Guidance for Fish Tissue Analysis for Mercury using Non-Lethal Methods for the Metal Mining Environmental Effects Monitoring Program

Final Version

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As part of the Environmental Effects Monitoring (EEM) requirements of the *Metal Mining Effluent Regulations* (MMER), effects on fish usability are monitored if mercury (Hg) exceeds 0.1 µg/l in a facility's effluent. This involves measuring mercury in fish tissue from fish collected in the receiving environment. Tissue analysis for mercury has been traditionally conducted by extracting a fillet from fish. Several recently published studies have demonstrated that non-lethal harvesting methods can produce accurate and reliable measures of fish muscle mercury concentrations provided appropriate analytical techniques are used (Tyus *et al.* 1999, Baker 2002, Baker *et al.* 2004, Peterson *et al.* 2005.). The use of non-lethal methodologies for mercury analysis are particularly attractive at sites where destructive sampling methods would be detrimental to fish populations, for example, at sites where fish density is low. The purpose of this guidance document is to describe appropriate non-lethal methodologies for tissue sampling and analysis for use in the Metal Mining EEM program.

Currently, the Metal Mining Guidance Document for Aquatic Environmental Monitoring (Environment Canada 2002) recommends that tissue analysis be conducted on 8 samples (to achieve 95% power) of a single species from one sex and age class during a lethal sampling study. This guidance should also be followed in a non-lethal survey with the exception of determining sex. It will not be possible to determine for most species if non-lethal sampling is used. However, several studies failed to find differences in mercury concentrations related to fish gender although males and females can differ in energy requirements (Lange *et al.* 1994; Henderson *et al.* 2003; Craig *et al.* 2004; Ward and Neumann 1999).

Baker *et al.* (2004) demonstrated that small tissue quantities collected with two different types of non-lethal biopsy tools (dermal punch and a Tru-Cut™ biopsy needle) provided accurate and precise estimates of mercury concentration in fish muscle relative to benchmark values from the traditional, fillet-style methods. The authors also found that the dermal punch method did not reduce survival of recaptured northern pike. Tyus *et al.* (1999) examined survival of rainbow trout and razorback sucker subjected to tissue collection using dermal punches, fin punches or liver punches and found no significant differences in growth or survival in any of the treated fish.

Recommended Methodology

Baker *et al.* (2004) noted that the reliability of the non-lethal technique depended on the biopsy tool, analytical methodology and tissue sample weight. The following recommended methodology for extraction of fish muscle tissue using a non-destructive approach is based on the work of Baker (2002) and Baker *et al.* (2004).

a) Practise - If at all possible, attempt to collect tissue from archived material or incidental mortalities before trying this method on a living fish. Becoming familiar with **a** technique will minimize possible handling and sampling stress.

b) Capture and anaesthetize fish - Prepare two holding containers, one with well-oxygenated water and another containing an anaesthetic (e.g. MS222). Capture fish by non-lethal means such as angling, short-set gill nets, electrofishing, etc. and place in the holding container. Transfer fish to the container containing the anaesthetic, one at a time, as necessary.

c) Obtain external fish measurements - Once anaesthetized, remove the fish from the water and determine length and weight. An aging structure (such as a pelvic fin ray) should be removed if appropriate.

d) Tissue Extraction - Two tools currently available for harvesting small tissue samples include dermal punches or the Tru-Cut™ biopsy needle.

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- Tru-Cut™. Remove two or three scales from the dorsal region of the fish just below the dorsal fin using a sterilized needle. The outer barrel is then inserted to a depth of about 1 cm into the fish muscle tissue beneath the scale at an oblique angle (to minimize penetration depth). The 2 cm long notched needle (inner barrel) is then extended into the flesh. The containment cover (i.e., sharp outer barrel) slides over the extended needle to cut the tissue and capture it within the notch. The needle is then withdrawn, the barrel opened and tissue slug removed with stainless steel (which should be acid washed between samples) or disposable plastic tweezers and placed in a small labelled vial. Samples obtained are approximately 25 mg. At least two tissue samples should be harvested and composited per fish to obtain a sufficient quantity to permit analysis. Baker *et al.* (2004) indicates that this procedure requires about 10 seconds for an experienced person to harvest a single sample.
- Dermal punch. The dermal punch harvests a larger quantity of tissue and, for this reason, it is the recommended harvesting method if only CVAAS is available for tissue analysis. This method can be used on fish greater than 200 mm in size. A few scales are removed and the dermal punch is placed on the skin. Moderate pressure and twisting action is applied to penetrate the epaxial musculature to harvest a small slug of tissue (approximately 60 mg of tissue). As with the biopsy approach, two samples should be harvested per fish and composited.

e) Sample Preservation - Samples should be frozen using dry ice or liquid nitrogen to prevent decomposition during storage and transport to an analytical laboratory. Samples should be freeze dried and weighed prior to analysis.

f) Infection Prevention - Tissue extraction methods, particularly the dermal punch, leaves an open wound that may lead to an increased likelihood of infection. Sterile crazy glue, such as Nexaband™, which acts like a waterproof bandage, should be used to close the wounds to decrease the chance of infection.

g) **Monitor and Reintroduce Fish-** Once the tissue samples are harvested, return the fish to the holding container until it appears to have recovered and swims normally. The fish is then released back into the receiving water body.

h) **Chemical Analysis** - Selection of an analytical methodology must consider the accuracy of chemical measurement for small tissue quantities. Cold vapor atomic absorption spectrophotometry (CVAAS) requires a minimum of 100 mg sample weight. Cold vapour atomic fluorescence spectrophotometry (CVAFS) has lower detection limits and is better suited to determining mercury concentrations in small tissue quantities. Combustion atomic absorption spectrometry with gold amalgamation is a simplified and rapid procedure for analyzing small tissue quantities for total mercury (Cizdziel *et al.* 2002).

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