FISH MERCURY DATABASE SUMMARY - 2001

BRITISH COLUMBIA

Prepared for

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Appendix A .............................................................................. Fish Mercury Raw Data Summary
ACKNOWLEDGEMENTS

I would like to thank Ed Hill for his assistance throughout my efforts to gather fish mercury concentration data from British Columbia freshwater lakes and reservoirs. His efforts are greatly appreciated. I would also like to acknowledge the many people who contributed to this study by providing reports, gray literature, unpublished data or other information to facilitate my attempts to gather as much fish mercury data as possible. In particular I would like to thank Bonnie Antcliffe (DFO, Vancouver), Julia Beatty – Spence (BC WALP, Nelson), Bruce Carmichael (BC WALP, Prince George), Ray Copes (Health and Welfare, Victoria) Bill Duncan (Cominco, Trail), Glynn Fox (BC WALP, Victoria), Bob Grace (BC WALP, Kamloops), Christine Garrett (Environment Canada, North Vancouver), Walter Kuit (Cominco, Vancouver), Mike Macfarkane (BC WALP, Victoria), Bev Raymond (Environment Canada, Vancouver), Iain Sharpe (BC WALP, Smithers), Patrick Shaw (Environment Canada, Victoria), and Greg Thomas (G3 Consulting, Vancouver).

Randy Baker (Aqualibrium Environmental Consulting Inc.) wrote this report.
EXECUTIVE SUMMARY

This report contains a compilation of mercury (Hg) concentration data in fish muscle between 1970 and 2001, from freshwater fish in lakes and reservoirs in British Columbia. Data were gathered from published reports and data summaries, BC Hydro unpublished data, consultant reports, and from data provided by various federal and provincial government agencies and universities. Data are reported alphabetically by the common name of each species, followed by waterbody (lake or reservoir) and year. Length and weight data (if available) accompany mercury concentration data for individual fish in parts per million (ppm) wet weight. In some cases, only mean values were available and reported for length, weight and Hg for particular species, waterbodies or years.

Key species for which data were widely available included bull trout (Salvelinus confluentus), lake trout (S. namaycush), lake whitefish (Coregonus clupeaformis), mountain whitefish (Prosopium williamsoni), and rainbow trout (Oncorhynchus mykiss). Limited data were available for Dolly Varden (Salvelinus malma), brook trout (S. fontinalis), cutthroat trout (Salmo clarki), kokanee (Oncorhynchus kisutch), lake sturgeon (Acipenser fulvescens), walleye (Stizostedion vitreum), northern pike (Esox lucius) and northern pikeminnow (Ptychocheilus oregonensis). We believe that this report contains the vast majority of available Hg data for BC freshwater fish.

This report builds on two previous reports prepared for BC Hydro that compiled and analysed all fish mercury data previously collected by BC Hydro, using standard analytical procedures, to provide an unbiased assessment of fish mercury concentrations in BC Hydro reservoirs (Baker, 1998; 1999). Note that only arithmetic mean fish size and mercury concentration data are reported in this document. Size-adjusted mercury concentrations were calculated for those species and reservoirs where sufficient data permitted and these are reported in Baker (1999).

A description of the collection, analysis and presentation protocol using size-adjusted fish Hg data, following procedures employed elsewhere in Canada, is contained in this report. We recommend that this protocol be followed in all future studies to ensure that comparisons of fish mercury concentration in BC are unbiased by differences in fish size, particularly if comparisons are made between lakes, or over time.

We have recently developed a non-lethal technique for harvesting muscle tissue from fish for Hg analysis and a description of this procedure is provided in this report. It is no longer necessary to sacrifice fish to determine Hg content in muscle.

Many of the data sets collected suffered from deficiencies such as small sample size, inadequate size range, or confusion between wet and dry weight concentrations. Despite these deficiencies, mercury concentrations for the vast majority of species in BC lakes and reservoirs were reasonably low and in nearly all cases, were less than the commonly used guideline concentration of 0.5 ppm for the commercial sale of fish. We recommend that all future monitoring programs use non-destructive techniques, and standard collection procedures and analytical protocols to ensure that comparisons of fish Hg among waterbodies and over time are unbiased by differences in fish size.
1.0 INTRODUCTION

1.1 Background
Mercury is a naturally occurring, widespread element that is commonly found in low concentrations in water, soil, vegetation, sediment and at all levels of the food chain in aquatic systems, even in pristine lakes and streams. The cycling of mercury in aquatic systems and the process by which mercury is accumulated and concentrated through the food chain has been studied extensively (see reviews by Beijer and Jernelov, 1979; Huckabee et al., 1979; Hecky et al., 1991; Bodaly et al., 1997). Mercury concentrations can become elevated in biota over what is considered background concentrations as a result of many factors. These include releases from industrial developments (e.g., mines, chlor-alkali facilities, manufacturing), from flooding (e.g., hydroelectric development), or in areas that geologically, have naturally elevated mercury concentrations, such as the Pinchi Fault area of British Columbia (Plouffe, 1995).

There are many means by which mercury (Hg) can enter food webs. The most important of these is via microbial pathways, whereby inorganic mercury ions become "methylated" and attached to a methyl (CH$_3$) group (i.e., to become methyl mercury, Hg-CH$_3$). This is the most toxic form of mercury and its abundance in aquatic biota increases through the food chain, with highest concentrations found in fish eating animals (e.g., bull trout, lake trout, loons, osprey, mink). Fish are also the main pathway of exposure by humans to mercury.

Fish mercury data are normally expressed on a wet weight basis as parts per million (ppm), which are equivalent to mg/kg, or µg/g, based on chemical analysis of fish muscle tissue. Nearly all mercury measured in fish (>95%) is methyl mercury (MeHg). At lower levels of the food web, such as benthos and zooplankton, a lesser amount of the total mercury in the tissue is in the methyl form (e.g., typically 30% to 50%).

The total amount of methyl mercury in aquatic biota tends to increase through the food web, with higher trophic levels having relatively greater methyl mercury concentrations. The process of increasing mercury content through food chains is known as "biomagnification". There are also significant differences in the concentration of mercury in different kinds of fish, which are consistent between contaminated and uncontaminated environments. Generally, piscivorous (i.e., fish eating) fish, such as lake trout (Salvelinus namaycush), northern pike (Esox lucius) and bull trout (Salvelinus confluentus), have higher concentrations of mercury than fish that consume plankton, such as kokanee (Oncorhynchus nerka) and lake whitefish (Coregonus clupeaformis) or fish that consume insects, such as rainbow trout (O. mykiss). In addition, there is a well-known relationship between fish size or age and mercury content (Scott and Armstrong, 1972; Bodaly et al., 1984; Strange et al., 1991; Somers and Jackson, 1993), as larger, older fish tend to have higher mercury concentrations than smaller, younger fish. This is partly due to differences in diet and the length of time of exposure.

Fish can take up methyl mercury directly from the water via their gills; however, the vast majority of methyl mercury in fish is acquired via dietary pathways (Harris and Snodgrass, 1993; Hall et al. 1997). The amount of methyl mercury incorporated in fish tissue depends on many
factors that can influence the rate of uptake, as well as the rate of clearance, or elimination of tissue mercury (Huckabee et al., 1979; Rodgers and Beamish, 1983). Examples of these factors are water temperature, fish metabolic rates, food types, and the level of contamination of the food web.

1.2 Mercury and Hydroelectric Development

Flooding of vegetation and terrestrial soils during creation of reservoirs for hydroelectric purposes causes mercury concentrations in aquatic biota to increase (Abernathy and Cumbie, 1977; Bodaly et al., 1984; Brouard et al., 1989; Hecky et al., 1987, 1991, and many others). The mercury and organic nutrients contained in the newly flooded soils, litter and vegetation contributes to increased microbial activity and hence, methyl mercury production (Wright and Hamilton, 1982). As a result, the rate of MeHg production is much greater in flooded versus unflooded environments, causing mercury concentrations to become elevated in aquatic biota, especially fish, a condition that can persist for decades (Schetagne et al., 1997).

The phenomenon of increased mercury concentrations in fish as a result of flooding was first documented in the early 1970s. In Canada, this relationship has been studied extensively, especially in Manitoba (Bodaly et al., 1984; Strange et al., 1991; Hecky et al., 1991), and Quebec (Verdon et al., 1991; Morrison and Therien, 1995), where flooding of lakes for hydroelectric purposes has caused fish mercury concentrations to become elevated. The duration that mercury concentrations in biota, especially fish, remain elevated above background is typically between 20 and 30 years, depending on environmental conditions (Bodaly et al., 1997). In some cases mercury concentrations in fish became high enough to result in the closure of commercial fisheries and alter traditional fishing activity patterns by First Nations. There is no widely applicable, successful strategy to mitigate against increased mercury in fish in new reservoirs.

In British Columbia, most of the large hydroelectric stations were constructed before the relationship between reservoir creation and increased fish mercury concentrations was understood, therefore, there are few historic data available. Consequently, it has been difficult to determine to what extent hydroelectric development has been responsible for observed mercury levels in fish and the long-term or historic trend in fish mercury concentrations in BC reservoirs. Currently, Williston Reservoir, which was formed in 1968, is the only BC reservoir where there is a consumption advisory for bull trout that was applied in 1991. Pinchi Lake (mining contaminated) also has a consumption advisory for lake trout.

A consumption advisory generally means that long-term consumption of the particular species for which there is an advisory, should be limited. The commonly referred to guideline concentration of 0.5 ppm is the threshold concentration for the commercial sale of fish. That is, if the mean concentration of a species bound for commercial sale exceeds 0.5 ppm, the sale is restricted. This guideline concentration for commercial sale in the United States is 1.0 ppm. Consumption advisories are applied by the appropriate agencies and are based on a combination of the fish mercury concentration and the consumption rate of fish by local people. Therefore, if the population mean mercury concentration exceeds 0.5 ppm, this does not necessarily imply that an advisory is warranted.
1.3 Objectives

The overall objective of this report was to provide under a single cover, a compilation of the vast majority of mercury data for freshwater fish collected from British Columbia reservoirs and lakes since 1970. This was initiated by BC Hydro following an investigation of fish mercury in the Upper Columbia Generation Area in 1998, that identified the need to compile and standardize the available fish mercury data when it was recognized that there was no recent compilation of data, and that existing data sets varied considerably in their treatment and presentation of fish mercury data.

The data presented in this report have been gathered from a wide variety of sources including published literature, federal and provincial government (Department of Fisheries and Oceans (DFO), BC Ministry of Water Air and Land Protection (BC WALP), Environment Canada, Health Canada) reports and files, unpublished data and gray literature, consulting firms, libraries (e.g., DFO, Department of Mines), universities (UBC, Simon Fraser), and industry (e.g., Cominco Ltd.).

During our initial evaluation of fish mercury data sets in the possession of BC Hydro, it was quickly recognized that the issue of mercury in fish was not well understood and that an ad hoc approach to mercury in fish has been taken by many studies in BC. Although there have been compilations of fish mercury data in British Columbia in the past (e.g., Peterson et al., 1970; Garrett et al., 1980; Rieberger, 1992), nothing comprehensive has been completed recently. This report therefore, represents an extensive, but not exhaustive compilation of historic freshwater fish mercury data in British Columbia, current to November 2000.

In addition, this document contains a description of the proper collection, analysis and presentation protocol for fish mercury data, to ensure that population mean concentrations are not biased by differences in fish size, which was a common problem in most studies. Standardized analytical procedures were followed for all available data sets in BC, for which adequate data sets were found (i.e., sufficient sample size and size range of fish) and results of these preliminary studies can be found in Baker (1999 and 2000). Also, we have recently developed a non-lethal tissue extraction technique for analysis of mercury content in fish using biopsy procedures. A description of this procedure is also included in this report and is further described in Baker et al. (2002a).
2.0 METHODS AND RESULTS

2.1 Sources of Fish Mercury Data

Since 1998, two reports have been completed for BC Hydro that have compiled and analysed fish mercury data in lakes and reservoirs of British Columbia. The first report reviewed the quality and quantity of the fish mercury database held by BC Hydro for BC Hydro reservoirs and selected reference lakes (Baker, 1998). The objective of this review was to provide an assessment of the adequacy of these data with respect to their ability to accurately represent historic fish mercury concentrations in BC reservoirs and possible temporal trends. This assessment focused on six fish species from 12 reservoirs and 20 reference lakes, which, in some cases, were collected over several years.

A second report, completed in 1999 (Baker, 1999), undertook a statistical analysis of all adequate data sets identified in the 1998 report, using standardized statistical procedures for fish mercury data, to provide an unbiased assessment of fish mercury concentrations in BC Hydro reservoirs. These data were put in context with fish data from non-impounded reference lakes in BC, and to a lesser extent, with reservoirs elsewhere in Canada. These reports did not attempt to relate observed fish mercury data to reservoir characteristics, such as age of the impoundment, water retention time, or any other physical parameter (such as the Pinchi Fault, a naturally merciferous area).

The objective of the current, final report was to complete the acquisition and presentation of fish mercury data sets from freshwater lakes and reservoirs in British Columbia. All supplementary data sets were assessed with respect to their adequacy and where appropriate, data were analysed according to standard procedures for fish mercury data to determine standardized mean mercury concentrations. These additional data were solicited from federal and provincial government agencies, regulators, consultants, industry representatives and academics. Individuals were asked to provide raw data contained in published reports, gray literature, unpublished documents or files, environmental impact assessment documents, or any other source they were aware of that may contain fish mercury data.

The current report represents a compilation of all of the raw data acquired from these sources and includes data collected by Health and Welfare Canada (1980), Garrett et al. (1980), BC Hydro (1990), Rieberger (1992) and recent fish mercury surveys on Carpenter Reservoir and Seton Lake (Baker (2001a) and Finlay Reach, Williston Reservoir (Baker et al., 2002b) on behalf of BC Hydro and six lakes in the Pinchi Lake region (Baker 2001b) on behalf of Cominco Ltd. All raw data from all data sources are presented in Appendix A.

2.2 Reporting Format and Limitations

reported here were received in non-electronic format and were contained in reports, publications, or other written documents such as internal files. In a few cases, data were received electronically and these were input directly into the raw data set.

Appendix A lists all raw data gathered, analysed and reported in Baker (1998), all supplementary data gathered since 1998 and are ordered alphabetically by common name of the fish species and secondarily by waterbody (lake, reservoir, or river). Data are then listed chronologically by year, followed by sample size, length (mm), weight (g) and mercury concentration (ppm wet weight). Where appropriate, comments relevant to a particular data set are made. Please make note of the following limitations of the raw data:

- Many of the historic data sets reported by Peterson et al. (1970) and Garrett et al. (1980) provide only mean mercury data, often with no length or weight data as original data could not be located. In these cases only mean data and sample size are reported. Mean data are bolded in Appendix A.
- All mercury data in Appendix A are reported as wet weight concentrations in parts per million (ppm or ug/kg). In a few cases dry weight data were received and were converted to wet weight concentrations assuming a moisture content of 78%. Data sets converted from dry weight to wet weight are indicated with an asterisk.
- For those data sets containing 5 records or more, mean length, weight and mercury concentration were calculated. These mean values are also bolded. Note that the mean mercury concentrations are not meant to represent standardized mean values, but are simply arithmetic mean concentrations, and are not adjusted for fish size.
- Length and weight data did not accompany fish mercury concentration data for some lakes, thus the utility of these data were somewhat limited.
- No assessment of the quality of the data collected (analytical laboratory, quality assurance/quality control, handling procedures) has been made.
- Each data set contains a reference where either the original data can be found, or where no raw data exist and only mean values could be found (e.g., Garrett et al., 1980), the appropriate reference is provided in Appendix A.

Mercury data sets are reported for important sport species in British Columbia including lake trout (Salvelinus namaycush), bull trout (S. confluentis), Dolly Varden (S. malma), cutthroat trout (Salmo clarki), and rainbow trout (Oncorhynchus mykiss). Some of the earlier data sets that reported Hg data for Dolly Varden were probably bull trout and we did not attempt to correct this. Data for lake whitefish (Coregonus clupeaformis) and mountain whitefish (Prosopium williamsoni) are reported because of their abundance and importance as food web species. Rieberger (1992) reported a few data sets for Arctic grayling (Thymallus arcticus) and some anadromous salmon species including sockeye and the landlocked form, kokanee (Oncorhynchus nerka), coho (O. kisutch), and chinook salmon (O. tschawytscha). These data are also included in this database. Note that mercury concentrations in anadromous fish were always very low. Additional important species for which a few data sets are reported include lake sturgeon (Acipenser fulvescens), walleye (Stizostedon vitreum), northern pike (Esox lucius) and northern
pikeminnow (*Ptychocheilus oregonensis*). This report does not contain all mercury data from freshwater fish in BC, but we believe that it contains the vast majority of available data.

### 2.3 Collecting, Analyzing and Presenting Fish Mercury Data

Mercury behaves differently than most other metals when present in the food web in that it tends to increase in concentration at progressively higher levels of the aquatic food chain, with highest concentrations being found in fish eating fish, birds and mammals. There is also a well-known positive correlation between fish size (length and weight) and mercury concentration in muscle tissue (Scott and Armstrong, 1972; Bodaly, et al., 1984; Somers and Jackson, 1993). Therefore, the mean mercury concentration in a sample of fish very much depends on the size of fish being measured, with larger fish having higher concentrations of mercury. This trend is much stronger for fish eating or piscivorous species such as lake trout or bull trout than non-fish eating species such as whitefish and rainbow trout. To eliminate the bias associated with differences in fish size, mercury concentrations must be measured over a wide size range. Then, appropriate statistical procedures are used to determine the mean mercury concentration for a specific fish size, usually near the size most frequently captured by anglers. This is called the size-adjusted or “standardized” mean mercury concentration. When this is done for multiple lakes or years, comparisons of standardized mean mercury concentration can be made that are unbiased by differences in fish size.

There is an established protocol that describes the sample size and size range of fish needed to derive a good statistical relationship between mercury concentration and fish size (Strange and Bodaly, 1998). Optimally, tissue from 25 – 35 fish is gathered from each species, ranging from small fish to large fish. Tissue samples are usually gathered during designed studies or opportunistically, such as during fishing derbies. In the vast majority of historic studies, acquisition of tissue samples for mercury analysis required that the fish be sacrificed. With technological advances made in analytical techniques, reliable estimates of fish mercury concentrations can now be made with very small sample sizes (<100 mg) that do not require sacrificing the fish. In the next section we describe a non-destructive means of acquiring tissue samples for mercury analysis that does not cause mortality of fish.

The standardized size used is usually consistent within species and approximates the mean size that a sport or commercial fisherman may encounter. Elsewhere in Canada, where there are substantial commercial fisheries for lake whitefish, northern pike and walleye for example, this approach is important in comparing mercury concentrations within species among lakes or years. Different species have different standardized sizes. Bull trout are relatively long lived and reach larger sizes than many other species and therefore have a larger standardized size (550 mm) than mountain whitefish or rainbow trout (350 mm), which are smaller, shorter-lived species. By using standardized sizes to compare mercury concentrations among lakes or between years, any bias associated with differences in fish sizes is eliminated. The strength of the relationship between mercury concentration and fish size depends on sample size and the distribution of fish size over the size range being tested. An example of the standard protocol to describe the optimal sample size per size interval in British Columbia for mountain whitefish, rainbow trout and bull trout from which to derive standardized mercury concentrations is as follows:
These standardized sizes fall near the middle of the size range of fish collected and have been used as standardized sizes elsewhere in British Columbia or Canada, which facilitates comparisons between lakes and years for the same species.

Length, weight, and age have been used to estimate the relationship between mercury and fish size. Although each have their merits, fish length is the most commonly used parameter because it is subject to less natural variability than weight and is much more easily measured than age. Thus, more precise estimates of standardized mercury concentrations are usually derived using length rather than weight data.

The following points describe why it is preferable to use length data versus age or weight data. Fish age is not normally used for the following reasons:

- When fish are collected in the field, age is not apparent, so the age range collected is not immediately known.
- Estimating the age of fish can be difficult, especially in larger older fish.
- Expertise in fish aging is required.
- Less confidence is placed in estimates of fish age than in length or weight measurements.

With regard to fish size, length is preferred over weight for the following reasons:

- Measuring fish length is quick and simple, and is less variable than estimates of weight, which can be more variable depending on the device used to measure weight.
- The range in fish length is smaller than fish weight, which reduces variability of the data and increases statistical confidence. For example, bull trout typically range in size from 150 mm to 800 mm, while the weight of the same fish ranges from 50 g to 8,000 g.
- Fish length is much less variable over time than fish weight. For example, fish captured in spring or fall may differ considerably (20% or more) in weight due to the

<table>
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<th>LENGTH INTERVAL (MM)</th>
<th>MOUNTAIN WHITEFISH</th>
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<td>&gt;700</td>
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<tr>
<td>Total</td>
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"Standardized Size" 300 mm 550 mm 350 mm
accumulation of body fat or state of maturity (e.g., females with eggs) that is not reflected to the same degree by differences in length.

- Fish length is independent of what a fish has recently eaten. For example, the weight of large piscivorous fish can differ by as much as 40% depending upon whether it has recently consumed another fish.

Each of the above factors can confound the relationship between mercury and weight and introduce variability. Using fish length eliminates or reduces this variability. The protocol for describing the relationship between mercury (ppm) and length (mm) is well known (Johnson, 1987; Bodaly et al., 1988; McMurtry et al., 1989) and is of the form:

$$\log_{10}[\text{Hg}] = a + b(\log_{10}[\text{Length}])$$

where “a” and “b” are the calculated intercept and slope respectively of the linear relationship. Size data are normally log\(_{10}\) transformed because growth of fish (irrespective of age, weight, or length data) is curvilinear, not linear. Fish grow quickly when they are young, with growth rates declining with increasing size and age. Therefore, it is inappropriate to apply linear regression techniques against non-linear data without first transforming the data (Ricker, 1975; Sokal and Rohlf, 1981) or using multivariate techniques (Somers and Jackson, 1993; Tremblay et al., 1998).

Once sufficient data have been collected within the size ranges list above, the following procedures should be followed:

- Compile the data and enter them into a spreadsheet and check for accuracy.
- Log\(_{10}\) transform the length (mm) and mercury (ppm wet weight) data.
- Plot log (length) and log [mercury] data and examine for linearity and presence of outliers;
- Calculate linear regression equations for each relationship to determine the significance of the regression: yes ($p<0.05$) or no ($p>0.05$); intercept (a), slope (b) and the goodness of fit ($r^2$). If the regression is not significant there is no relationship between mercury and fish size and it is inappropriate to apply any further statistical procedures.
- Graphical comparisons of the mercury data can be made where appropriate.

Analysis of covariance (ANCOVA) can then be used to determine whether standardized mercury concentrations among years or lakes differ significantly from one another. ANCOVA adjusts for differences in size and allows for unbiased comparisons of mercury concentrations to be made at a common size (i.e., the standardized length). The first test of covariance is for equality of slopes among groups. That is, ANCOVA compares the linear regression relationships for log[mercury] and log(length) for a particular species between years or lakes. If the slopes (i.e., the rate of mercury accumulation averaged over the entire size range of fish being tested) are not significantly different from each another ($p>0.05$), then one is justified in proceeding to the next step. That is, to test whether differences in standardized mean mercury concentrations are significantly different between groups of fish being tested. In other words, provided that slopes are similar, the next test that ANCOVA performs is for equality of intercepts, which can be applied to a standard length (e.g., 550 mm for bull trout). In instances where standardized mercury concentrations are being compared among several lakes, a pair-wise comparison (e.g.,
Tukey’s test) is used to determine whether mercury concentrations among the different lakes being tested are significantly higher or lower than one another.

If slopes are significantly different ($p<0.05$) from each other, then the relationship between the rate of mercury accumulation and fish size is not consistent between the two populations, and one is not generally justified in comparing differences in intercepts or standardized mean mercury concentrations. However, depending on the degree and nature of the slope differences, some qualified comparisons can be made.

Using this simple framework and analytical procedure provides managers and researchers with a consistent methodology that allows for changes in fish mercury concentrations to be accurately and unbiasedly tested among lakes and reservoirs over time.

### 2.4 Non-Destructive Tissue Extraction Techniques

Historically, the vast majority of fish mercury studies required that the fish be sacrificed to acquire a sufficiently large tissue sample (10 g) for chemical analysis. The development of more modern analytical techniques (i.e., cold vapor spectrophotometry) require only very small tissue quantities, usually less than 50 mg, which can be harvested using one of several small tissue extraction tools. We have recently tested two different tissue biopsy extraction tools to harvest multiple tissue samples from the same fish and compared the results against the traditional fillet style sample (Baker et al., 2002a). We showed that mercury concentrations from small tissue samples harvested with biopsy tools were statistically similar to mercury concentrations from fillet style samples, regardless of where the muscle tissue was harvested on the fish within the dorsal epaxial muscle). Thus, very small tissue samples can be reliably used to accurately measure fish mercury concentrations with little or no mortality. Several experimental studies have shown that very little (<0.5%) mortality to fish results from correct application of this procedure (e.g., Moy and Dredge, 1979; McAndrew 1891; Van Meter, 1995).

The following describes a methodology for harvesting small tissue samples from fish using two different tools, a Tru-Cut™ biopsy needle and a dermal punch. Both are effective at harvesting small tissue quantities and their use is encouraged in studies where mortality of fish is a concern. The procedure is as follows:

- Prepare two tubs: one holding tub with well-oxygenated fresh water and another tub containing an anesthetic, such as clove oil.
- Once two or three fish of an appropriate size are captured (e.g., by angling, short set gill nets, electrofishing) and placed in the holding tub, transfer fish to the tub containing the anesthetic.
- Once anaesthetized, remove the fish from the water and quickly measure for fork length (mm) and weight (+/- 25 g). If desired, an aging structure (e.g., pelvic fin ray) can be removed, or a Floy tag placed in the fish.
- Extract a tissue sample using a tissue extraction tool, in this case a biopsy needle. A Tru-Cut biopsy needle consists of a double barrel (inner and outer), and a 14 gauge x 7.6 cm cannula with a 20 mm notch within which tissue is harvested.
• Dip the needle in alcohol to sterilize it before it is used to remove two or three scales from the dorsal musculature of the left side of the fish just below the dorsal fin.

• Insert the needle about 1 cm into the fish, beneath a scale, at an oblique angle into the muscle tissue to avoid pushing the needle too deeply.

• Extend the 2 cm long notched needle (inner barrel) into the flesh. Slide the containment cover (i.e., sharp outer barrel) over the extended needle to cut the tissue and capture it within the notch.

• Withdraw the needle, slide open the barrel and remove the tissue slug with stainless steel or plastic tweezers and place in a small, labeled vial. Approximately 30 mg of tissue is harvested with each application. Two tissue samples should be harvested and composited per fish. An experienced person requires only about 5 – 10 seconds to harvest a tissue sample.

• Once a tissue sample has been successfully harvested, return the fish to the holding tub until it appears to have recovered and swims normally. Release the fish. Each needle can be used to extract about 25 tissue samples, or until it becomes dull.

• Ideally, tissue samples should be weighed (nearest 0.01 g) during collection. If this is not possible, tissue samples must be accurately weighed by the laboratory prior to analysis.

• Place the tissue sample on dry ice or ice and freeze as soon as is practicable. Because of their small size, biopsy samples should be analysed as soon as possible after collection to avoid loss of moisture.

With regards to chemical analysis, most commercial laboratories are equipped to analyse mercury in wet weight tissues using cold vapor atomic absorption spectrophotometry (CVAAS). If tissues are analysed wet using CVAAS, a minimum of 100 mg sample weight is required. Samples should be analysed as soon as possible after collection to avoid moisture loss. Some laboratories are capable of analyzing tissue samples using cold vapor atomic fluorescence spectrophotometry (CVAFS). CVAFS has lower detection limits and is better suited to determining mercury concentration in small tissue quantities.

If possible, tissue samples should be freeze-dried and weighed prior to analysis. Freeze-drying ensures that no bias in results is caused from differential moisture loss. For dried samples, results will be reported in terms of dry weight (ppm or µg/g dw). Concentrations must be converted from dry weight to wet weight values using the moisture content of the fish (if known), or assuming an approximate value of 78%, a value that we have determined from previous studies.

A dermal punch can also be used instead of a Tru-Cut needle. A dermal punch is a simple, inexpensive and effective tool that is effective on fish greater than 200 mm in size. To use the punch, remove a few scales, place the punch on the skin and apply a moderate pressure and twisting action to penetrate the underlying epaxial musculature. A small slug of tissue is harvested within the distal end of the punch that is then removed and placed on a glass slide. Using a clean scalpel, cut off the epidermis and place only the muscle tissue in a vial. If only CVAAS is available for analysis of tissues, we recommend that the dermal punch be used because a larger tissue weight is harvested than the Tru-Cut needle. Again, two samples should
be collected and composited. If possible, dermal punch samples should also be freeze-dried before analysis.

The advantages of the dermal punches are: they collect a relatively larger amount of tissue (60 mg); they are disposable; fairly cheap; and their use is easily mastered. One of the disadvantages of using the punches is that it leaves an open wound after removal of tissue that can lead to an increased likelihood of infection (especially for large punches). However, Nexaband™, a sterile Crazy Glue should be used to close the wounds to decrease chances of infection. This glue works well with fish tissue and polymerizes to form a thin, flexible waterproof bandage upon contact with water.

According to the literature we surveyed, when properly conducted, mortality of fish using biopsy techniques is very low. In previous controlled studies of biopsy procedures, mortality of fish was less than 0.5% (Uthe, 1971; Crawford et al., 1977; Mair, 1989). It is possible that some stress related mortality to fish may occur, however tissue extraction is not expected to exacerbate this.
3.0 CONCLUSION

There has been a considerable amount of information collected on fish mercury concentrations in British Columbia lakes and reservoirs since 1970. However, good summaries exist in only a few older documents (Peterson et al., 1970; Garrett et al., 1980; Reuben, 1989) and nothing has been recently produced. This document provides an up-to-date summary of the vast majority of mercury data available for freshwater fish in British Columbia through 2001.

Many data sets were widely scattered, difficult to find and were often contained within much larger documents, such as environmental impact assessments for proposed mine developments or within ministry files. We also observed that very few studies have been conducted that specifically target mercury concentrations in fish, with only a few exceptions (e.g., Reid and Morley, 1975; Antcliffe et al. 1997; Foster and Gadbois, 1998, Baker 2001a; Baker, 2001b; Baker et al., 2001b).

During the course of this work it was recognized that in many cases there was a lack of understanding of some fundamental concepts with regard to mercury concentrations in fish. For example in some cases there was confusion in reporting between wet and dry weight concentrations. More frequently, no account of differences in fish size was made, and the confounding effect of different fish sizes makes comparisons of arithmetic mean mercury concentrations less meaningful.

The majority of mercury data sets we examined suffer from at least one of the following deficiencies:

- In most cases, it appeared as if mercury data were collected opportunistically, during the course of other studies, without a specific objective relating to mercury. This results in small size ranges and/or sample sizes of fish captured, limiting the utility of the data.

- Mercury data sets were frequently represented by only a small sample size, or were gathered from a narrow size range of fish preventing derivation of mercury – size relationships.

- In some cases there was an absence of length and/or weight data and very few age data accompany the mercury data.

- Mercury data were not collected with specific objectives in mind, especially long-term studies.

- Comparisons or evaluations of mercury contamination were generally made using arithmetic mean mercury concentrations that are biased by differences in fish size between populations or years.

- In some data sets there was confusion between dry weight and wet weight concentrations.

- Quantitative protocols for examining mercury concentrations in fish populations established elsewhere in Canada (e.g., Strange and Bodaly, 1998: Tremblay et al. 1998) have not been employed in British Columbia.
Fortunately, despite these deficiencies, mercury concentrations for the vast majority of species in BC lakes and reservoirs were reasonably low. Thus, although most studies conducted to date provided “ballpark” estimates of mercury contamination in fish, in nearly all cases these estimates are far enough below the guideline concentration for commercial sale (i.e., 0.5 ppm), that there is very little cause for concern. Nevertheless, it is recognized that environmental contamination by mercury continues to be a persistent issue in some areas in British Columbia. We recommend that all future monitoring programs should use the standard protocols and procedures described here to ensure that comparisons of mercury in fish are made among species and reservoirs, and over time, that are unbiased by differences in fish size. Non-destructive techniques should be used wherever possible.
4.0 REFERENCES


APPENDIX A

Fish Mercury Raw Data Summary