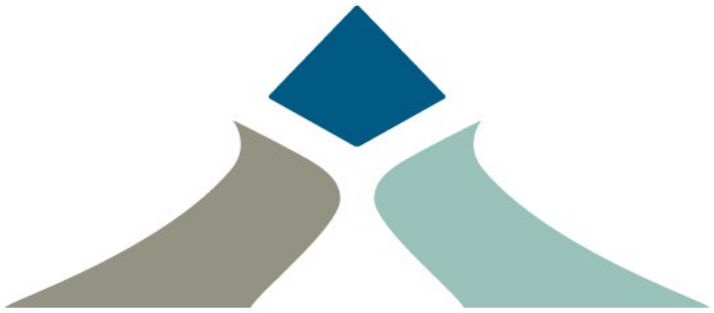
Guidance on Conducting Studies of Selenium in Fish Tissue

Supporting Requirements of the *Metal and Diamond Mining Effluent Regulations*



The Mining Association of Canada

ADVOCACY STEWARDSHIP COLLABORATION

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Version date: September 2023

Preface

The *Metal and Diamond Mining Effluents Regulations* (MDMER) were introduced in 2018 as an amendment to the 2002 *Metal Mining Effluent Regulations*. The MDMER introduced new requirements to conduct studies of selenium in fish tissue if conditions specified in the Regulations are met. This guidance document has been developed to support owners and operators of mines that are required to conduct such studies. The document is freely available on the MAC website and has been widely distributed outside of MAC member companies.

This guidance document has been developed by the Mining Association of Canada (MAC), in collaboration with subject matter experts from MAC members and MAC associate members. The guidance document has been developed taking into account the MDMER requirements for Environmental Effects Monitoring (EEM), best practices and the state-of-the-science related to selenium in fish tissue, including the most recent selenium technical support materials from the United States Environmental Protection Agency (US EPA).

The intent of this guidance document is to provide fit for purpose guidance for the conduct of MDMER studies of selenium in fish tissue. Such guidance is needed given the specific nature of the MDMER requirements. Guidance from other sources, such as the US EPA, while useful for some aspects, is not appropriate for supporting implementation of broader MDMER requirements.

This document has been reviewed by subject matter experts from a range of organizations outside MAC and MAC membership. In July 2022, the final draft was sent for review and comments were provided by subject matter experts from Environment and Climate Change Canada, Natural Resources Canada, the Selenium Working Group of the North American Metals Council, Kilgour and Associates, Lorax Environmental, and WSP. Comments submitted were considered in finalizing the document. MAC extends thanks to all of those who reviewed the document and provided comments.

While this guidance is written as a stand-alone document, it is aligned with, and is intended to be used in conjunction with guidance prepared by Environment Canada: *Metal Mining Technical Guidance for Environmental Effects Monitoring* (EC, 2012).

List of Abbreviations and Acronyms

CoC	Chain of Custody
CPUE	Catch per unit effort
dw	Dry weight
EC	Environment Canada
ECCC	Environment and Climate Change Canada (name changed from Environment Canada in 2015)
EEM	Environmental Effects Monitoring, as required in the MDMER
g	Grams
MDL	Method Detection Limit
MDMER	Metal and Diamond Mining Effluent Regulations
mm	Millimetres
RDL	Reportable Detection Limit
RIAS	Regulatory Impact Analysis Statement
SeFT	Selenium in Fish Tissue
US EPA	United States Environmental Protection Agency
µg/g	Micrograms per gram
µg/L	Micrograms per litre
QA/QC	Quality assurance/Quality control
YOY	Young of the year

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1 Introduction

1.1 Objective

This guidance document is intended to support implementation of requirements in the *Metal and Diamond Mining Effluent Regulations* (MDMER) to conduct studies of selenium in fish[1] tissue as part of the Environmental Effects Monitoring (EEM) requirements of the Regulations. This guidance document is specifically tailored to the requirements of the MDMER and is not intended to provide direction for other types of studies of selenium in fish tissue, such as studies to meet other legal requirements, research studies, etc.

Consistent with this objective, this guidance does not go beyond the scope of the MDMER requirements. For example, there is no discussion of the possible role of analysis of selenium in fish tissue as part of EEM investigation of cause studies since the Regulations do not define an "effect" with respect to concentrations of selenium in fish tissue, and there are no triggers to conduct investigation of cause studies on the basis of selenium concentrations in fish tissue.

The document provides guidance to inform the collection of high-quality data on selenium in fish tissue that is collected in a consistent manner and facilitates meaningful, valid comparisons/compilations of results from different sites.

This guidance document addresses:

- MDMER-specific considerations, including the integration of fish tissue sampling with other EEM requirements, particularly, fish population studies.
- Technical considerations such as selection of tissues for analysis, collection of samples, and analytical considerations.

Development of this guidance document was undertaken to ensure that "fit for purpose" guidance is available to owners/operators of mines required to conduct studies of selenium in fish tissue, and to consulting firms conducting such studies on behalf of owners/operators. Such "fit for purpose" guidance is needed given the specific nature of the MDMER requirements, described below. Guidance from other sources, such as the United States Environmental Protection Agency (US EPA), is useful for some aspects such as methodologies for collection of tissue samples; but is not appropriate for supporting implementation of MDMER requirements.

The state of science and practice with respect to monitoring of selenium in fish tissue is continuing to evolve. This guidance document reflects practices at the time of writing (2023) and may be updated as appropriate in the future to reflect evolving science and practices, as well as any future changes in the MDMER requirements.

[1] Under the *Fisheries Act*, the definition of fish includes finfish as well as shellfish and crustaceans (see *Fisheries Act*, subsection 2(1) for the full definition). This guidance document is focused on the collection and use of finfish for studies of selenium in fish tissue. However, in some situations, owner/operators may consider collection and use of non-finfish.

1.2 Overview of MDMER Requirements

The MDMER are regulations under Canada's *Fisheries Act*. The MDMER were introduced in 2018 as an amendment to the 2002 *Metal Mining Effluent Regulations* and introduced a new requirement to conduct a study of selenium in fish tissue if selenium concentrations in effluent exceed specified criteria.

The MDMER requirement for studies of selenium in fish tissue study of selenium in fish tissue requirement of the MDMER is part of the EEM requirements, as described in Schedule 5, Part 2, Biological Monitoring Studies — Required Studies. Specified criteria are linked to concentrations of selenium in effluent measured as part of the effluent characterization requirements of the MDMER (Schedule 5, sections 4 and 8). Criteria are described in Schedule 5, paragraph 9 (1)(d), which requires a study respecting fish tissue selenium, if:

> "(i) effluent characterization reveals a concentration of total selenium in the effluent that is equal to or greater than 10 µg/L,
> (ii) effluent characterization reveals an annual mean concentration of total selenium in the effluent that is equal to or greater than 5 µg/L, based on a calendar year, or
> (iii) the method detection limit used in respect of selenium for the analysis of any effluent sample is equal to or greater than 10 µg/L, or the method detection limit used in respect of selenium for the analysis of at least two of four effluent samples in a calendar year is equal to or greater than 5 µg/L."

If a study of selenium in fish tissue is required, then, in accordance with Schedule 5, paragraph 10(b), the EEM study design must provide a description of how the study will be conducted that includes:

> "(i) a description of and the scientific rationale for
> (A) the fish species selected, taking into account the abundance of the species most exposed to effluent,
> (B) the sampling areas selected within the exposure area and the reference area,
> (C) the sampling period selected,
> (D) the sample size selected, and
> (E) the field and laboratory methodologies selected"

The study design requirements are part of broader requirements for study design described in Schedule 5, section 10, that include requirements for site characterization (paragraph a), identifying the month in which the samples will be collected (paragraph d), and a description of the quality assurance and quality control measures that will be implemented (paragraph e).

It is important to highlight that the requirement in the MDMER is to measure total selenium concentrations in muscle or whole-body fish tissue samples, while sampling ovaries or eggs is included "if practicable" (Schedule 5, subclause 12(1)(e)(iv)). Given this requirement, this guidance document gives priority to collection and analysis of muscle or whole-body samples, recognizing that this may not be wholly consistent with other guidance (e.g., US EPA) which

emphasizes the importance of sampling ripe or late stage-development eggs/ovaries. This is discussed further in Section 3.2.

It is important to emphasize that the MDMER do not define an effect (e.g., criterion or effect size) associated with concentrations of selenium in fish tissue and do not require a comparison of results between exposure and reference areas. The MDMER also do not require comparison of results with any published standards/criteria for selenium in fish tissue, such as those published by Environment and Climate Change Canada (ECCC, 2022, Federal environmental quality guidelines for selenium) and the United States Environmental Protection Agency (US EPA, 2021a). Therefore, the statistical analyses that would be used to conduct such comparisons are not addressed in this guidance document.

Requirements of the MDMER specific to studies of selenium in fish tissue are limited to reporting of:

- The type of fish tissue studied and the scientific rationale for the selection of that tissue (Schedule 5, paragraph 12(g)).
- Total selenium (dry weight) reported in µg/g (Schedule 5, subclause 12(1)(e)(iv)).
- Percentage of the moisture content of the sample (Schedule 5, subclause 12(1)(e)(iv)).

Owners/operators must also report the mean, median, standard deviation, standard error, and minimum and maximum values of selenium concentrations in fish tissue collected from the sampling areas (Schedule 5, paragraph 12(1)(e)).

Other relevant reporting requirements in Schedule 5, section 12 must also be met, particularly if studies of selenium in fish tissue are not conducted in conjunction with other EEM studies. These include:

- A description of any deviation from the study design (Schedule 5, paragraph 12 (a)).
- Latitude and longitude of sampling areas and a description of sampling areas (Schedule 5, paragraph 12(b)).
- Dates and times when samples were collected (Schedule 5, paragraph 12(c)).
- Sample sizes (Schedule 5, paragraph 12(d).
- Identification of the sex of the fish sampled and of the presence of any lesions, tumours, parasites, or other abnormalities (Schedule 5, paragraph 12(g)).

1.3 Basis for the Guidance

This guidance document is predicated on the view that, to the extent practicable, sampling of fish tissue for selenium analysis should be aligned with the conduct of fish population studies, including the timing of sample collection and the species/individual fish collected. This approach will:

- Facilitate potential site-specific comparison of results of the fish population study with the selenium concentrations in fish tissue, which would not be possible if different species and/or timing for sample collection were used for studies of selenium in fish tissue relative to the fish population study.
- Avoid further harm/mortality to fish populations beyond that already associated with sampling to conduct fish population studies or monitoring to meet other regulatory requirements.

This approach is consistent with assumptions made by ECCC in developing the MDMER. The Benefits and Costs section of the Regulatory Impact Analysis Statement (RIAS) published with the MDMER on May 30, 2018, stated that "[i]t is assumed that affected mines currently conducting fish population studies will not incur costs to prepare an SeFT [study of selenium in fish tissue] study design, as these mines likely already have an existing study design *and will be able to conduct the fish population and SeFT studies in conjunction*." [emphasis added]

This guidance is intended to be practical and hands on. It incorporates learnings from field experience in fish tissue sampling and reflects the importance of the need to be flexible to adapt to site-specific conditions and challenges inherent in field sampling.

This guidance is intended to be used in conjunction with Environment Canada's (EC) 2012 *Metal Mining Technical Guidance for Environmental Effects Monitoring* (EC, 2012), referred to in this document as the EC Technical Guidance.

Based on 2019 to 2021 effluent characterization data for selenium provided to MAC by ECCC in 2022, approximately one third of mines subject to the MDMER are required to conduct studies of selenium in fish tissue.

1.4 Disclaimer

Consistent with the objective and disclaimer of the 2012 EC Technical Guidance, the objective of this document is to provide guidance to owners and operators on how to meet the MDMER requirements for studies of selenium in fish tissue. This is not a legal interpretation of the Regulations. To understand the legal requirements of the MDMER, owners and operators should refer to the Regulations, available at:
https://laws-lois.justice.gc.ca/eng/Regulations/SOR-2002-222/index.html

2 Monitoring Strategy

Developing a monitoring strategy for a study of selenium in fish tissue involves making decisions on numerous site-specific components in the study design, including which species and tissue(s) to sample, timing of sampling, the number of replicate samples to collect per study area, and the potential use of composite samples to meet analytical sample weight requirements. Considerations surrounding these study design decisions are discussed in this guidance document alongside practical information on field sampling methodologies, laboratory sample requirements and analyses, and data analysis requirements. When designing a monitoring strategy, it is important to consider both the requirements of the MDMER and site-specific requirements, such as provincial/territorial requirements related to tissue chemistry monitoring programs.

As discussed above, this guidance document advocates that it is preferable to integrate studies of selenium in fish tissue with an EEM fish population study or other fish monitoring programs already being conducted at the site. This approach is recommended to enable the practitioner to:

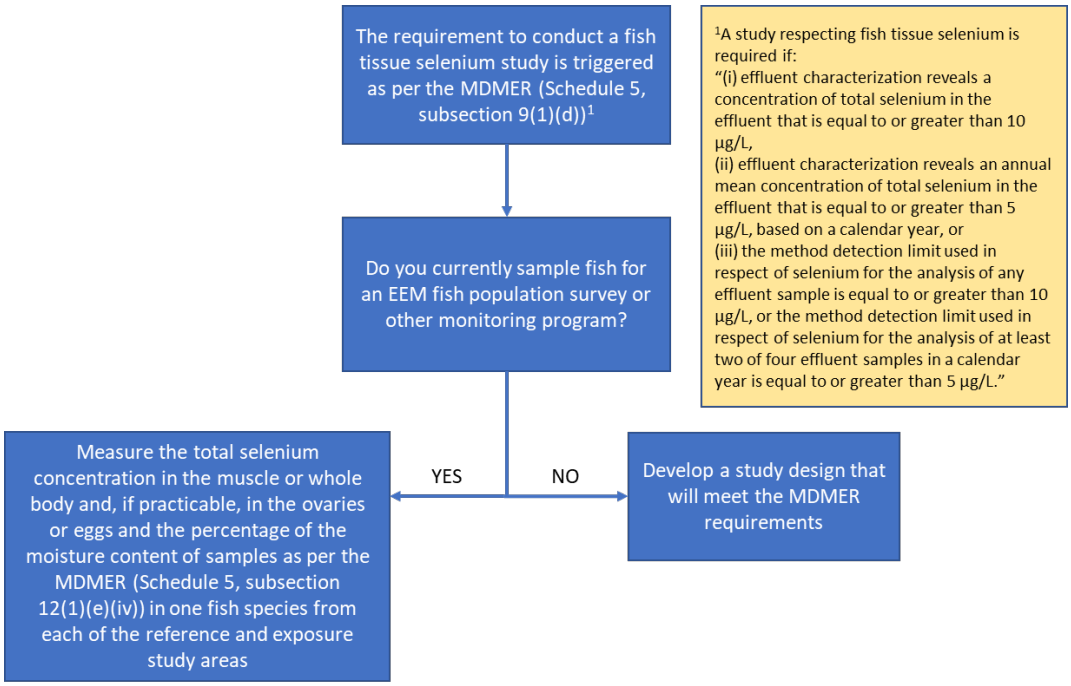
- Enable temporal comparisons between monitoring phases, including comparisons to historical fish tissue selenium concentrations, if available.
- Avoid an increase in resources associated with completing an additional sampling program during a different time of the year.
- Avoid additional fish mortality and optimize sampling to achieve multiple objectives (e.g., mercury fish tissue sampling, if required).

Avoiding additional fish mortality caused by monitoring should be of paramount importance when designing a monitoring strategy for studies of selenium in fish tissue to avoid additional stress on fish communities (Kambeitz *et al*., 2019). If fish are already being sacrificed for a lethal EEM fish population survey or for a fish tissue chemistry program, then those fish should also be used for the study of selenium in fish tissue. If fish are not currently being sacrificed as part of other site-specific monitoring programs, then consideration should be given to designing the study of selenium in fish tissue to minimize the number of fish being sacrificed to meet this MDMER requirement (Kambeitz *et al*., 2019). Alternatively, non-lethal fish tissue sampling methods such as muscle plugs (i.e., biopsy punches) should be considered.

The requirement in the MDMER is to measure total selenium concentrations in muscle or whole-body fish tissue samples, while sampling ripe or late stage-development eggs/ovaries is included “if practicable” (Schedule 5, subclause 12(1)(e)(iv)). Although egg-ovary data can provide valuable information on integrative dietary accumulation, transfer, and deposition of selenium in female fish, as well as potential selenium reproductive impairment (US EPA, 2021a,b), the monitoring strategy should consider relevant factors if choosing to include sampling of ripe or late stage-development eggs/ovaries as part of the EEM program. These considerations are discussed in Section 3.

Figure 1 provides a decision tree that illustrates a simplified approach to meeting the MDMER requirements for studies of selenium in fish tissue (if triggered) and is based on the premise of prioritizing the use of fish already collected for an existing monitoring program.

Figure 1: Monitoring strategy decision tree to meet the MDMER requirements to study selenium in fish tissue.



3 Study Design

As stated in Section 1.2, in accordance with Schedule 5, paragraph 10(b) of the MDMER, the EEM study design must provide a description of how the study will be conducted, including the fish species, sampling areas within the exposure and reference areas, sampling period, sample size, and field and laboratory methodologies selected.

The detailed study design information that must be provided for a fish tissue selenium study is the same as the information provided for an EEM fish population study. While these requirements do not explicitly address selection of the fish tissue to be sampled, it is assumed the methodologies would depend on the tissue selected and, therefore, such details should be included as part of the study design. Species selection, tissue selection, replicates and sample size are discussed in Sections 3.1, 3.2, and 3.3, respectively.

If a study of selenium in fish tissue is required, then there are four scenarios for development of study designs:

1. A study of selenium in fish tissue is conducted in conjunction with an EEM fish population study. In this scenario:
   - The sampling period would be the same for both studies.
   - The same exposure and reference areas would be used for both studies.
   - The species used for the study of selenium in fish tissue would be one of the species used for the EEM fish population study.

2. An EEM fish population study is required but the owner/operator decides not to conduct the study of selenium in fish tissue in conjunction with the EEM fish population study. For example, the owner/operator decided that the study of selenium in fish tissue would use eggs/ovaries (in addition to muscle or whole-body samples) and fish need to be collected at a different time of year than for the fish population study in order to collect eggs/ovaries that are ripe or in late stage-development. In this scenario, it is recommended that the fish species and study areas (i.e., exposure and reference areas) selected be the same as those used for the EEM fish population study, unless:
   - Other (e.g., provincial/territorial) regulatory requirements necessitate use of different species and/or exposure and reference areas; or
   - The species and/or exposure and reference areas used for EEM fish population studies have been determined to be unsuitable for studies of selenium in fish tissue.

3. A study of selenium in fish tissue is being conducted for the first time and an EEM fish population study has not previously been required, either because this is the first phase of EEM at the site, or because the effluent concentration at 250 m from the final discharge point is ≤1% (Schedule 5, paragraph 9(1)(a)). In this scenario, it is recommended that owners/operators:
   - Consult Section 3.2 of the EC Technical Guidance for selection of exposure and reference areas.

- Consult Section 3.3 of the EC Technical Guidance and Section 3.1 below for selection of species.
- Consider requirements of any other fish monitoring programs that have been, or are, in place (e.g., EEM mercury tissue study or provincial/territorial requirements).
- Refer to historical and/or publicly available data to determine the species most likely to be consistently abundant in both the reference and exposure areas.

4. A study of selenium in fish tissue is required, but an EEM fish population study is not required in that EEM phase based on the results of the previous two biological monitoring studies (Schedule 5, paragraph 9(1)(a)). In this scenario, it is recommended that the study of selenium in fish tissue be conducted using:
   - The same sampling period (i.e., timing) as the last EEM fish population study.
   - The same exposure and reference areas as the last EEM fish population study.
   - One of the fish species used in the last EEM fish population study.

3.1 Species Selection

One fish species is required for studies of selenium in fish tissue. Species selection should consider existing EEM requirements and make efforts to be consistent with previous studies' species selection. Species selection should also consider guidance in Section 3.3 of the EC Technical Guidance. Potential sampling effects to the fish population and community should also be considered by assessing species abundance and the benefits of collecting large-bodied vs small-bodied fish (e.g., sampling small-bodied fish may have less sampling effects on the population if they are more abundant than large-bodied fish). The need to sacrifice additional fish (i.e., beyond those used in the EEM fish population study) should be avoided, if possible.

In cases where a study of selenium in fish tissue is being conducted in conjunction with the EEM fish population study (Scenario #1 or #3 above), the study of selenium in fish tissue should use one of the sentinel species collected for that study. If two sentinel species are being sampled for the fish population study, it is recommended that the most consistently abundant species captured within both exposure and reference areas be used for the study of selenium in fish tissue. This approach will help minimize sampling impact on fish populations and increase the likelihood of collecting an adequate number of fish to satisfy sample size requirements for the study of selenium in fish tissue. Records from previous EEM studies can be queried to compare Catch Per Unit Effort (CPUE) results, and, where sentinel species have varied across time due to limited catch in some study years, the sentinel species that has been most commonly or consistently caught, should be selected.

The MDMER requirement to sample muscle tissue or whole-body may be met by sampling:

- Sexually mature (i.e., adult) males. Selenium concentrations in male tissues are stable regardless of reproductive state (US EPA, 2021b). If insufficient males are caught, then sexually mature females should be used.

- If collecting non-lethal samples or the sex cannot be determined, then sexually mature fish may be used (i.e., adults).
- If collecting adult fish is not possible then immature fish (i.e., juvenile) may be collected. Immature fish may be identifiable as male or female, or be of undetermined sex.
- If collecting adult or immature fish is not possible then young of the year (YOY) fish may be collected. YOY fish will most likely be of undetermined sex.

If collecting ovary or egg samples, sexually mature (i.e., adult) females should be collected. The expected stage of gonadal development at the time of sampling is also important (see Section 3.2.2).

In instances where the EEM fish population study includes both a large-bodied and a small-bodied fish species, a number of factors should be considered in determining which species to use for the study of selenium in fish tissue, including:

- Species most commonly or consistently caught as sexually mature, non-YOY life stages.
- Potential for detrimental sampling impact on the fish population and/or community.
- Ease of sampling.
- Type of tissue to be collected and the ability to provide adequate sample volumes for chemical analysis (including the potential to collect muscle plugs, which may reduce fish mortality).
- Timing of reproduction and gonad development of each species relative to the study timing (if ovaries or eggs are to be collected).
- Home range/mobility of the species and the degree to which individuals captured may be representative of conditions in the exposure or reference areas (i.e., considering the potential for fish movement between exposure and reference areas).

In many cases, and depending on the fish tissue type being sampled, the study of selenium in fish tissue will require that fish captured are sacrificed to collect tissue samples. The use of biopsy punches (i.e., muscle plugs) allows for non-lethal tissue chemistry sampling from most large-bodied fish species. If the EEM fish population study is being carried out using non-lethal methods and endpoints, then the use of muscle plugs and/or expressed eggs should be considered to minimize fish mortality. If another concurrent monitoring program requires lethal fish collections, then the fish species used in the concurrent monitoring program should be strongly considered for use in the study of selenium in fish tissue study.

As per the *Fisheries Act*, subsection 2(1), the definition of “fish” in the Act is not limited to finfish and includes shellfish and crustaceans. This guidance document is focused on the collection and use of finfish for studies of selenium in fish tissue, and it is expected that in most cases finfish would be used. Based on 2019 to 2021 data on fish species and tissues used for studies of selenium in fish tissue provided to MAC by ECCC in 2022, all species used during that time period were finfish. However, in some cases, collection of finfish may not be possible, either because of an absence of finfish in the exposure and/or reference area, or because of restrictions on the collection of finfish. In such scenarios, the owner/operator may consider

alternatives to the collection and use of finfish. The owner/operator should discuss alternatives with ECCC during study design development to confirm that the sampling program will meet the MDMER requirements.

3.2 Selection of Tissue Type to be Collected

As described in the MDMER, and as illustrated in Figure 1, muscle or whole-body tissue are to be sampled for the study of selenium in fish tissue, while eggs or ovaries may be sampled "if practicable" (Schedule 5, subclause 12(1)(e)(iv)). Although the MDMER do not specify that eggs or ovaries collected for selenium analysis should be in a late stage of development or are ripe, it is strongly recommended that, if eggs or ovaries are used to meet the MDMER requirements for studies of selenium in fish tissue, they should be ripe. The rationale for this is discussed in Section 3.2.2.

Although ripe eggs or ovaries provide the most direct means to assess potential reproductive effects due to selenium, there are a number of practical issues (e.g., inability to collect fish at the right time for a given species to obtain ripe ovaries) that may preclude the collection of good quality data from ripe eggs or ovaries. Samples of muscle or whole-body tissue from adult fish serve as robust alternatives for assessing exposure to selenium if gonadal tissues cannot be collected (US EPA, 2021b).

3.2.1 Muscle and Whole-body Tissue Samples

Selenium concentrations in muscle (e.g., fillet) or whole-body tissues (i.e., including all organs) will provide representative information on selenium bioaccumulation and ecological exposure.

Concentrations of selenium in muscle and whole body reflect integrative dietary accumulation and deposition of selenium in fish tissues over time and space in fish population(s) at a given site. Muscle and whole-body tissue samples are relatively easy to collect and seasonal considerations are less stringent relative to collecting ripe eggs or ovaries. Seasonal collection of muscle or whole-body fish tissue samples should be consistent with the timing of existing EEM fish population studies and should align with guidance in Section 3.5 of the EC Technical Guidance.

If the sex of the fish can be determined, it is preferable to use male fish for muscle or whole-body samples, since the selenium concentrations in their tissues are more stable on a seasonal/annual basis compared to females and will provide high quality data (US EPA, 2021b). Determining the sex is not always possible at the time of sample submission to the analytical laboratory and for small bodied-fish, pooling of individual fish to form composite samples is often required to achieve adequate sample size for analysis. In such circumstances, pooling of small-bodied fish of unknown sex is not anticipated to adversely affect data quality (Mo *et al*., 2020).

When collecting muscle tissue from large-bodied fish, biopsy punches or muscle plugs can be a viable alternative to collecting whole muscle fillets and also has the advantage of reducing fish mortality. Muscle fillet or whole-body tissue sampling requires fish to be sacrificed, while muscle plugs can be collected non-lethally (recognizing that there may still be some mortality using this method). Stahl *et al*. (2021) reported that there were no statistically significant differences in selenium concentrations between tissue plug and homogenized fillet results.

When developing a study design for a study of selenium in fish tissue that will be conducted in conjunction with an EEM fish population study or other fish study, tissue selection for selenium analysis should consider other sampling activities that will be necessary to meet other monitoring requirements. For example, in lethal EEM fish population studies, fish are dissected to remove and weigh the liver and gonads. Without these organs, the remaining fish carcass would not be considered a whole-body sample for selenium analysis. In these cases, muscle fillet samples may be collected from these fish, or the organs (and other tissues) removed may be submitted with the carcass as a whole-body sample for selenium analysis. Thus, when fish collected are to be used for multiple purposes, the study design should address this to ensure that suitable samples are collected to meet these different purposes. If organs have been removed from whole-body samples in previous studies, then it may be beneficial to remain consistent in the methodology to allow identification of temporal trends.

3.2.2 Ripe Egg/Ovary Tissue Samples

Toxicity data indicate that selenium concentrations in ripe fish eggs and ovaries are the most robust measurement endpoint directly tied to adverse reproductive effects due to selenium (Chapman *et al*., 2010; ECCC, 2022; US EPA, 2021a).

In sexually mature female fish, selenium is deposited in the ovaries during egg development. The accumulated selenium is transferred into eggs during final oocyte maturation (Janz *et al*., 2010). The length of time over which vitellogenesis and associated selenium accumulation occurs is variable among species. Egg maturation and selenium deposition can occur over months for some species (e.g., rainbow trout, *Oncorhynchus mykiss* and lake trout, *Salvelinus namaycush*), while in others (e.g., fathead minnow, *Pimephales promelas*) it may be more rapid. Some species spawn multiple times per season, referred to as asynchronous spawning, while other species skip spawning entirely in some years (i.e., not all adults spawn every year). For asynchronous spawners, egg maturation may occur well before, immediately prior to, or during, the spawning season. For skip spawners, egg maturation may not occur in one individual while proceeding normally in other individuals in the same population.

Given the relationship between egg development and selenium accumulation, selenium concentrations in ovary and egg tissue can vary widely throughout the year. Selenium concentrations in egg/ovary when those tissues are not developing or are in early-stage

development[2] may not be representative of selenium accumulation and concentrations when egg/ovary tissues are in late-stage development[3] or are ripe[4] (Brown-Peterson, 2011, US EPA, 2021a,b). Therefore, best practice is to collect egg/ovary samples when they are ripe or in late-stage development (US EPA, 2021a,b), and sampling eggs/ovary tissues at other times in gonadal development is not recommended for selenium analysis.

The time of year when eggs/ovaries are ripe or in late-stage development and females are ready to spawn depends primarily on the species. For a given species, the timing will also depend on the location and environmental factors such as the duration of daylight hours (photoperiod), water temperature, and water flow. Some of these environmental factors vary depending on weather conditions. As a result, the exact time of year when females of a given species will spawn typically varies from year to year and can be difficult to accurately predict at the time when study designs are developed. More specifically, this timing may be difficult to accurately predict because:

- The time-window within which spawning may occur can span several months while spawning itself typically only lasts for a few weeks.
- For asynchronous or skip spawners, spawning recurs multiple times per year or not at all.
- The time period when eggs/ovaries of a selected species are ripe or in late-stage development:
  - is not known for some species; and,
  - may vary between locations, including between exposure and reference areas used for EEM.

The EC Technical Guidance, Section 3.5, Table 3-5 provides recommended timing of sampling for fish species routinely employed within EEM fish population studies. These sampling windows have been established to detect differences in gonadal development in support of assessing endpoints used in EEM fish population studies. However, these recommended timings may not represent the optimum timing for sampling eggs/ovaries for selenium analysis. For example, for species that spawn synchronously in the spring (e.g., rainbow trout), Table 3-5 recommends sampling in late fall. For spring-spawning species, eggs and ovaries would not be ripe or in late-stage development in late fall (e.g., rainbow trout oocyte diameter may only be 40-50% of the final size at spawning) (Tyler *et al*., 1990) and thus, would not be suitable for a study of selenium in fish tissue. Therefore, the recommended sampling times provided in Table 3-5 of the EC Technical Guidance are not recommended for use in designing and conducting studies of selenium in fish egg/ovary tissues.

[2] Ovaries in early-stage development are small, often orange and have a granular appearance, but individual oocytes are not visible macroscopically at this stage.
[3] Ovaries in late-stage development are large and take up a significant portion of the body cavity, and individual oocytes are readily visible but are not released with slight pressure to the abdomen.
[4] Egg/ovaries are ripe when fish have completed the final stages of reproductive development and are ready to release the eggs. The ripe stage is typically brief, lasting for only a few days prior to spawning.

A further challenge associated with sampling when eggs/ovaries are ripe or in late stage development is that, for some species, this may occur at times of year when it is not safe or possible to collect fish, such as when fish are spawning under ice (e.g., burbot, *Lota lota*) or when water flows are very high due to freshet conditions (e.g., white sucker, *Catostomus commersonii*).

The use of egg/ovary tissue for selenium analysis may be challenging in a routine monitoring program such as EEM and may not be practicable. Even in cases where fish can be safely collected, there is the potential that eggs/ovaries may not be ripe or in late-stage development in both exposure and reference areas at the time that field work is conducted, despite best efforts to accurately predict when females will be spawning. If females are predicted to be spawning at a different time of year than the time during which the EEM fish population study is conducted, then targeting eggs/ovaries in the study of selenium in fish tissue may result in considerably higher fish mortality, with limited likelihood of successfully collecting suitable eggs/ovaries for selenium analysis.

These factors should be carefully considered in the study design stage when determining the tissue type(s) to be collected for studies of selenium in fish tissue.

3.3 Replicates and Sample Size

As required in the MDMER, Schedule 5, clause 10(b)(i)(D), the sample size selected for a study of selenium in fish tissue must be included in the study design, but the Regulations do not specify a required sample size. This guidance document recommends a minimum number of replicate samples of five fish (or five composite samples) collected from both the exposure and reference areas. These samples must be of the same species and same tissue type and of similar size, and they should be collected within the same sampling period. This aligns with US EPA technical guidance (US EPA, 2021b). For metal mines conducting studies of mercury in fish tissue, the EC Technical Guidance recommends using at least eight fish. However, a lower minimum number is recommended for studies of selenium in fish tissue for two reasons:

- A smaller sample size reduces fish mortality while allowing for the collection of quality data that meets the objectives of the MDMER requirements for studies of selenium in fish tissue.
- The MDMER do not provide a definition of an effect associated with selenium in fish tissue. In contrast, studies of mercury in fish tissue require statistical analysis of results to determine if there is an effect on fish tissue from mercury, as defined in Schedule 5, subsection 1(1). Thus, a higher level of statistical certainty is required for studies of mercury in fish tissue, and a higher number of replicates is, therefore, recommended to support statistical analyses.

Each replicate may be a sample from an individual fish (e.g., whole fish, fillet, single muscle plug, ripe ovaries) or each may be a composite of samples from a number of fish (Section 4.3).

Tissue from any individual fish should be used in one replicate only (i.e., individual sample or contribute tissue to a composite sample, but not both).

In cases where the weight of tissue samples is too small to meet minimum laboratory requirements (typically either whole-body or egg/ovary) based on correspondence with the laboratory (see Section 5), composite samples may be created that incorporate tissue from two or more fish. The use of individual samples for chemical analysis is preferred and given that current analytical methods have very small sample weight requirements, the use of individual samples is likely to be possible in most cases. However, composite samples have been demonstrated to provide an accurate representation of selenium concentrations in the fish making up the composite sample and are sufficient in the context of MDMER requirements for studies of selenium in fish tissue (e.g., US EPA, 2021b).

If there are other relevant requirements for sampling fish tissue for selenium analysis (e.g., provincial/territorial requirements) then the number of replicates collected may be increased accordingly, to ensure that a single round of sampling can be conducted to meet all relevant requirements, while minimizing fish mortality.

Another factor that may influence the number of replicates is the amount of effort expended. If collection of fish for a study of selenium in fish tissue is not coupled with an EEM fish population study, then the reasonable effort expended to collect the recommended number of replicates may vary depending on study area, fishing methods, and species. If the target number of replicates per area cannot be achieved, consultation with the ECCC EEM coordinators may be required. It is recommended that reasonable sampling effort be defined and included in the study design.

During the development of the study design, the laboratory conducting the analyses of selenium concentrations in fish tissue should be contacted to confirm sample weights required for the intended analyses (i.e., selenium and percentage of moisture in the tissue), as sample size requirements can vary between labs. Sample submission requirements, chain of custodies and other specifics should also be confirmed. An understanding of required sample weight is needed to allow for:

- Necessary quality control procedures and/or samples to be run (Section 5.3).
- Measurement of moisture content (as a percentage) if freeze drying is not used (Section 5.1).
- Desired method detection limits (MDLs) to be met.
- Additional parameters to be analyzed, if necessary, to meet other monitoring requirements (e.g., mercury, other metals, pesticides, dioxins/furans, lipids).

These needs should be identified during the development of the study design and then communicated clearly to both field and laboratory staff. Communication of such details should ensure that sufficient sample weights are obtained, and the correct analyses are requested of, and performed by, the analytical laboratory.

4 Field Sampling Considerations

4.1 Supporting Information

Supporting information should be documented from fish collected for selenium analyses to provide data that can be used to determine if factors such as age, sex, condition, and/or spawning condition may potentially influence selenium concentrations. In addition, although not required by the Regulations, it is best practice to obtain supporting information when sampling fish, particularly if the sampling is lethal, in order to maximize data availability. If the same fish are being sampled for the study of selenium in fish tissue and the EEM fish population study, then data from supporting information endpoints will already be available; however, the endpoints measured will differ depending on whether the EEM fish population study is conducted using lethal or non-lethal methods. For situations in which fish are only being collected for the study of selenium in fish tissue, Table 1 provides recommendations related to the collection of supporting information based on guidance provided in Section 3 of the EC Technical Guidance for the EEM fish population study and best practices. Collection of such supporting information is recommended, regardless of the fish tissue type being used for selenium analysis.

4.2 Tissue Collection and Preparation

Fish sampling (i.e., collection) methods are addressed in Section 3.8 of the EC Technical Guidance. Once fish are captured, they should be stored in buckets (small-bodied fish only), livewells or pens until sample collection begins. If fish have experienced injury or undue stress as a result of fishing method (e.g., gillnets), then fish should be sacrificed as soon as practicable after capture, to avoid suffering. If fish health endpoints to be measured include supporting information such as tissue colour assessments, then time elapsed between sacrifice and assessment should be considered when interpreting data from those fish. Supporting information described in Table 1 should be documented for each fish. If fish collected are also being used for a fish population study, then those required endpoints should also be documented (e.g., gonad and liver weight).

If fish are being held prior to sampling, they should be carefully observed for signs of physical damage or other sources of stress and should be handled as little as possible using dip nets and soft material gloves. Holding water temperature and dissolved oxygen should be monitored to ensure that ambient conditions (i.e., same as the in-lake/stream/river environment) are maintained according to animal use permit commitments, where applicable. Fish density should be kept as low as reasonably possible in the holding containers to minimize stress to the fish. If non-lethal sampling is occurring, fish should be released following processing at or near the site of capture.

Table 1: Recommended supporting information to be collected from fish used for tissue sampling.

Endpoint	Precision/Information	Are Data Collected for Lethal or Non-Lethal Surveys?
Taxonomy	Species	Non-lethal and lethal
Length (fork or total)[1]	+/- 1 mm	Non-lethal and lethal
Total body weight (fresh)	+/- 1.0%	Non-lethal and lethal
Carcass weight[2]	+/- 1.0%	Lethal
Gonad weight (if fish are sexually mature)	+/- 0.1 g for large-bodied fish species and 0.001 g for small-bodied fish species	Lethal
Abnormalities	Presence of any lesions, tumours, parasites, or other abnormalities	External only for non-lethal, external and internal for lethal
Sex	Male or female adult, male or female immature, or indeterminant	Lethal, unless sex can be identified externally
Age	+/- 1 year	Lethal
Spawning condition (if possible)	Resting, early- or late-stage development, ripe, spent	Lethal, unless spawning condition can be identified externally during the spawning period
Stomach contents[3]	Species identification to the extent possible	Lethal

[1] If caudal fin is forked, use fork length (from the anterior-most part of the fish to the fork of the tail); otherwise, use total length, and report type of length measurement conducted.

[2] A carcass is considered the whole eviscerated body of a fish.

[3] Recording stomach contents can provide valuable supporting information but is challenging and is not always practical or necessary depending on study objectives. Stomach contents may be submitted to a specialist laboratory for genus or species level identification (e.g., for benthic invertebrate contents) if this endpoint is critical to a monitoring program.

For collection of whole body or carcass, muscle fillet and ovary samples, fish should first be humanely sacrificed, and necropsy procedures should commence immediately following sacrifice (Wolf *et al*., 2004).

Dissections should be conducted on clean surfaces suitable for sampling fish tissue for chemical analysis (e.g., acetone-washed, baked aluminum foil or plastic-wrapped surface), and using appropriately cleaned instruments. All surfaces and instruments should be cleaned between fish, including those used for the collection of muscle plugs. All other surfaces, including containers for sample storage, should be new or appropriately cleaned to prevent cross-contamination of samples during collection, storage, and transportation to the laboratory.

For more detailed guidance on the sampling protocols detailed in Sections 4.2.1 through 4.2.4, please see US EPA (2021b).

4.2.1 Whole-Body Samples

Whole-body samples should include all organs. If organs have been removed to measure endpoints for EEM fish population studies (e.g., liver or gonads removed to determine their weight as per Schedule 5, section 2 of the MDMER), then the specimen is not suitable for whole-body analysis. This is because removal of any part of the body produces a sample that is not comparable to other whole-body samples and would not represent the selenium concentration present in the whole body of the fish. However, if the organs that were removed can be returned to the sample prior to submission for selenium analysis with the rest of the carcass, then the whole-body sample criteria would be met. If there are exceptions to this, for reasons such as temporal comparisons to non-whole-body samples, then the rationale should be documented. Alternatively, if the fish is large enough, a muscle fillet may be collected (Section 4.2.2).

In some cases, particularly for small-bodied fish, it may not be possible to collect a muscle fillet sample and submitting the removed organs together with the rest of the carcass may not be representative of the whole fish (e.g., due to loss of tissue during the extraction of the liver or gonads). In these cases, to avoid further fish mortality, the carcass may be eviscerated (i.e., all internal organs removed) and sent for selenium analysis. In such cases, the samples should be clearly labelled as eviscerated. When data are submitted to ECCC (Section 5.3), comment fields should be used to clarify that these samples are eviscerated fish, rather than whole body or muscle.

4.2.2 Muscle Samples

Muscle samples may include fillets or muscle plugs (Section 3.2). The samples are typically collected after supporting information has been documented and any other measurements have been taken.

Fillets should be collected from a consistent location for all fish samples (e.g., dorsal portion of the musculature above the lateral line).

Sampling muscle plugs or biopsy punches provides an alternative to lethal techniques for the collection of small volumes of muscle tissue. Muscle plugs can be collected if fish are of adequate size for a plug of appropriate weight to be collected while minimizing the potential for adverse effects on the fish. When muscle plugs are to be collected, the following steps should be taken:

- Using a pre-cleaned utensil, remove a small area of scales (if present) from above the lateral line. The white oval on Figure 2 indicates the preferred sampling area in the dorsal muscle.
- Insert the biopsy punch through the skin at the descaled area with a slight twisting motion to the prescribed depth of the punch, penetrating the skin and muscle tissue.

- Using a slight tilting motion to sever the tissue at the end of the punch while leaving the sample intact, remove the punch.
- Apply air pressure on the opposite end of the biopsy punch from the tissue sample (e.g., using a laboratory pipette bulb) to discharge the tissue sample directly into a pre-cleaned sample container or, if it must be further processed (e.g., compositing), onto a cleaned surface. If necessary, use clean forceps to remove the tissue from the punch.
- Apply sterile cyanoacrylate adhesive (e.g., vet bond tissue adhesive) to seal the wound.
- If anesthesia was used, allow the fish to recover in a safe location before returning the fish to the waterbody from which it was collected.
- Do not attempt to reuse a biopsy punch that has contacted a specimen or any non-clean surface.

If collection of muscle plugs is planned, practitioners may also refer to Baker *et al*. (2004). In addition, practitioners should contact the analytical laboratory in advance for any specific considerations related to muscle plug samples. Refer to Stahl *et al*. (2021) for further information.

Figure 2: Recommend part of the fish to target for collection of muscle plugs.

4.2.3 Eggs

If eggs are to be collected for the determination of selenium concentrations, some unique quality assurance and quality control (QA/QC) procedures need to be implemented. Prior to collecting eggs:

- All material used for egg collection and storage should be carefully cleaned and dried.
- The area surrounding the urogenital opening of females selected should be dried (e.g., with paper towels) to ensure that eggs do not come in contact with water.
- Precautions should be taken before and during the process to avoid fecal, blood or urine contamination of the egg samples.

To express the eggs, gentle pressure should be applied from behind the pectoral fins towards the anus. Check that eggs are released “clean” (e.g., without feces) before starting collection, to avoid contamination of the entire egg batch. Eggs from each fish should be individually collected into appropriate containers. Samples should be covered to prevent sun exposure and should be kept cool. Collected eggs should be inspected and any eggs with adhered feces, urine or blood should be discarded (Janz and Muscatello, 2008). Eggs should then be weighed prior to laboratory submission (i.e., fresh/wet weight should be recorded).

4.2.4 Ovaries

To remove ovaries, the body cavity is first opened ventrally. Typically, an incision is made starting from the anal pore and cut anteriorly towards the head. If the sex of fish has not been confirmed, the sex should first be determined by macroscopic inspection of the gonads. For females, the ovaries should then be examined to confirm that the specimens are in late-stage development or ripe. Indicators that the female is in late-stage development or ripe are:

- Ovaries are yellow orange in colour.
- Increased blood flow causes the ovaries to become highly vascularized and appear reddish.
- Individual oocytes are readily apparent macroscopically during late-stage development but are not released after applying slight pressure to the abdomen until the fish is ripe.

If the specimen is not ripe or in late-stage development, ovary tissue should not be used for selenium analysis. The whole fish, ovaries included, may alternatively be used for whole-body analysis.

If the specimen is ripe, then the ovaries should be removed by severing the oviducts and mesenteric attachments, dissecting in a caudal to cranial direction (Wolf *et al.*, 2004). Once collected, ovaries should be weighed prior to laboratory submission (Section 4.4).

4.3 Composite Samples

As noted in Section 3.3, composite samples (incorporating tissue from two or more fish) may be used for selenium analyses. Compositing samples is typically only recommended if individual fish tissue samples are too small (e.g., <1 g) to provide enough tissue to achieve laboratory MDLs.

If composite samples are to be used, then tissue samples should be collected from each area (i.e., exposure and reference) and combined to yield at least five composite samples to meet the intended sample size (see Section 3.3). Each composite sample should be comprised of tissue from two or more individual fish (the number of fish per composite sample will be determined by the individual samples sizes and the analytical sample weight requirements). Composite samples should:

- Consist of the same species and be comprised of a similar number of individual fish.
- Be comprised of fish of similar length, weight, sex (if possible), and age or age class (if possible), so that the composite samples for a particular species are comparable between sampling areas. The smallest individual in a composite should be no less than 75% of the total length (size) of the largest individual in the same composite (i.e., the “75% rule”, US EPA, 2000). Supporting information per Table 1 (Section 4.1) should be documented for each individual fish in the composite.
- Be collected as close to the same time as possible, and no more than one week apart.
- Be of sufficient initial weight at the time of submission to the analytical laboratory to yield a composite homogenate sample that supports all necessary analyses as outlined in Section 5.1 and 5.3.

The general approach regarding minimum sample weight determination should be reasonable, cost-effective and provide maximum reliability in results. It is strongly recommended that sample weight requirements are confirmed with the laboratory in advance of sampling. Sample requirements as low as 0.2 g wet weight have become common (Ashby *et al.*, 2023).

As per Section 3.3, a minimum of five samples (i.e., individual and/or composite) is recommended for each species in each sampling area. Greater precision in the estimated standard error is gained by increasing the number of composite samples per sampling area rather than by increasing the number of fish in each composite. The appropriate number of individuals per composite should be determined on a case-by-case basis and depends on the size of the fish caught, not only in one area but between the reference and exposure areas, to ensure that sample weight requirements are met (Section 5.3.3).

4.4 Sample Storage, Preservation and Transportation

Each sample (muscle, muscle plug, whole body, carcass, egg, or ovary) should be individually packaged (e.g., placed in plastic bags, vials, bottles, or wrapped in aluminum foil) and labelled

to ensure sample integrity and to prevent contamination. Sample containers must be airtight to avoid the loss of moisture prior to sample processing and should be waterproof. Samples should be packaged as soon as possible after collection. Composite samples may be packaged separately with compositing instructions provided to the laboratory on the Chain of Custody (CoC) form. Alternatively, composite samples may be packaged together to prevent them becoming separated. All sample containers (both lid and vial/bottle) should be properly labelled, using markers with indelible ink.

Samples should be kept cool or frozen as soon as possible after packaging (kept at 4 °C or below). To maintain sample integrity during storage and transportation at or below 4 °C, ice, freezer packs, or refrigeration may be used. If the time from sample collection and packaging to arrival at the lab will exceed 24 hours, then samples should be frozen and maintained at -20 °C or below. To maintain frozen samples, dry ice or a freezer may be used.

Sample condition, including temperature, should be documented by the laboratory upon receipt of the samples and reported with the analytical report (CoC forms may be included as an appendix to the report). This information can be used to validate sample condition during transport, when samples are not delivered directly (i.e., by hand) to the laboratory.

Samples should be transported to the laboratory and processed/analyzed as soon as possible after collection. The longer a sample is frozen, the greater the potential that the tissue may degrade (i.e., suffer freezer burn or moisture loss). Maximum recommended storage time for frozen samples is 6 months at -20 °C (Janz and Muscatello, 2008). If moisture loss occurs before samples are dried, this could potentially affect the percentage of moisture measurement and selenium concentrations reported as dry weight.

5 Laboratory Analyses

As described in Section 1.2, Schedule 5, subclause 12(1)(e)(iv) of the MDMER states that the EEM interpretive report must contain total selenium (dry weight) reported in µg/g and the moisture content of the sample reported as a percentage. Thus, for each replicate submitted to the laboratory, there are two parameters that must be measured:

- Total selenium (µg/g dry weight)
- Moisture content (%)

5.1 Determination of Moisture Content

To prepare samples for determination of moisture content and chemical analysis, freeze-drying is recommended. This technique allows the same sample to be used for both the moisture content and the subsequent selenium analyses. This is particularly important when dealing with small sample weights, such as small-bodied fish species, ovaries, eggs, or muscle plugs. To facilitate sample handling and to ensure that no sample material is lost, samples may be placed in small plastic bags, weighed in the bag, then freeze-dried and weighed again to determine the moisture content. The bag containing the sample can then be sent for selenium analysis.

If freeze-drying is not used, the sample weight needed should be clarified with the laboratory before sample collection, as larger samples are typically needed (e.g., 2.5 to 5 g). In this case, separate sub-samples of each replicate should be used for moisture content and selenium analyses. If this approach is used, it is important to ensure that sub-samples are representative of the entire replicate. It is recommended that each replicate be homogenized and then sub-sampled to provide separate portions for moisture content and selenium analyses. This approach is only recommended when sample weights are adequate to provide suitable sub-samples for both analyses.

In some cases (i.e., small sample size and freeze-drying is unavailable), it may not be possible to determine both selenium concentration and moisture content of replicates, as per the MDMER requirements. In such cases, the moisture content may be estimated based on previous moisture content results for the same species, location, and time of year, or by using values for the same species in published literature, such as Appendix B of US EPA (2021b). If estimates of moisture content are used, this should be clearly stated when data are submitted to ECCC, together with the assumptions used to develop those estimates.

The moisture content can have a considerable influence on the selenium concentration in tissues expressed in dry weight (dw). For example, a wet weight selenium concentration in tissue of 0.2 µg/g would be equivalent to 0.5 and 0.8 µg/g dw based on a percentage of moisture content of 60% and 75%, respectively. The equation used to convert from wet weight to dry weight measurements is:

Dry weight concentration = Wet weight concentration x (100 / (100 - % moisture)).

5.2 Sample Preparation for Selenium Analysis

Samples for selenium analysis must be in an aqueous solution for most analytical methods. Thus, prior to selenium analysis, tissue samples must be homogenized and digested into an aqueous solution. Samples may be homogenized using a blender (wet samples) or a mortar and pestle (freeze-dried or dried samples). If oven drying, do not dry samples for selenium analysis above 60 °C as selenium can volatilize (Ohlendorf *et al*., 2011, Janz and Muscatello, 2008). Recognized methods of digestion procedures, such as those published by the US EPA[5] or in Standard Methods 2020 (#3030), should be used to ensure sample integrity and full digestion of tissue and appropriate sample preparation blanks and reference material should be analyzed as part of QA/QC procedures. Strong acid digestion should then be completed using a closed-vessel microwave digestion system (ideal to prevent sample loss and potential contamination) or an open-vessel digestion procedure that is compatible with, or recommended for, the analytical method being used. Acids used for digestion should include a strong oxidizer to ensure complete conversion of any volatile selenide species to the less volatile, water-soluble selenate form. To ensure complete oxidation of the tissue, a final digestion using hydrogen peroxide (typically 30%) is typically used to fully extract selenium from the tissue sample. The instruments and reagents used for homogenization and digestion should be clean and of high purity, so as not to contaminate the sample.

5.3 Analytical Methods and Tissue Requirements

5.3.1 Analytical Methods

Ohlendorf *et al*. (2011) reported that the “choice of analytical methods, with careful consideration of matrix interferences and digestion and extraction problems, is important for a well-designed selenium bioaccumulation sampling program.”

It is important that sample preparation and analytical methods for samples for exposure and reference areas be the same to ensure that results are comparable. If methods change between EEM phases, those changes should be documented in the EEM interpretive report and considered in the subsequent study design.

To achieve the required analytical MDLs, and to circumvent issues commonly encountered with analytical instruments that use optical systems, the analysis of the final digest solution for total selenium is typically conducted by inductively coupled plasma-mass-spectrometry (ICP-MS). For situations in which the sample weight is very small (<0.2 g wet weight), analysis using an instrument providing an even greater degree of sensitivity may be appropriate, or necessary. Typically, this would be achieved through the use of a Triple Quadrupole ICP-MS (TQ-ICPMS or

[5] See US EPA (2021b), Appendix L

ICPMS-QQQ), High Resolution ICP-MS (HR ICP-MS), or another instrument capable of dealing with matrix interference (e.g., collision reaction cell technologies).

5.3.2 Detection Limits

As per MDMER requirements, selenium concentrations in fish tissue are to be reported on a dry-weight basis along with the moisture content of the sample. Moisture content in tissues may vary among both species and tissues and could be influenced by variability associated with sample collection and handling. Therefore, reporting on a dry-weight basis is important to normalize the data to allow for comparison.

There is no required reportable detection limit (RDL) for selenium in fish tissue stated in the MDMER. It is best practice for the RDL to be a factor of five times less than the reported concentrations to ensure the concentrations are reliably quantifiable (BC MOE, 2020). Additionally, the MDLs will scale up or down according to the sample weight (see below). For example, the MDL will typically increase as the weight of the sample decreases. To the extent practical, separate MDL studies should be conducted at different weights to assess the MDL. Consult the laboratory to obtain the MDLs for selenium for the target sample weights anticipated in the study.

As an example, for illustration purposes only, Table 2 below shows a sample weight provided and the potential MDL, which may vary, based on the amount of sample available. In this example, 0.2 g is the minimum sample weight required by the lab. With less than 0.2 g wet weight provided, the MDLs are affected and increase. Consult with the laboratory of choice for analysis to confirm sample weight requirements and MDLs. Additional considerations regarding sample weight are outlined in Ashby *et al.* (2023).

Table 2: Example of method detection limits for selenium in fish tissue for different sample weight (dry values based on 75% moisture).

Wet Sample Weight (g)	Dry Sample Weight (g)	Se MDL (µg/g wet weight)	Se MDL (µg/g dry weight)
20	4	0.01	0.04
1.0	0.2	0.01	0.04
0.2	0.04	0.01	0.04
0.1	0.02	0.02	0.08
0.05	0.01	0.04	0.16

The laboratory sample method is validated with a specific target weight (0.02 g dry weight in the example above). If a higher sample weight is provided, it will be sub-sampled to provide the required validated weight. More sample weight does not equate to lower MDLs. Sample weights

less than the validated weight (for example, 0.04 g dry weight) will result in a proportional increase in the MDL.

5.3.3 Tissue Requirements

The weight of tissue that is typically required for ICP-MS analysis is 0.2 to 2.5 g (wet weight) to achieve the lowest RDLs possible and maintain accuracy and precision, but practitioners should confirm with the laboratory prior to collecting samples. If samples collected in the field are larger, then sub-sampling is typically done in the laboratory. Sub-sampling this tissue should be completed in a consistent manner, such as sampling the same portion of muscle or homogenizing the tissue and then sub-sampling.

If moisture is being determined using an oven, larger sample weights would likely be required by the laboratory as separate tissue samples would be needed for analysis of selenium and percent moisture. Consult with the laboratory to determine the weight required.

Field studies should aim to collect samples that are large enough to meet laboratory weight requirements for analysis. When considering the sample weight required, a review of past fish tissue data for the location is useful, if available, to ensure that it will be possible to detect selenium with the sample weight collected. If, during the collection of fish in the field, it is determined that samples will be too small (e.g., individual fish are too small for whole-body analysis) then consideration should be given to collecting composite samples (Section 4.3).

However, if samples submitted to the laboratory are determined to be near or below the minimum recommended weight, it may be necessary to ensure that as much sample as possible is available for analysis by dropping one or more QC samples from the study (e.g., matrix spikes, duplicates), which could result in an increase in the MDL. It may also impede the laboratory's ability to repeat the sample analysis from the original material to confirm reported values. In such cases, it is recommended that laboratories be asked to conduct additional QC analysis using certified reference materials and/or laboratory control samples that are at weights comparable to those of the samples submitted.

5.3.4 Accreditation, Reporting Standards and Laboratory QA/QC

The laboratory selected should be accredited for the analyses being conducted. In Canada, accreditation may be either of the following forms:

1. Accreditation issued in accordance with the International Organization for Standardization standard ISO/IEC 17025, entitled "General Requirements for the Competence of Testing and Calibration Laboratories", by an accredited body that is a signatory to the International Laboratory Accreditation Cooperation (ILAC) Mutual Recognition Arrangement, such as through the Canadian Association of Laboratory Accreditation; or
2. Under province of Quebec's *Environment Quality Act*, CQLR, c. Q-2

The Laboratory's Scope of Accreditation provides an outline of the analytical method used, and applicable sample matrix (i.e., tissue/biota). The laboratory's accreditation status and selenium analysis method should be reviewed and confirmed during preparation of the study design.

As part of the analysis and accreditation, QA/QC samples may include matrix spike, duplicate, method blank, method spike, and certified reference material. If large-bodied fish muscle (fillet) samples are collected, additional field duplicates may be included (i.e., fillets collected from the dorsal musculature of each side of the fish submitted as duplicate samples); these QA/QC samples are unrelated to laboratory accreditation.

5.3.5 Alternative Analytical Methods

Analytical methods continually evolve, and new techniques will likely become available to determine the concentration of selenium in tissues. If the measurement of selenium beyond the standard MDMER requirements is required/desired, such as in an EEM Investigation of Cause study, or to assess the ecological risk due to selenium, then alternative or complementary methods may be used in addition to those outlined above. Refer to the North American Metals Council website (NAMC, 2022), the US EPA (2021b), Ohlendorf *et al.* (2011) or Chapman *et al.* (2010) for more information.

The use of laser ablation with ICP-MS (LA ICP-MS) analysis was compared with traditional ICP-MS protocols by Ashby *et al.* (2023). The results indicated LA ICP-MS protocol supported lower sample weights (e.g., muscle plugs, eggs) than other protocols as the sample is completed on a pelletized sample rather than requiring a digestion procedure (Ashby *et al.,* 2023). Furthermore, traditional protocols tended to overestimate concentrations of selenium in small weight samples, and this was not observed in the LA ICP-MS protocol (Ashby *et al.,* 2023)

6 Data Analyses and Reporting

As stated in Section 1.2, the EEM interpretive report submitted to ECCC must include, for each replicate collected from exposure and reference areas, the fish species and tissue type, the total selenium concentration (µg/g dry weight) and the moisture content (%). The interpretive report must also include the mean, median, standard deviation, standard error and minimum and maximum values for the exposure and reference areas. Since the MDMER do not define an "effect" associated with selenium in fish tissue, there are no requirements to conduct a statistical analysis comparing results from exposure and reference areas or to compare results with guidelines/criteria for concentrations of selenium in fish tissue (e.g., Beatty & Russo, 2014; DeForest *et al.*, 2012; ECCC/HC, 2017; ECCC, 2022; US EPA, 2021a).

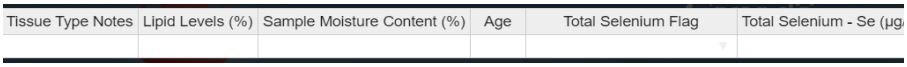
In addition to submitting the interpretive report, raw data must be submitted to ECCC using the EEM electronic reporting system (EEMER).

An Excel-based template is available from ECCC to facilitate uploading of data. Reporting fields included in the template are listed below, with mandatory fields presented in italics. Further information on these reporting fields is provided in the Environmental Effects Monitoring Electronic Reporting (EEMER) System User Guide (ECCC, 2023), available upon request from ECCC.

- *Station ID*
- Specimen ID
- *Collection date*
- *Collection method*
- *Analysis date*
- *Taxon*
- Taxon notes
- *Sex* (options: Female - adult, Male – adult, Female – immature, Male – immature, Indeterminable)
- Fish in composite [based on ECCC guidance for mercury in fish tissue fields, provide the total number of individual subsamples used in each composite sample]
- *Tissue type* (options: whole body, muscle, egg, ovary, liver, hepatopancreas, liver/bile composite, bile, N/A, other)
- Tissue type notes
- Lipid levels (%)
- Sample Moisture (%)
- Age (years)
- Total selenium flag (if measurement is below the reportable detection limit)
- *Total Selenium - Se (µg/g dw)*
- Comments

Below is an example of some of the key fields within EEMER. [Note: the screenshot below is too wide to be displayed in a single line so it is split over two lines.]

	Station Id	Specimen Id	Collection Date (YYYY-MM-DD)	Collection Method	Analysis Date (YYYY-MM-DD)	Taxon	Taxon Notes	Sex	Fish in Composite	Tissue Type
1										



Tissue Type Notes	Lipid Levels (%)	Sample Moisture Content (%)	Age	Total Selenium Flag	Total Selenium - Se (µg

Additionally, when reporting the results of selenium in tissue within the biological data report in EEMER, owners/operators must identify the trigger that was exceeded to require the selenium in tissue study and the date of the trigger with the concentration of reported. The three options include:

- Annual mean concentration of total selenium in the effluent ≥5 µg/L;
- Concentration of total selenium in the effluent ≥10 µg/L; or,
- The MDL of selenium for the analysis of any effluent sample is equal to or greater than 10 µg/L, or the MDL used in respect of selenium for the analysis of at least two of four effluent samples in a calendar year is equal to or greater than 5 µg/L.

7 Summary

The 2018 MDMER introduced new requirements for owners/operators of mines subject to the Regulations to conduct studies of selenium in fish tissue if conditions described in Schedule 5, paragraph 9 (1)(d) are met. These studies are to be conducted in reference and exposure areas using muscle or whole-body fish tissue samples, and ovaries or eggs if practicable. The Regulations do not define an effect associated with concentrations of selenium in fish tissue and do not require a comparison of results between exposure and reference areas.

The EEM requirements of the Regulations also require fish population studies if conditions described in the Regulations are met. To minimize additional mortality to local fish populations (i.e., beyond that already associated with sampling to conduct EEM studies), it is recommended that, to the extent practicable, sampling of fish tissue for selenium analysis should be aligned with the conduct of the fish population studies. This alignment may include the timing of sample collection and the species selected, resulting in a sharing of individual fish collected between the population and the selenium studies.

In cases where owners/operators are required to conduct studies of selenium in fish tissue, it is recommended that they apply the guidance provided in this document to:

- Develop a monitoring strategy (Section 2).
- Prepare a study design (Section 3).
- Conduct field studies (Section 4), including the collection and preparation of tissue samples or composite samples, and the collection of supporting information.
- Conduct laboratory analyses (Section 5).
- Analyze data and report to ECCC (Section 6).

This guidance document should be used in conjunction with the 2012 *Metal Mining Technical Guidance for Environmental Effects Monitoring* (or future updates, if provided) available on the ECCC website. In addition, practitioners may also consult other relevant guidance, notably from the US EPA (e.g., US EPA 2021b), for some specific technical aspects such as tissue sample collection and preparation. However, given the specific MDMER requirements, guidance from the US EPA is not considered appropriate for use as a primary reference for the design of studies of selenium in fish tissue to meet MDMER requirements given the aspects of the US EPA guidance that do not align with the MDMER (e.g., MDMER emphasis on muscle or whole body tissue samples).

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