10.1	0.6	1.6	-	_i	1.07
54	S	M	10	302	312	275	6.4	0.4	1.3	1	9 i	0.99
55	S	F	11	344	305	303	11.1	10.3	2.4	-	_i	1.21
56	S	F	5	258	286	231	5.0	8.8	1.7	5	_i	1.10
57	S	F	5	277	300	258	5.3	4.1	1.4	5	_i	1.03
58	S	F	5	217	265	199	3.2	5.8	1.5	6	9	1.17
59	S	F	11	318	295	286	7.9	8.2	2.2	•	-	1.24
60	S	M	9	318	306	284	10.6	0.4	2.4	-	-	1.11

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
401	Slocan	М	3	129	226	120	-	•	-	-	-	1.18
	(July 14,1992)											
402	S	M	11	291	297	268	-	-	1.4	-	Α	1.11
403	S	F	9	269	296	238	-	-	1.4	1 ^b	-	1.04
404	S	M	4	168	253	156	-	•	0.8	-	-	1.04
405	S	F	7	288	294	261	•	-	2.4	-	Bh	1.13
406	s	M	5	186	260	-	-	•	0.9	-	-	1.06
407	S	M	4	143	242	131	•	-	0.7	•	•	1.01
408	S	F	-	170	247	153	-	•	1.0	-	-	1.13
409	S	M	3	164	246	142	-	•	0.6	-	•	1.10
410	S	M	4	187	247	172	-	-	0.4	-	-	1.24
61	Genelle	F	17	506	387	462	7.7	8.9	5.1	_c	1,C	0.87
	(July 9,1992)											
62	G	F	18	354	364	324	7.3	3.0	3.8	6 ^c	-	0.73
63	G.	F	16	407	354	380	6.5	4.6	1.7	-	-	0.92
64	G	M	16	496	388	458	11.9	0.8	4.5	-	1,6	0.85
65	(July 10,1992)	F	20	678	370	618	9.7	15.1	5.8	4d	1 ^e	1.34
66	G	F	17	510	357	449	16.9	12.8	4.5	6	0	1.12
67	G	M	3	403	320	358	9.0	1.9	3.5	_c	-	1.23
68	G	F	16	423	342	383	8.6	11.0	3.2	-	8	1.06
69	G	M	15	388	357	370	7.3	0.6	1.7	1 ^d	1,D	0.85

APPENDIX 1. continued.

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
70	Genelle (July 10, 1992)	М	17	609	407	575	12.1	3.0	3.0	1	Е	0.90
71	G	M	3	212	266	224	10.0	5.5	1.7	-	Ge	1.13
72	G	F	3	217	260	197	5.0	0.3	1.3	2	-	1.23
73	\mathbf{G}	F	17	757	406	686	19.8	20.0	3.9	-	•	1.13
74	G	F	18	443	356	408	8.6	10.0	2.5	1	1	0.98
75	G	M	16	349	356	352	6.8	-	0.6	-	-	0.77
76	G	M	18	324	342	303	5.5	1.1	-	1	8	0.81
77	G	F	16	604	373	545	18.7	15.3	4.8	-	G	1.16
78	G	F	18	808	425	731	16.5	13.0	7.5	-	-	1.05
79	G	F	3	354	293	326	4.3	3.7	1.4	-	-	1.41
80	G	F	3	278	283	255	2.9	1.3	0.9	-	-	1.23
81	G	F	3	397	306	348	7.0	13.2	2.4	-	-	1.39
82	G	M	16	327	345	305	6.2	0.6	1.9	1	-	0.80
83	G	F	16	264	316	243	6.0	2.9	2.1	1	-	0.84
84	G	F	19	448	387	422	5.4	2.7	2.2	1		0.77
85	G	M	14	387	335	348	12.1	0.5	2.3	_	-	1.03
86	G	M	10	504	346	455	8.1	9.7	2.8	-	-	1.22
87	G	F	19	429	374	385	10.2	7.0	3.0	1	-	0.82
88	G	F	16	365	347	323	5.7	2.0	2.9	8	5,17	0.87
89	G	M	4	442	316	389	9.3	22.0	2.8	-	-	1.40

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
90	Genelle (July10,1992)	F	19	664	398	597	13.6	18.3	6.0	-	_h	1.05
91	Ğ	F	10	547	347	487	12.0	23.5	4.3	-	_i	1.31
92	G	F	11	515	350	464	8.5	17.5	4.0	•	_i	1.20
93	G	F	18	393	354	362	7.1	6.4	2.0	1	_i	0.89
94	G	F	8	447	335	408	6.1	10.4	3.4	1,2	_i	1.19
95	G	F	20	429	340	382	10.8	11.3	4.0	-	_i	1.09
96	G	M	16	392	330	364	8.1	3.4	2.3	-	_i	1.09
97	G	F	17	469	365	424	10.1	10.5	3.3	1,2	_i	0.96
98	G	M	16	432	358	402	7.2	0.9	2.6	1	_i	0.94
99	G	F	20	475	373	417	18.9	10.0	3.5	1	_i	0.92
100	G	F	17	495	360	458	5.4	5.8	3.9	1	_i	1.06
101	G	F	15	525	371	490	5.8	9.5	2.2	1	_i	1.03
102	G	F	14	527	379	468	17.5	7.9	2.8	1,2	_i	0.97
103	\mathbf{G}	F	15	845	432	766	17.1	12.9	5.2	_	1 ⁱ	1.05
104	G	F	11	591	356	535	5.8	25.3	4.0	1	_i	1.31
105	G	F	18	491	380	455	6.7	12.1	3.4	•	8 ⁱ	0.89
106	G	F	8	605	400	544	12.6	12.9	5.7	2	- i	0.95
107	G	M	10	583	361	543	9.1	2.4	2.5	2	1 i	1.24
108	G	F	17	519	361	473	8.4	12.6	2.9	3	.i	1.10
109	G	M	4	435	323	393	7.8	7.2	2.8	-	1 ⁱ	1.29

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
110	Genelle (July 10,1992)	F	19	471	352	436	5.5	8.7	3.5	1	.i	1.08
111	G	F	19	644	399	587	13.5	11.8	4.9	-	1	1.01
112	(July 13,1992)	M	. 17	331	340	300	12.8	0.7	1.5	1	•	0.84
113	G	M	3	234	264	204	3.8	7.0	1.1	_d	-	1.27
114	G	М	3	353	298	318	5.7	1.4	2.6	-		1.33
115	. G	F	4	377	297	341	9.0	7.4	3.6	-	-	1.44
116	\mathbf{G}	F	16	471	352	430	•	10.7	3.4	-	-	1.08
117	G	M	3	281	285	253	6.3	0.6	0.9	-	-	1.21
118	G	F	23	435	404	402	-	5.0	3.3	1 ^d	8, D^{d,i}	0.66
119	G	M	17	461	428	437	-	-	2.0	1	1 , C	0.59
120	G	F	19	878	422	743	-	37.0	13.8	-	-	1.17
501	G	M	-	356	355	334	-	-	-	-	-	0.80
502	G	F	•	507	376	464	•	-	-	-	-	0.95
503	G	M	-	433	352	402	-	-	-	-	-	0.99
504	G	F	-	712	408	637	-	-	-	-	-	1.05
505	G	F	-	580	408	521	-	-	-	-	•	1.05
506	G	F	17	426	356	378	-	-	-	7	H	0.94
121	Beaver (July 15,1992)	M	18	-	360	334.9	6.0	0.3	1.3	1 ^c	1,E ⁱ	-
122	В	M	11	485	342	441.0	7.6	19.8	3.1	-	1 ⁱ	1.21

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
123	Beaver (July 15,1992)	М	6	553	333	476.0	14.6	32.3	3.6	•	_i	1.50
124	В	F	7	689	354	612	13.2	19.8	8.5	_c	_i	1.55
125	В	F	18	521	368	473	11.5	5.1	4.5	1	_i	1.05
126	В	F	4	373	307	329	8.1	10.6	3.8	-	_i	1.29
127	В	F	3	321	290	294	7.6	3.9	1.3	-	_i	1.32
128	В	F	2	303	293	262	6.1	8.1	1.9	•	4 ⁱ	1.20
129	В	F	3	360	302	314	11.7	10.0	3.7	-	_i	1.31
130	В	F	4	413	292	370	6.9	10.8	4.5	•	_i	1.66
131	В	F	18	441	367	396	12.4	13.5	4.7	•	_i	0.89
132	В	F	19	536	380	480	13.2	12.0	4.4	1	1 ⁱ	0.98
133	В	F	10	605	372	510	12.2	34.2	3.6	3 ^c	1 ⁱ	1.18
134	В	F	3	422	309	364	12.8	6.8	2.8	•	$\mathbf{D^{i}}$	1.43
135	В	M	3	418	290	369	6.3	20.9	2.0	7 g	Fi	1.71
136	В	M	11	310	355	297	6.6	0.3	0.8	1,4 ^b	_i	0.69
137	В	M	2	278	265	243	8.3	1.5	2.6	-	_i	1.49
138	В	М	3	376	293	327	8.0	15.2	1.9	-	F ⁱ	1.49
139	В	F	8	406	333	367	15.3	9.0	2.2	1,5 ^{b,d}	_i	1.10
140	В	F	20	418	362	382	10.6	7.2	3.4	1	_i	0.88
141	В	M	3	389	295	319.5	6.6	19.3	2.7	_d	3	1.52
142	В	F	3	250	260	205.6	7.0	0.1	0.8	-	•	1.42

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
143	Beaver (July 15,1992)	М	10	567	346	497	15.0	34.3	3.1	-	-	1.37
144	В	M	17	491	365	445	16.0	1.3	3.9	1,3 ^b	G	1.01
145	В	F	5	433	296	387	13.0	13.0	3.6	2,7	•	1.67
146	В	M	5	665	343	560	14.5	3.2	67.3	2	F	1.65
147	В	M	2	299	277	267	5.6	1.1	11.7	-	3	1.41
148	В	F	18	411	367	364	14.8	9.4	2.4	1	3	0.8
149	В	F	3	518	319	452	7.1	27.5	3.3	-	-	1.60
150	В	F	18	349	355	317	8.9	5.1	2.4	1	-	0.78
151	В	M	3	253	265	223	5.7	1.1	1.5	-	-	1.36
152	В	F	3	430	295	385	6.9	11.7	3.1	3	-	1.67
153	В	M	10	598	351	529	11.1	37.3	3.3	-	F	1.38
154	В	M	3	421	303	365	7.0	18.4	2.6	2	-	1.51
155	В	F	10	507	354	461	10.1	14.4	3.8	-	-	1.14
156	В	F	4	551	338	490	6.3	17.9	4.5	2	-	1.43
157	В	M	3	344	300	316	7.9	1.8	2.1	-	-	1.27
158	В	M	6	489	325	434	9.6	24.1	2.4	2	•	1.42
159	В	M	4	402	306	363	6.5	9.3	1.4	-	-	1.40
160	В	F	4	410	325	368	10.1	13.2	3.1	2	-	1.19
161	В	F	4	465	305	409.6	6.7	19.8	1.7	-	-	1.64
162	В	M	16	415	354	382	12.6	0.8	2.9	1	1	0.94

Fish No.	Site (Date Sampled)	Sex	Age (yr)	Wet Weight (g)	Fork Length (mm)	Gutted Weight (g)	Stomach Weight (g)	Gonad Weight (g)	Liver Weight (g)	External Signs	Internal Signs	Condition Index
163	Beaver (July 15,1992)	F	11	564	374	505	14.2	16.0	3.9	•	1	1.08
164	В	F	3	322	280	283	7.0	2.3	2.0	•	_	1.47
165	В	F	4	491	339	421	13.3	16.3	6.5	2	_	1.26
166	В	M	7	499	338	452	6.2	16.2	3.0	2	_	1.29
167	В	M	4	431	305	322	10.8	18.7	2.7	-	-	1.52
168	В	М	4	303	271	276	4.7	7.8	1.2	-	-	1.52
169	В	M	2	347	283	311	6.6	4.4	2.0	2	-	1.53
170	В	M	3	408	305	362	8.5	17.8	2.5	•	-	1.44
171	В	F	12	584	363	533	-	-	-	-	-	1.22
172	В	F *	12	667	352	599	-	-		-	-	1.53
173	В	F	3	578	347	497	-	-	-	•	•	1.38
174	В	F	15	542	323	490	-	-	-	1		1.61
175	В	F	16	467	376	414	_	-	-	1	1	0.88
176	В	F	8	578	353	513	-	-	-	-	-	1.31
177	В	F	5	467	325	413	-	-	-		-	1.36
178	В	F	5	580	335	513	-	-	-	-	-	1.54
179	В	F	5	440	315	382	-	-	-	-	-	1.41
180	В	M	3	350	300	313	-	-	_	-		1.30

Notes: "-" indicates not determined

a: for key to external and internal abnormalities see Appendix 2

b: moribund

c: dead

d: picture taken

e: lots of body fat

f: little body fat

g: scoliosis

h: cyst identified as Eustrongyloides larvae

i: cultured on Lowenstein-Jensen medium

j: condition index =

(wet weight (g) x fork length (mm))⁻³ x 10⁵

k: Letters were used to indicate abnormalities

#10 - 17 (see Appendix 2)

A= abnormality #10 E= 14 B= 11 F= 15 C= 12 G= 16 D= 13 H= 17

APPENDIX 2. Rating of Abnormalities found in Mountain Whitefish (*Prosopium williamsoni*) sampled from two sites within the Columbia River and a reference site within the Slocan River, July 6 - 15, 1992, to obtain Cumulative Disease Severity (CDS).

Rating Code: 1 = light 2 = moderate	3 = severe
Abnormality	Rating
External	
1. thin	2
2. dark gills	1 2
pale gills mechanical damage	1
5. hemorrhagic spot:at fins	2
6. hemorrhagic spot on belly	2
7. other	1
8. neoplastic disease	3
Internal	
1. enlarged spleen	2
2. parasite lesion/cyst	1
3. adhesions 4. pale liver	1
5. grey liver	1
6. brown liver	1
7. small liver	2
8. liver lesion	2
9. lumpy spleen	1
10. cyst in spleen	1
11. cyst in pyloric caeca 12. cyst in liver	1 1
	1
13. cyst in kidney 14. cyst in ovary	1
15. ripe	Ō
16. hemorrhagic area	2
17. other	11
Gill	
1. aneurysms	1
2. protozoan parasites	1
metazoan parasite focal hyperplasia	1 2
	2
5. cysts 6. lamellar clumping	3
7. other	1
Liver	
1. inflammatory foci	3
2. melanosis/hemosiderin deposits	2 2
3. diffuse inflammation	2 1
4. parasite cysts	
5. foamy areas (collection of foam cells)6. cirrhosis	2 3
7. cysts	
8. tumour	2
9. hemorrhagic area	2 2
10. granulomas	
11. other	1

Abnormality	Rating
Kidney	
protozoan melanin deposits interstitial hypercellularity (hyperplasia) other parasite	1 1 2 1
5. tumour6. granulomas: head kidney7. granulomas: renal interstitium8. melano-macrophage activity	3 2 2 2
9. lymphocytosis (lymphoid infiltration) 10. other	3 1
Pyloric caeca	
hypercellular submucosa granulomas in mesentery foamy lesion in pancreas tumour	2 2 2 3
5. metazoan parasites6. other	1 1
Hindgut	
hypercellular submucosa metazoan parasite lymphoid infiltration cysts	2 1 2 2
5. neoplasm 6. other	3 1
Other	
1. tumour 2. spleen: melanin deposits 3. spleen: granulomas 4. other	3 1 2
Myxobacteria (Gram stain)	
no bacteria l. long thin gram negative rods	0 2
Other bacteria (TSA culture)	
0. no growth 1. ERM bacteria	0 2
Growth on Lowenstein Jensen +/or acid-fast ba	cteria
no bacteria acid fast or growth	0 3

APPENDIX 2. continued.

Summary of Abnormalities and Cumulative Disease Severity found in Gross Examination and Histological Evaluation of Mountain Whitefish (*Prosopium williamsoni*), sampled from the Slocan River, and two sites within the Columbia River, July 6 - 15, 1992.

			Nu	mber of Abnormali	ties
Organ Examined	Abnormality	Abnormality Rating	Slocan (n=69)	Genelle (n=61)	Beaver (n=60)
External	1. thin 2. dark gills 3. pale gills 4. mechanical damage 5. hemorrhagic spot at fins 6. hemorrhagic spot at belly 7. other 8. neoplastic disease Total: Cumulative Disease Severity: al 1. enlarged spleen es 2. parasite lesion/cyst 3. adhesions 4. pale liver 5. grey liver 6. brown liver 7. small liver 8. liver lesion 9. lumpy spleen 10. cyst in spleen 11. cyst in pyloric caeca 12. cyst in kidney	2	3	21	12
		1	2	6	9
		2	1 .	1	3
	4. mechanical damage	1	0	1	1
	5. hemorrhagic spot at fins	2	4	0	1
	6. hemorrhagic spot at belly	2	2	2	0
	7. other	1	0	1	2
	8. neoplastic disease	3	0	1	0
	Total:		12	33	28
	Cumulative Disease Severity:		22	59	44
Gross Internal	1. enlarged spieen	2	4	10	7
Abnormalities		1	1	0	0
		1	1	Ŏ	3
		1	3	Ō	1
	5. grev liver	1	1	0	0
		1	0	2	Õ
		2	1	0	ő
		2	1	4	ő
	9. lumpy spleen	1	3	0	0
		1	1	0	0
		1	1	0	0
		1	0	2	0
	13. cyst in kidney	1.	0	2	1
	14. cyst in ovary	1	1	1	1
	15. ripe	0	0	0	4
	16. hemorrhagic area	2	0	2	1
	17. other	1	0	1	0
	Total:		18	24	18
<u> </u>	Cumulative Disease Severity:		24	40	22
Histological Evaluation					
Gill	1. aneurysms	1	6	5	2
	2. protozoan parasites	1	1	3	Ö
	3. metazoan parasites	1	2	0	1
	4. focal hyperplasia	2	0	0	. 0
	5. cysts	2	0	1	1
	6. lamellar clumping	3	0	0	i
	7. other	1	0	0	0
	Total:		9	9	5
	Cumulative Disease Severity:		9 -	10	8

APPENDIX 2. continued.

		_	Nu	mber of Abnormali	ties
Organ Examined	Abnormality	Abnormality Rating	Slocan (n=69)	Genelle (n=61)	Beaver (n=60)
Liver	1. inflammatory foci	3	5	26	!1
	2. melanosis/hemosidern deposits	2	0	8	9
	3. diffuse inflammation	2	1	0	1
	4. parasite cysts	1	0	7	4
	5. foamy areas	2	0	8	0
	6. cirrhosis	3 2	0	3 1	0
	7. cysts 8. tumour	3	0	2	0 0
			_	_	
	9. hemorrhagic area 10. granulomas	2 2	0 0	0 3	7
	11. other	1	Ö	1	í
	Total:			50	
			6	59	34
	Cumulative Disease Severity:	·	17	141	74
Kidney	1. protozoan	1	1	0	0
	2. melanin deposits	1	0	5	5
	3. interstitial hypercellularity	2	0	2	3
	4. other parasite	1	0	1	0
	5. tumour	3	0	2	0
	6. granulomas: head kidney	2	0	2	2
	7. granulomas: renal interstitium 8. melano-macrophage activity	2 2	0 0	0	0 1
	• •		•		-
	9. lymphocystosis 10. other	3 1	0	0 0	2 0
		1 '			
	Total:		1	13	13
	Cumulative Disease Severity:		1	22	23
Pyloric Caeca	1. hypercellular submucosa	2	0	2	1
	2. granulomas in mesentery	2	0	2	2
	3. foamy lesion in pancreas	2	0	1	0
	4. tumour	3	0	2	0
	5. metazoan parasites	1	0	0	1
	6. other	1	0	0	0
	Total:		0	7	4
	Cumulative Disease Severity:		0	16	7
Hindgut	1. hypercellular submucosa	2	1	4	0
	2. metazoan parasites	1	Ö	Ó	. 0
	3. lumphoid infiltration	2	0	1	0
	4. cysts	2	0	0	0
	5. neoplasm	3	1	2	0
	6. other	1	0	1	0
	Total:		2	8	0
	Cumulative Disease Severity:		5	17	0
Other	1. tumour	3	0	1	0
Oulei	2. spleen: melanin deposits	1	0	1	1 .
	3. spleen: granulomas	2	ő	i	Ô
	4. other	1	0	1	0
	Total:		0	4	1
	IOIAI:				

APPENDIX 2. continued.

			Nu	mber of Abnormali	ties	
Organ Examined	Abnormality	Abnormality Rating	Slocan (n=69)	Genelle (n=61)	Beaver (n=60)	
Myxobacteria	1. no bacteria	0	64	55	60	
•	2. long thin gram-negative rods	2	5	6	0	
	Total:		5	6	0	
	Cumulative Disease Severity:		10	12	0	
Other Bacteria	1. no growth	0	68	61	60	
	2. ERM bacteria	2	1	0	0	
	Total:		1	0	0	
	Cumulative Disease Severity:		2	0	0	
Growth on						
Lowenstein Jensen	1. no bacteria	0	64	52	56	
+/or acid-fast bacteria	2. acid fast or growth	3	5	9	4	
	Total:		5	9	4	
	Cumulative Disease Severity:		15	27	12	

APPENDIX 3. Raw Dioxin and Furan Data for Individual Fish. Dioxin and Furan Concentrations (pg/g) for Individual Mountain Whitefish.

					Lo	cation =	SLOC	AN RIVE	R						
Sample	S02	803	S06	S07	S08	S09	S10	S11	812	S 13	S15	S 16	S18	S20	S52M
2,3,7,8-TCDD	0.16	0.11	0.16	0.13	0.16	0.16	0.23	0.10	0.13	0.12	0.09	0.11	0.07	0.10	0.10
Total TCDD	0.16	0.11	0.16	0.13	0.16	0.16	0.23	0.10	0.13	0.12	0.09	0.11	0.07	0.10	0.10
1,2,3,7,8-PCDD	0.26	0.21	0.23	0.26	0.25	0.31	0.19	0.10	0.22	0.21	0.10	0.11	0.05	0.10	0.11
Total PCDD	0.26	0.21	0.23	0.26	0.25	0.31	0.19	0.10	0.22	0.21	0.10	0.11	0.05	0.10	0.11
1,2,3,4,7,8-HxCDD	0.38	0.28	0.38	0.36	0.34	0.38	0.32	0.11	0.25	0.28	0.17	0.16	0.11	0.10	0.14
1,2,3,6,7,8-HxCDD	0.38	0.28	0.38	0.36	0.34	0.38	0.32	0.11	0.25	0.28	0.17	0.16	0.11	0.10	0.14
1,2,3,7,8,9-HxCDD	0.38	0.28	0.38	0.36	0.34	0.38	0.32	0.11	0.25	0.28	0.17	0.16	0.11	0.10	0.14
Total HxCDD	0.38	0.28	0.38	0.36	0.34	0.38	0.32	0.11	0.25	0.28	0.17	0.16	0.11	0.10	0.14
1,2,3,4,6,7,8-HpCDD	0.43	0.42	0.60	0.51	0.72	0.63	0.45	0.20	0.40	0.52	0.18	0.20	0.24	0.23	0.30
Total HpCDD	0.43	0.42	0.60	0.51	0.72	0.63	0.45	0.20	0.40	0.52	0.18	0.20	0.55	0.23	0.30
OCDD	1.60	3.00	0.70	1.00	0.99	0.79	2.40	0.40	2.10	0.61	0.49	0.26	0.78	0.40	0.29
2,3,7,8-TCDF	0.17	0.24	0.19	0.23	0.47	0.18	0.45	0.10	0.09	1.60	0.57	0.21	0.40	0.16	0.37
Total TCDF	0.17	0.24	0.19	0.23	0.47	0.18	0.45	0.10	0.09	1.60	0.57	0.21	0.55	0.16	0.37
1,2,3,7,8-PCDF	0.16	0.18	0.17	0.18	0.20	0.21	0.17	0.10	0.15	0.14	0.10	0.09	0.08	0.10	0.09
2,3,4,7,8-PCDF	0.16	0.18	0.17	0.18	0.20	0.21	0.17	0.10	0.15	0.14	0.10	0.09	0.08	0.10	0.09
Total PCDF	0.16	0.18	0.17	0.18	0.20	0.21	0.17	0.10	0.15	0.14	0.10	0.09	0.13	0.10	0.09
1,2,3,4,7,8-HxCDF	0.30	0.27	0.35	0.30	0.32	0.38	0.42	0.10	0.24	0.28	0.18	0.14	0.08	0.10	0.12
1,2,3,6,7,8-HxCDF	0.30	0.27	0.35	0.30	0.32	0.38	0.42	0.10	0.24	0.28	0.18	0.14	0.08	0.10	0.12
2,3,4,6,7,8-HxCDF	0.30	0.27	0.35	0.30	0.32	0.38	0.42	0.10	0.24	0.28	0.18	0.14	0.10	0.10	0.12
1,2,3,7,8,9-HxCDF	0.30	0.27	0.35	0.30	0.32	0.38	0.42	0.10	0.24	0.28	0.18	0.14	0.08	0.10	0.12
Total HxCDF	0.30	0.27	0.35	0.30	0.32	0.38	0.42	0.10	0.24	0.28	0.18	0.14	0.10	0.10	0.12
1,2,3,4,6, 7,8 -HpCDF	0.34	0.35	0.44	0.51	0.44	0.52	0.32	0.14	0.33	0.33	0.16	0.18	0.12	0.10	0.19
1,2,3,4,7,8,9-HpCDF	0.34	0.35	0.44	0.51	0.44	0.52	0.32	0.14	0.33	0.33	0.16	0.18	0.12	0.10	0.19
Total HpCDF	0.34	0.35	0.44	0.51	0.44	0.52	0.32	0.14	0.33	0.33	0.16	0.18	0.12	0.10	0.19
OCDF	0.42	0.41	0.58	0.76	1.00	0.73	0.37	0.33	0.61	0.56	0.30	0.21	0.12	0.24	0.31
(1) Total Dioxin/Furan	4.22	5.47	3.80	4.24	4.89	4.29	5.32	1.68	4.52	4.65	2.34	1.67	2.58	1.63	2.0
(1) TEQ (N.D. = D.L.)	0.642	0.545	0.658	0.627	0.690	0.733	0.741	0.294	0.516	0.671	0.381	0.346	0.252	0.296	0.33
(2) TEQ (ENV. CAN.)	0.019	0.027	0.019	0.023	0.047	0.000	0.047	0.000	0.002	0.160	0.057	0.021	0.053	0.016	0.03
(3) TEQ (B.C. MIN.)	0.330	0.286	0.338	0.325	0.368	0.366	0.394	0.147	0.259	0.415	0.219	0.183	0.153	0.156	0.18

^{*} M = mean of blind duplicate samples

APPENDIX 3. continued.

				Locati	on = GEN	ELLE							
Sample	G68	G69	G71	G72	074	G76	079	G80M	G82M	G85	G86M	G88	G89
2,3,7,8-TCDD	2.70	13.00	0.56	0.38	8.80	2.60	0.53	0.55	1.65	6.60	12.00	1.60	2.20
Total TCDD	2.70	13.00	0.56	0.38	8.80	2.60	0.53	0.55	1.65	6.80	12.00	1.60	2.20
1,2,3,7,8-PCDD	0.07	0.12	0.37	0.14	0.13	0.09	0.14	0.11	0.15	0.20	0.32	0.24	0.30
Total PCDD	0.07	0.12	3.60	0.14	0.13	0.09	0.14	0.11	0.15	0.20	0.32	0.24	0.30
1,2,3,4,7,8-HxCDD	0.14	0.15	0.29	0.16	0.19	0.11	0.28	0.14	0.15	0.20	0.20	0.40	0.35
1,2,3,6,7,8-HxCDD	0.14	0.15	39.00	0.16	0.47	0.11	0.28	0.14	0.15	0.20	0.44	0.40	0.35
1,2,3,7,8,9-HxCDD	0.14	0.15	5.50	0.16	0.19	0.11	0.28	0.14	0.15	0.20	0.20	0.40	0.3
Total HxCDD	0.14	0.15	320.00	0.16	0.47	0.11	0.28	0.14	1.65	0.20	0.44	0.40	0.3
1,2,3,4,6,7,8-HpCDD	0.11	0.22	250.00	0.20	0.25	0.12	0.61	0.25	0.55	0.22	0.33	0.64	0.6
Total HpCDD	0.11	0.22	600.00	0.20	0.25	0.12	0.61	0.30	0.55	0.22	0.33	0.64	0.6
OCDD	0.64	0.88	65.00	0.85	0.29	0.42	0.87	0.72	0.78	0.61	0.86	0.68	0.8
2,3,7,8-TCDF Total TCDF	30.00 30.00	100.00 100.00	15.00 25.00	4.40 4.40	120.00 120.00	10.00 10.00	14.00 14.00	16.00 16.50	2.85 2.85	18.00 18.00	305.00 305.00	2.40 2.40	82.0 82.0
1,2,3,7,8-PCDF	0.20	0.99	1.40	0.10	0.94	0.10	0.10	0.10	0.15	0.20	1.60	0.21	0.2
2,3,4,7,8-PCDF	0.51	3.10	2.30	0.10	2.50	0.10	0.10	0.10	0.15	0.20	4.15	0.21	0.2
Total PCDF	0.71	4.10	28.00	0.10	3.50	0.10	0.10	0.10	0.15	0.20	6.20	0.21	0.2
1,2,3,4,7,8-HxCDF	0.07	0.19	2.50	0.14	0.21	0.12	0.16	0.13	0.20	0.20	0.25	0.33	0.3
1,2,3,6,7,8-HxCDF	0.07	0.19	0.82	0.14	0.21	0.12	0.16	0.13	0.20	0.20	0.25	0.33	0.3
2,3,4,6,7,8-HxCDF	0.07	0.19	2.90	0.14	0.21	0.12	0.16	0.13	0.20	0.20	0.25	0.33	0.3
1,2,3,7,8,9-HxCDF	0.07	0.19	0.10	0.14	0.21	0.12	0.16	0.13	0.20	0.20	0.25	0.33	0.3
Total HxCDF	0.07	0.19	300.00	0.14	0.21	0.12	0.16	0.13	0.27	0.20	0.25	0.33	0.3
1,2,3,4,6,7,8-HpCDF	0.07	0.19	55.00	0.30	0.22	0.12	0.22	0.22	0.39	0.20	0.36	0.49	0.4
1,2,3,4,7,8,9-HpCDF	0.07	0.19	0.10	0.30	0.22	0.12	0.22	0.22	0.39	0.20	0.36	0.49	0.4
Total HpCDF	0.07	0.19	160.00	0.30	0.22	0.12	0.22	0.22	0.39	0.20	0.36	0.49	0.4
OCDF	0.06	0.21	9.10	0.13	0.30	0.13	0.97	0.26	0.51	0.30	0.71	0.63	0.6
1) Total Dioxin/Furan	34.57	119.06	1511.26	6.80	134.17	13.81	17.88	19.02	8.94	26.93	326.460	7.62	88.
(1) TEQ (N.D. = D.L.)	6.073	24.788	11.701	1.058	22.338	3.785	2.215	2.360	2.232	8.757	45.008	2.345	10.9
(2) TEQ (ENV. CAN.)	5.966	24.600	11.690	0.821	22.144	3.600	1.930	2.151	1.936	8.401	44.821	1.840	10.4
(3) TEQ (B.C. MIN.)	6.019	24.694	11.696	0.939	22.241	3.693	2.073	2.255	2.084	8.579	44.915	2.093	10.6

^{*} M = mean of blind duplicate samples

APPENDIX 3. continued.

			I	ocation =	BEAVE	R CREEK	:						
Sample	T121	T125	Т126	T130	T131	T135	T140	T142	Т147	T162M	T164	T166	T167
2,3,7,8-TCDD	1.60	4.20	1.30	3.70	4.20	1.20	1.20	0.20	0.32	4.15	0.48	7.20	1.80
Total TCDD	1.60	4.20	1.30	3.70	4.20	1.20	1.20	0.20	0.32	4.15	0.48	7.20	1.80
1,2,3,7,8-PCDD	0.25	0.22	0.18	0.12	0.20	0.20	0.11	0.20	0.22	0.11	0.23	0.40	0.21
Total PCDD	0.25	0.22	0.18	0.12	0.20	0.20	0.11	1.80	0.22	0.11	0.23	0.40	0.21
1,2,3,4,7,8-HxCDD	0.33	0.39	0.26	0.14	0.40	0.21	0.11	0.10	0.37	0.12	0.31	0.30	0.26
1,2,3,6,7,8-HxCDD	0.33	0.39	0.26	0.14	0.40	0.21	0.11	0.10	0.37	0.12	0.31	0.30	0.26
1,2,3,7,8,9-HxCDD	0.33	0.39	0.26	0.14	0.40	0.21	0.11	0.10	0.37	0.12	0.31	0.30	0.26
Total HxCDD	0.33	0.39	0.26	0.14	0.40	0.21	0.11	1.10	0.37	0.12	0.31	0.30	0.26
1,2,3,4,6,7,8-HpCDD	0.51	0.44	0.38	0.32	0.82	0.56	0.24	0.30	0.67	0.24	0.52	0.45	0.4
Total HpCDD	0.51	0.44	0.38	0.59	0.82	0.56	0.48	3.60	0.67	0.24	0.52	0.45	0.4
OCDD	0.63	0.69	0.70	0.65	1.50	1.00	0.50	6.10	1.10	0.49	0.59	0.93	0.5
2,3,7,8-TCDF	0.79	68.00	78.00	190.00	79.00	54.00	55.00	6.30	5.50	18.50	12.00	170.00	73.0
Total TCDF	0.79	68.00	78.00	190.00	79.00	64.00	55.00	9.60	5.50	18.50	12.00	170.00	73.0
1,2,3,7,8-PCDF	0.21	0.33	0.15	0.59	0.10	0.12	0.39	0.10	0.18	0.10	0.19	1.00	0.40
2,3,4,7,8-PCDF	0.21	0.96	0.15	1.00	1.10	0.39	0.51	0.79	0.18	0.35	0.19	1.80	0.48
Total PCDF	0.21	0.96	0.15	1.60	1.10	0.39	0.90	7.20	0.18	0.35	0.19	2.80	0.89
1,2,3,4,7,8-HxCDF	0.44	0.39	0.25	0.16	0.20	0.20	0.12	1.20	0.31	0.10	0.34	0.30	0.3
1,2,3,6,7,8-HxCDF	0.44	0.39	0.25	0.16	0.20	0.20	0.12	1.20	0.31	0.10	0.34	0.30	0.3
2,3,4,6,7,8-HxCDF	0.44	0.39	0.25	0.16	0.20	0.20	0.12	0.86	0.31	0.10	0.34	0.30	0.30
1,2,3,7,8,9-HxCDF	0.44	0.39	0.25	0.16	0.20	0.20	0.12	0.10	0.31	0.10	0.34	0.30	0.30
Total HxCDF	0.44	0.39	0.25	0.16	0.26	0.20	0.12	19.00	0.31	0.10	0.34	0.30	0.30
1,2,3,4,6, 7 ,8-HpCDF	0.52	0.45	0.29	0.16	0.90	0.46	0.15	8.00	0.59	0.14	0.36	0.60	0.3
1,2,3,4,7,8,9-HpCDF	0.52	0.45	0.29	0.16	0.90	0.46	0.15	0.20	0.59	0.14	0.36	0.60	0.3
Total HpCDF	0.52	0.45	0.29	0.16	0.90	0.46	0.15	17.00	0.59	0.14	0.36	0.60	0.3
OCDF	0.46	0.64	0.36	0.16	1.20	0.67	0.12	0.42	0.96	0.25	0.49	0.39	0.4
(1) Total Dioxin/Furan	5.74	76.38	81.87	197.28	89.58	68.89	58.69	66.02	10.22	24.44	15.51	183.37	78.2
(1) TEQ (N.D. * D.L.)	2.211	11.894	9.461	23.403	12.984	7.060	7.117	1.788	1.335	6.315	2.142	25.578	9.67
(2) TEQ (ENV. CAN.)	1.679	11.480	9.100	23.233	12.650	6.795	6.977	1.437	0.870	6.175	1.680	25.150	9.36
(3) TEQ (B.C. MIN.)	1.945	11.687	9.281	23.318	12.817	6.928	7.047	1.612	1.102	6.245	1.911	25.364	9.51

^{*} M = mean of blind duplicate samples

APPENDIX 3. continued.

Comparison of Results of Blind Duplicate Analyses Completed by Axys.

Congener	S18	S18	G74	G74	G80	G80	T141	T141
2 3 7 8 TCDD	0.07	0.13	8.80	9.00	0.55	0.48	0.12	1.20
1 2 3 7 8 PCDD	0.07	0.12	0.46	0.32	0.11	0.13	0.20	0.12
1 2 3 4 7 8 H6CDD	0.11	0.14	0.19	0.15	0.14	0.14	0.40	0.11
1 2 3 6 7 8 H6CDD	0.11	0.14	0.47	0.50	0.14	0.14	0.40	0.11
1 2 3 7 8 9 H6CDD	0.11	0.14	0.19	0.15	0.14	0.14	0.40	0.11
1 2 3 4 6 7 8 H7CDD	0.24	0.37	0.25	0.22	0.25	0.19	1.50	0.24
08CDD	0.78	1.00	0.64	0.44	1.10	0.72	6.10	0.50
2 3 7 8 TCDF	0.40	0.40	120.00	120.00	16.00	15.00	56.00	55.00
1 2 3 7 8 P5CDF	0.08	0.09	0.94	0.91	0.13	80.0	0.40	0.39
2 3 4 7 8 P5CDF	0.08	0.09	2.50	2.30	0.18	0.13	0.53	0.51
1 2 3 4 7 8 H6CDF	0.08	0.15	0.21	0.16	0.13	0.14	0.30	0.12
1 2 3 6 7 8 H6CDF	0.08	0.15	0.21	0.16	0.13	0.14	0.30	0.12
2 3 4 6 7 8 H6CDF	0.10	0.20	0.55	0.16	0.13	0.14	0.30	0.12
1 2 3 7 8 9 H6CDF	0.08	0.15	0.21	0.16	0.13	0.14	0.30	0.12
1 2 3 4 6 7 8 H7CDF	0.14	0.15	0.22	0.19	0.31	0.17	0.32	0.15
1 2 3 4 7 8 9 H7CDF	0.12	0.15	0.22	0.19	0.22	0.17	0.32	0.15
08CDF	0.12	0.19	0.41	0.24	0.26	0.19	0.76	0.12
T4CDD (TOTAL)	0.07	0.13	8.80	9.00	0.55	0.48	2.00	1.20
P5CDD (TOTAL)	0.05	0.12	0.13	0.09	0.11	0.13	0.20	0.11
H6CDD (TOTAL)	0.11	0.14	0.47	0.50	0.14	0.14	0.40	0.11
H7CDD (TOTAL)	0.55	0.20	0.25	0.22	0.30	0.19	2.20	0.48
T4CDF (TOTAL)	0.55	0.40	120.00	120.00	16.50	15.00	58.00	55.00
P5CDF (TOTAL)	0.13	0.09	3.50	3.20	0.10	0.08	1.10	0.90
H6CDF (TOTAL)	0.10	0.15	0.21	0.16	0.13	0.14	0.30	0.12
H7CDF (TOTAL)	0.12	0.15	0.22	0.19	0.22	0.17	0.32	0.15

APPENDIX 3. continued.

Comparison of Analytical Results for Dioxins and Furans from Axys and IOS Laboratories.

Congener	S08AX	S08IOS	G86AX	G86IOS	G89AX	G89IOS	T164AX	T164IOS	T166AX	T166IOS
2 3 7 8 TCDD	<0.16	<0.14	11.00	9.89	2.20	2.12	0.48	0.41	7.20	6.72
1 2 3 7 8 PCDD	< 0.25	<0.11	0.53	0.39	<0.3	0.18	< 0.23	0.06	<0.4	0.24
1 2 3 4 7 8 H6CDD	< 0.34	<0.18	< 0.20	<0.11	< 0.35	< 0.09	< 0.31	<0.12	< 0.30	<0.11
1 2 3 6 7 8 H6CDD	< 0.34	<0.20	0.67	0.77	< 0.35	0.25	< 0.31	<0.12	< 0.30	0.26
1 2 3 7 8 9 H6CDD	<0.34	<0.20	< 0.20	<0.11	< 0.35	<0.11	< 0.31	<0.12	< 0.30	< 0.12
1 2 3 4 6 7 8 H7CDD	<0.72	0.18	< 0.30	0.18	< 0.64	0.14	< 0.52	<0.15	< 0.45	0.22
08CDD	<0.99	<0.18	0.96	0.28	<0.81	0.17	<0.59	<0.17	<0.93	<0.20
2 3 7 8 TCDF	0.47	0.82	310.00	293.42	82.00	99.43	12.00	13.53	170.00	188.81
1 2 3 7 8 P5CDF	< 0.20	<0.08	1.60	1.1	<0.20	0.28	< 0.19	<0.05	1.00	0.6
2 3 4 7 8 P5CDF	< 0.20	<0.08	4.20	2.99	< 0.20	0.48	<0.19	0.1	1.80	1.37
1 2 3 4 7 8 H6CDF	< 0.32	<0.09	< 0.30	<0.07	<0.36	<0.06	< 0.34	<0.06	<0.30	< 0.06
1 2 3 6 7 8 H6CDF	< 0.32	<0.09	< 0.30	<0.06	< 0.36	<0.05	< 0.34	<0.06	< 0.30	< 0.06
2 3 4 6 7 8 H6CDF	< 0.32	<0.11	< 0.30	<0.06	<0.36	<0.06	< 0.34	<0.08	< 0.30	< 0.06
1 2 3 7 8 9 H6CDF	< 0.32	<0.14	< 0.30	<0.08	< 0.36	< 0.08	< 0.34	<0.09	< 0.30	< 0.09
1 2 3 4 6 7 8 H7CDF	< 0.44	<0.11	< 0.30	<0.08	<0.45	<0.06	< 0.36	<0.06	<0.60	<0.09
1 2 3 4 7 8 9 H7CDF	<0.44	<0.15	< 0.30	<0.11	<0.45	<0.09	< 0.36	<0.11	< 0.60	<0.14
08CDF	<1.00	<0.14	<0.48	<0.16	< 0.69	<0.12	<0.49	<0.14	< 0.39	< 0.17

APPENDIX 4. Summary Data for PCB Concentrations (ng/g) for Mountain whitefish Muscle.

		-	Sub	totals - By	Congener	Family (r	1g/g)	-	·· · · · · · · · · · · · · · · · · · ·	Total PCB	Arochlor
Fish ID	diCB	triCB		pentaCB				nonaCB	decaCB	(ng/g)	(ng/g)
				1.7	1.9	1.3	0.2	0.2	0.1	5.7	6.3
CR92S02	0.1	0.1 0.1	0.1 0.1	1.7	1.1	0.6	0.1	0.2	0.1	3.4	2.9
CR92S03	0.1 0.1	0.1 0.1	0.1	1.4	1.0	0.6	0.1	0.2	0.1	3.7	3.4
CR92S06 CR92S07	0.1	0.1	0.1	0.5	0.6	0.2	0.2	0.3	0.1	2.2	1.9
CR92S08	0.1	0.1	0.1	1.7	2.1	1.3	0.3	0.3	0.1	6.1	6.2
CR92S09	0.1	0.1	0.3	2.3	1.3	0.6	0.1	0.2	0.1	5.1	4.7
CR92S10	0.1	0.1	0.1	0.7	0.6	0.3	0.1	0.2	0.1	2.3	2.5
CR92S11	0.1	0.1	0.1	0.8	0.4	0.2	0.1	0.2	0.1	2.1	2.3
CR92S12	0.1	0.1	0.4	2.5	2.2	1.0	0.2	0.2	0.1	6.8	6.1
CR92S13	0.1	0.3	1.0	3.9	4.7	3.2	0.5	0.1	0.1	13.9	12.0
CR92S15	0.1	0.1	0.2	2.0	3.8	1.8	0.5	0.1	0.1	8.7	13.0
CR92S16	0.1	0.1	0.1	0.9	0.7	0.2	0.1	0.1	0.1	2.4	2.8
CR92S18	0.1	0.1	0.3	3.7	6.1	1.9	0.4	0.1	0.1	12.8	20.0
CR92S20	0.1	0.1	0.1	0.8	1.1	0.4	0.1	0.1	0.1	2.9	4.0
CR92S52	0.1	0.1	0.1	0.7	1.3	0.6	0.2	0.1	0.1	3.3	4.8
CR92T121	0.1	0.1	0.5	15.9	42.7	31.9	7.8	0.7	0.1	99.8	150.0
CR92T125	0.1	0.4	3.6	26.3	61.9	45.8	15.9	1.9	0.1	156.0	250.0
CR92T126	0.1	0.1	0.8	3.9	8.2	4.7	0.9	0.1	0.1	19.0	24.0
CR92T130	0.1	0.1	2.1	8.4	16.5	10.3	3.1	0.4	0.1	41.1	57.0
CR92T131	0.1	0.2	1.4	12.7	37.9	27.7	11.6	1.5	0.1	93.2	160.0
CR92T135	0.1	0.1	2.2	7.8	10.5	4.5	1.4	0.2	0.1	26.9	37.0
CR92T140	0.1	0.2	2.0	7.4	11.9	6.4	1.2	0.1 .	0.1	29.4	40.0
CR92T142	0.2	0.1	0.6	3.1	3.5	2.2	0.7	0.1	0.1	10.6	11.0
CR92T147	0.2	0.1	0.5	3.3	3.9	2.3	0.4	0.1	0.1	10.9	11.0
CR92T162	0.2	0.1	2.5	51.3	88.5	91.4	30.9	6.1	0.2	271.2	330.0
CR92T164	0.2	0.2	1.9	6.9	6.1	3.4	1.4	0.2	0.1	20.4	20.0
CR92T166	0.2	0.3	5.5	37.2	36.3	24.0	6.5	0.9	0.1	111.0	120.0
CR92T167	0.2	0.1	1.6	7.1	8.3	5.1	1.5	0.3	0.1	24.3	23.0
CR92G68	0.1	0.1	0.8	6.3	15.4	12.5	4.2	0.2	0.1	39.7	53.0
CR92G69	0.1	0.1	5.1	40.4	84.7	60.5	14.5	0.6	0.1	206.1	300.0
CR92G71	0.1	0.2	1.3	4.9	6.3	3.0	0.7	0.1	0.1	16.7	21.0
CR92G72	0.1	0.2	1.2	3.9	5.8	3.2	0.6	0.1	0.1	15.2	18.0
CR92G74	0.1	0.1	2.9	15.5	46.1	44.2	12.3	0.9	0.1	122.2	140.0
CR92G76	0.1	0.1	1.4	13.2	61.1	74.7	21.3	0.9	0.1	172.9	220.0
CR92G79	0.2	0.1	1.0	8.0	7.5	4.0	1.0	0.2	0.1	22.1	23.0
CR92G80	0.1	0.5	1.2	4.4	5.7	3.1	0.7	0.1	0.1	15.9	18.0
CR92G82	0.1	0.1	0.5	14.5	56.0	52.3	10.6	0.5	0.1	134.7	200.0
CR92G85	0.2	0.1	3.2	44.1	71.9	63.9	15.8	0.9	0.1	200.2	250.0
CR92G86	0.1	1.0	9.5	44.0	61.9	37.9	7.3	0.4	0.1	162.2	210.0
CR92G88	0.1	0.1	0.1	3.1	10.1	8.3	1.8	0.1	0.1	23.8	36.0
CR92G89	0.1	0.1	2.9	9.2	15.3	8.1	1.3	0.1	0.1	37.2	54.0

NOTE: Total PCBs represent sums of individual PCB compounds, excluding NOS PCBs. Where the sum is zero (i.e. non-detected), the largest individual compound detection limit is used as a conservative estimate.

APPENDIX 4. continued.

Summary Data for Non-Ortho Substituted PCB Concentrations (pg/g) in Mountain Whitefish Muscle.

Fish #		Congener		Total
	77	126	169	NOS PCB
S02	3.7	4.4	2.8	10.9
S03	5.9	3.7	2.7	12.3
S06	3.6	3.4	3.1	10.1
S07	2.9	3.5	2.7	9.1
S 08	8.6	4.4	4.3	17.3
S 09	5.7	3.4	3.8	12.9
S 10	5.0	3.4	5.9	14.3
S 11	2.9	3.4	3.1	9.4
S12	2.2	3.4	3.2	8.8
S13	22.0	5.3	4.1	31.4
S15	6.1	3.3	2.5	11.9
S16	4.2	2.0	2.0	8.2
S18	6.4	3.3	3.0	12.7
S20	4.5	3.0	48.0	55.5
S52	4.4	2.2	2.8	9.4
G68	10.0	5.7	7.8	23.5
G69	12.0	20.0	11.0	43.0
G71	11.0	2.4	1.8	15.2
G72	9.5	1.6	1.9	13.0
G74	25.5	74.5	13.5	113.5
G76	8.4	19.0	6.3	33.7
G79	24.0	25.0	15.0	64.0
G80	14.5	14.5	6.0	35.0
G82	5.6	20.0	5.5	31.1
G85	21.0	26.0	10.0	57.0
G86	46.0	28.0	7.7	81.7
G88	5.0	78.0	9.3	92.3
G89	25.0	16.0	8.1	49.1
T121	5.2	23.0	10.0	38.2
T125	22.0	22.0	9.7	53.7
T126	17.0	21.0	10.0	48.0
T130	43.0	20.0	14.0	77.0
T131	20.0	1.0	9.0	30.0
T135	27.0	14.0	10.0	51.0
T141	25.5	14.0	9.1	48.6
T142	12.0	12.0	8.6	32.6
T147	14.0	21.0	10.0	45.0
T162	22.0	27.0	9.3	58.3
T164	25.0	14.0	12.0	51.0
T166	38.0	53.0	6.8	97.8
T167	25.0	10.0	12.0	47.0

APPENDIX 4. continued.

Comparison of Blind Duplicate Analyses of PCBs by Axys.

Congener	S 18	S18*	G74	G74*	G80	G81*	T141	T141
Arochlor	20	17	140	130	18	18	40	25
Di CB	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tri CB	0.1	0.1	0.1	0.1	0.5	0.2	0.2	0.1
Tetra CB	0.3	0.5	2.9	2.3	1.2	1.1	2	1.7
Penta CB	3.7	3.4	15.5	14.4	4.4	4.3	7.4	7.4
Неха СВ	6.1	4.7	46.1	42.1	5.7	5.8	11.9	8.3
Hepta CB	1.9	1.8	44.2	39.9	3.1	3.1	6.4	5.9
Octa CB	0.4	0.5	12.3	11.2	0.7	0.7	1.2	1.6
Nona CB	0.1	0.1	0.9	0.8	0.1	0.1	0.1	0.2
Deca CB	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PCB 77 (pg/g)	6.7	6	28	23	15	14	24	27
PCB 126 (pg/g)	3.9	2.6	29 (NDR)	120 (NDR)	12 (NDR)	17 (NDR)	14 (NDR)	14 (NDR)
PCB 169 (pg/g)	2.8	3.2	15	12 (NDR)	<5.6	<6.4	<10	<8.2

NDR = Peak detected but did not meet quantification criteria.

All units are ng PCB/g tissue except where indicated.

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APPENDIX 4. continued.

Comparison of PCB Measurements from Axys and IOS (pg/g).

Congener	S08AX	S08IOS	G86AX	G86IOS	G89AX	G89IOS	T164AX	T164IOS	T166AX	T166IOS
CoPlanar PCBs (pg/g)										
3,3'4,4'-TeCB (PCB 77)	8.6	8.28	46	316.2	25	24.2	25	21.6	38	33
3,3'4,4',5-PeCB (PCB 126)	4.4	8.28	28(ndr)	112.8	16(ndr)	6.57	14(ndr)	2.9	53(ndr)	13.35
3,3'4,4',5,5'-HxCB (PCB 169)	4.3	2.39	7.7	36.9	<8.1	3.51	12(ndr)	0.55	6.8(nd	r) 3.48
Mono-Ortho PCBs (pg/g)										
2,3',4,4'-TeCB (PCB 66)			900	904.6	300	425.7	200	320.9	700	834.6
2,3',4,4',5-PeCB (PCB 118)			7500	7523.9	2000	1760.4	1400	1117.6	8500	7054
2,3,4,4',5-PeCB (PCB 114)			200	200.2	<100	50.07	<100	29.02	200	207.7
2,3,3',4,4'-PeCB (PCB 105)			2000	3748.3	400	843.9	400	493.9	2300	3694
2,3,3',4,4',5-HxCB (PCB 156)			1500	1855.3	300	419.1	100	197.4	1000	1751
2,3,3',4,4',5'-HxCB (PCB 157)			300	364.9	<100	82.1	<100	39.4	200	357.8
2,3,3',4,4',5,5'-HpCB (PCB 189)			100	220.6	<100	41.3	16.83	16.7	100	163.9

APPENDIX 5. Means, Standard Deviations, Sample Numbers and Ranges of Mixed Function Oxidase Activities from Mountain Whitefish collected from the Columbia and Slocan Rivers during July, 1992.

		EROD	АНН	Cytochrome P-450
Slocan River Males	N	8	8	8
	Mean	0.0166	0.0565	0.1639
	Standard Deviation	0.0047	0.0182	0.0922
	Range	0.011-0.026	0.033-0.087	nd-0.277
	Nango	0.011 0.020	0.000	114 0.277
Slocan River Females	N	11	11	11
	Mean	0.0169	0.0455	0.1405
	Standard Deviation	0.0115	0.0253	0.0619
	Range	0.004-0.036	0.021-0.095	0.044-0.28
Columbia River Males				
(Genelle)	N	7	7	7
	Mean	0.2076	0.2217	0.2420
	Standard Deviation	0.3827	0.3147	0.0840
	Range	0.015-1.061	<0.001-0.905	0.153-0.38
Columbia River Females				
(Genelle)	N	9 .	9	9
	Mean	0.0331	0.0607	0.1712
	Standard Deviation	0.0277	0.0405	0.0723
	Range	0.003-0.088	0.003-0.132	0.068-0.28
Columbia River Males				
(Beaver)	N	9	9	9
	Mean	0.0406	0.0950	0.2393
	Standard Deviation	0.0565	0.0851	0.0492
	Range	0.009-0.189	0.028-0.308	0.188-0.32
Columbia River Females				
(Beaver)	N	10	10	10
	Mean	0.0160	0.0339	0.1509
	Standard Deviation	0.0078	0.0181	0.0383
	Range	0.009-0.030	<0.001-0.067	0.105-0.21