A Review of Environmental Management Criteria for Selenium and Molybdenum

MEND Report 10.1.1

This work was done on behalf of MEND and sponsored by:
The Mining Association of Canada (MAC) and MEND

February 2008

A REVIEW OF ENVIRONMENTAL MANAGEMENT CRITERIA FOR SELENIUM AND MOLYBDENUM

Report prepared for:

THE MEND INITIATIVE
MEND Secretariat
555 Booth Street
Ottawa, Ontario
K1A 0G1

Report prepared by:

ECOMETRIX INCORPORATED
14 Abacus Road
Brampton, Ontario
L6T 5B7

Ref. 04-1159.1
November 2007

ACKNOWLEDGEMENTS

This review was initiated in response to a preliminary report on metal leaching at neutral pH by the senior reviewer of this report, and was published as MEND Report 10.1 – Review of Water Quality in Neutral pH Drainage: Examples of Emerging Priorities for the Mining Industry in Canada. MEND is gratefully acknowledged for financially supporting this and the previous initiatives.

Several individuals provided thoughtful comments on the draft version of this document and are gratefully acknowledged. These include:

- Dr. Peter Chapman, Golder Associates;
- Ms. Peri Mehling, MESH Environmental;
- Ms. Sue Moodie, CCSG Associates;
- Dr. Keith Finley, Duke Energy;
- Ms. Brenda Dixon, Natural Resources Canada, and
- the MEND Secretariat

This document benefited from the comments and suggestions from all reviewers. Although these comments and suggestions were appropriate, it was not always feasible to directly address them in the text of this report for various reasons. For example, in some instances the information was not readily available for inclusion here.

All errors and deficiencies remain the responsibility of the senior reviewer.
The main technical authors of this report include:

- Mr. Neil Morris – with major contributions on selenium; and
- Dr. Dean Fitzgerald – with major contributions on molybdenum, update on the selenium literature, and addition of information to the case histories, and overall report compilation.

The senior reviewer was Dr. R.V. (Ron) Nicholson who initiated the review partially in response to his “exposure” to molybdenum and selenium as major players in Ecological Risk Assessments at mining operations in Canada.

R.V. (Ron) Nicholson
ECOMETRIX INCORPORATED
rnicholson@ecometrix.ca
26 November 2007

EXECUTIVE SUMMARY

Selenium and molybdenum are naturally occurring trace elements that are often released to the environment at relatively low concentrations as a result of mining industry discharges. At present, Canada's Metal Mining Effluent Regulations (i.e., MMER) do not identify limits for either of these elements. In order to independently establish discharge limits for selenium and molybdenum, it is possible to use water quality guidelines established for the protection of aquatic life e.g., 0.001 mg/L for selenium and 0.073 mg/L for molybdenum. This simple approach, however, is problematic in that recently established regulatory limits to protect water quality are very low, reflecting extreme scenarios that are very unlikely to be encountered in the receiving environments at most mine sites in Canada. The regulatory limits for selenium and molybdenum follow a risk-based approach that involves numerous assumptions that are multiplicative in their conservatism. While these limits will ensure environmental protection, they are very likely overly restrictive for most sites. A more site-specific approach, based on ecological risk assessment, to resolve effluent limits is advisable in most cases.

The establishment of risk-based limits (generic or site-specific) for selenium and molybdenum is problematic due to a series of unique aspects of these two elements, including:

- the role of both elements as essential nutrients to plants and animals, and the unique processes that govern their physiological regulation;
- the relatively narrow range between exposure levels that are sufficient and required, from a nutritional perspective, and those that are potentially toxic;
- the variability in partitioning, bio-uptake, and toxicity of selenium and molybdenum, depending on the specific form of these elements;
- the possibility for toxic effects of *aquatic* releases of selenium and molybdenum on *terrestrial* wildlife, owing to direct and indirect foodchain exposure pathways; and
- the overall complexity of interactions in the receiving environment that determine the chemical form of selenium or molybdenum, and the partitioning to various environmental compartments, including aquatic and terrestrial foodchains.

Selenium is one of the few elements with an established case history of significant impacts to both aquatic and terrestrial biota owing to initial loading of this element to the water column. For this reason, it has been the subject to more restrictive regulation and focused research to understand the ecological risk implications in Canada and elsewhere. It is important to understand that the documented cases of selenium-related impacts (e.g. agricultural drainage reservoirs in seleniferous regions of the western USA, and coal-affected reservoirs in various locations) are quite unique. While much has been learned from these cases, an assumption that all receiving environments will behave the same is not justified or warranted. This is evidenced by the many cases, including mine sites in

Canada, that display exceedances of conservative regulatory thresholds for selenium exposure accompanied by an absence of measurable effects.

The regulatory limits for selenium have arisen from findings at problematic sites. This understanding of environmental behaviour and toxicity of selenium represents site specificity in terms of the media and organisms involved. Thus, the application of these cases for environmental protection elsewhere needs to be cautious and consider key modifying factors. Site data are presented to demonstrate different approaches for site assessment and role of modifying factors shaping the risk from selenium.

There is not a body of evidence for molybdenum to suggest that similar significant impacts have ever occurred that are comparable to those attributed to selenium. The concerns regarding potential foodchain impacts of molybdenum released to aquatic systems are largely theoretical, and are based on the potential toxic effects to a particularly sensitive group of animals (i.e., ruminant). Most research and site data indicate an extremely low likelihood that molybdenum releases to water would result in any detrimental effect(s) on sensitive species of terrestrial wildlife associated with the aquatic environment. Some of these site data are presented to clarify this view.

Overall, generic extrapolation of the conservative concerns and toxicological characterizations of selenium and molybdenum are not an effective means to determine whether or not measurable effects might occur for a given aquatic release of these elements. Application of site-specific risk assessment (SSRA) is the best means to determine whether or not a given release of either selenium or molybdenum is likely to have impacts. This represents an emerging consensus among many researchers and informed stake-holders.

The SSRA approach can be applied to provide a degree of confidence that adverse effects will be avoided without undue conservatism or excessive preventative management efforts. For selenium, the SSRA needs to consider virtually all environmental transport processes and pathways that may ultimately be linked to the environment and food web. Generic default values of various transport parameters are identified herein, but site-specific values are strongly recommended. Critical elements of the SSRA process for selenium include:

- Characterization of the receiving environment, as the uptake of selenium into the food web is highly dependent on habitat features. Assessment of sediments is critical, as this environmental compartment can represent the key link between selenium in the physical environment and the foodchain.
- Careful selection of bioaccumulation factors (BAFs) that are appropriate for the scenario under consideration. Site-specific values should be used when feasible.
- Use of tissue-based toxicity thresholds requires a confident understanding of uptake, and must consider the receptor's of relevance at a site and their diet.

For molybdenum, the SSRA process is not likely to require the same level of detail and pathway delineation as required for selenium. This is primarily because molybdenum exhibits a much lower propensity to bioaccumulate than selenium, and exposure via the food web is typically low relative to toxic thresholds. The primary concern is the exposure of ruminants via consumption of plants with high molybdenum content.

For the environmental management of either element, the SSRA needs to effectively consider all exposure pathways of relevance. The SSRA should not be a stand-alone tool, especially for selenium. The SSRA should be part of an overall management plan that includes appropriate site characterization, release planning, and targeted and effective environmental monitoring programs. These latter points are illustrated in case studies of mine sites in Canada.

RÉSUMÉ

Le sélénium et le molybdène sont des éléments traces d'origine naturelle qui sont souvent libérés dans l'environnement dans des concentrations relativement peu élevées par l'industrie minière. À l'heure actuelle, le *Règlement sur les effluents des mines de métaux* (REMM) du Canada n'identifie pas de limites pour l'un ou l'autre de ces éléments. Pour établir de façon indépendante des objectifs de rejet pour le sélénium et le molybdénium, il est possible d'utiliser des critères de qualité de l'eau pour la protection de la vie aquatique, par exemple 0.001 mg/L pour le sélénium et 0.073 mg/L pour le molybénium. Cette approche simple est problématique parce que les limites réglementaires récemment établies pour protéger la qualité de l'eau sont très peu élevées, ce qui reflète des scénarios extrêmes qui ne se produiront probablement pas dans les milieux récepteurs à la plupart des sites miniers au Canada. Les limites réglementaires pour le sélénium et le molybdène s'inspirent d'une approche basée sur les critères génériques qui comporte de nombreuses hypothèses multiplicatives de par leur conservatisme. Ces limites assureront la protection de l'environnement, mais elles sont très probablement exagérément restrictives pour la plupart des sites. Une approche particulière au site, basée sur l'évaluation des risques écologiques est souhaitable dans la majorité des cas pour résoudre la question des limites des effluents.

L'établissement de limites à partir des critères de qualité de l'eau génériques est problématique dans le cas du sélénium et du molybdène, à cause d'aspects uniques à ces deux éléments, notamment :

- les deux éléments sont des nutriments essentiels pour les plantes et les animaux, et des processus uniques régissent leur régulation physiologique;
- la marge relativement mince entre les niveaux d'exposition qui sont suffisants et requis, du point de vue de la nutrition, et les niveaux qui peuvent éventuellement être toxiques;

- la variabilité dans la répartition, la bio-absorption et la toxicité du sélénium et du molybdène, selon la forme de ces éléments;
- la possibilité que les rejets de sélénium et de molybdène *dans l'eau* aient des effets toxiques sur la faune *terrestre*, en raison des voies d'exposition directes et indirectes dans la chaîne alimentaire; et
- la complexité générale des interactions dans le milieu récepteur qui déterminent la forme chimique du sélénium ou du molybdène et la répartition entre les divers milieux environnementaux, notamment les chaînes alimentaires aquatiques et terrestres.

Le sélénium est l'un des rares éléments pour lesquels des impacts importants à la fois sur les biotes aquatiques et terrestres ont été démontrés via la colonne d'eau. C'est pourquoi le sélénium a fait l'objet d'une réglementation restrictive et de recherches sur les risques écologiques au Canada et ailleurs. Il est important de comprendre que les exemples documentés d'impacts attribuables au sélénium (p. ex. réservoirs de drainage agricole dans les régions sélénifères de l'ouest des États-Unis et réservoirs touchés par les effets du charbon) sont plutôt uniques. Bien que ces exemples aient permis d'augmenter de beaucoup les connaissances, toute hypothèse selon laquelle tous les milieux récepteurs auront la même réaction n'est pas justifiée ou fondée, comme le montrent les nombreux cas, dont des sites miniers au Canada, où sont enregistrés des dépassements de seuils réglementaires d'exposition au sélénium alors que les seuils sont conservateurs et qu'il n'y a aucun effet mesurable.

On doit l'établissement de limites réglementaires pour le sélénium aux constatations faites aux sites problématiques. Cette compréhension du comportement environnemental et de l'écotoxicité du sélénium représente la régio-spécificité en termes des milieux et des organismes en cause. Par conséquent, en appliquant ces cas à la protection de l'environnement ailleurs, il faut faire preuve de prudence et tenir compte des facteurs modificatifs clés. Les données de ces sites sont présentées pour démontrer diverses approches d'évaluation spécifique au site et le rôle des facteurs modificatifs déterminant le risque issu du sélénium.

Il n'existe aucune preuve que le molybdène a eu des impacts importants comparables à ceux qui sont attribués au sélénium. La crainte que les rejets de molybdène dans les réseaux aquatiques aient des impacts sur la chaîne alimentaire est en grande partie théorique et découle des éventuels effets toxiques du molybdène sur un groupe d'animaux particulièrement sensibles (c.-à-d. les ruminants). La plupart des recherches et des données sur les sites problématiques indiquent une probabilité extrêmement faible que le molybdène contenu dans les effluents déversés dans les plans d'eau aient des répercussions nuisibles sur les espèces sensibles de la faune terrestre ayant besoin du milieu aquatique. Certaines des données spécifiques aux sites problématiques sont présentées pour clarifier ce point de vue.

En fin de compte, une extrapolation générale des préoccupations conservatrices et la caractérisation toxicologique du sélénium et du molybdène ne sont pas efficaces pour déterminer la présence d'effets mesurables dans le cas d'un rejet de ces éléments dans un plan d'eau. L'évaluation des risques particuliers au site (ERPS) constitue le meilleur moyen de déterminer si un rejet de sélénium ou de molybdène aura vraisemblablement des impacts. Cette façon de procéder représente un consensus émergent parmi de nombreux intervenants avertis et chercheurs.

L'ERPS peut permettre d'assurer jusqu'à un certain point que les effets nuisibles seront évités sans qu'il soit nécessaire d'avoir recours à un conservatisme indu ou à des mesures excessives de gestion préventive. Dans le cas du sélénium, l'ERPS nécessite de prendre en compte tous les processus de transport dans l'environnement et toutes les chaînes de pénétration dans l'environnement qui peuvent être en dernier ressort liés à l'environnement et au réseau trophique. Les valeurs générales implicites des divers paramètres du transport sont identifiées dans le présent document, mais il est fortement recommandé d'utiliser les valeurs spécifiques au site. Pour le sélénium, les éléments cruciaux de l'ERPS comprennent :

- la caractérisation du milieu récepteur, parce que l'absorption du sélénium par le réseau trophique dépend beaucoup des caractéristiques de l'habitat – l'évaluation des sédiments est essentielle parce que ce milieu environnemental peut représenter le principal lien entre le sélénium dans le milieu physique et la chaîne alimentaire;
- une sélection attentive des facteurs de bioaccumulation qui conviennent au scénario étudié – il faut autant que possible utiliser les valeurs spécifiques au site;
- l'utilisation de seuils de toxicité basés sur les tissus nécessite une solide connaissance de l'absorption ainsi que la prise en compte des récepteurs du site et de leur régime alimentaire.

Dans le cas du molybdène, l'ERPS ne nécessitera probablement pas le même niveau de détail et de délimitation des chaînes de pénétration dans l'environnement que dans le cas du sélénium. Cela s'explique principalement par le fait que le molybdène a beaucoup moins tendance à bioaccumuler comparativement au sélénium et par le fait que l'exposition au molybdène par le biais du réseau trophique est généralement peu élevée par rapport aux seuils de toxicité. La principale préoccupation consiste en l'exposition des ruminants par le biais de la consommation de plantes renfermant beaucoup de molybdène.

Pour la gestion de l'un ou l'autre élément à des fins de protection de l'environnement, l'ERPS doit tenir compte efficacement de tous les modes d'exposition pertinents. L'ERPS ne doit pas être utilisée seule, tout particulièrement dans le cas du sélénium. L'ERPS doit faire partie d'un plan de gestion global qui inclut une caractérisation appropriée du site, une planification des rejets, et des programmes de suivi des effets sur l'environnement ciblés et efficaces. Ces derniers points sont illustrés dans des études de cas portant sur des sites miniers situés au Canada.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table No.

LIST OF FIGURES

Figure No.

APPENDIX

LIST OF ACRONYMS USED IN THIS DOCUMENT

ANZFA:	Australia and New Zealand Food Authority
ATSDR:	U.S. Agency for Toxic Substances and Disease Registry
BAF:	Bioaccumulation factor
BCF:	Bioconcentration factor
CCME:	Canadian Council of Ministers of Environment
COPC:	constituents of potential concern
dw:	dry weight
EC:	effect concentration
ERA:	ecological risk assessment
ENEV:	estimated no effect value
FAV	Final Acute (toxicity) Value
fw:	fresh weight
FCV	Final Chronic (toxicity) Value
GMCV	Genus Mean Chronic (toxicity) Values
Kd:	water-sediment partitioning coefficient
IAEA:	International Atomic Energy Agency
LD:	lethal dose
LOAEL	lowest observed adverse effect level
MMER:	Canada's Metal Mining Effluent Regulations
MWLAP:	Ministry of Water, Land, and Air Protection (formerly the Ministry of Land and Water Protection (of British Columbia)
NOAEL:	no observed adverse effect level
RAIS:	Risk Assessment Information System used by Oak Ridge National Laboratory
SMAV	Species Mean Acute (toxicity) Value
SSRA:	site-specific risk assessment
STD:	submarine tailings disposal
TF_{ing}:	ingestion transfer factor
TMDL:	total maximum daily load limits
U.S. DOI:	United States Department of Interior
WSS:	Winter Stress Syndrome

1.0 INTRODUCTION

1.1 Environmental Protection Requirements

The major activities at mine sites (mining, milling, waste management) all have the potential to generate environmental releases of constituents of potential concern (COPC) directly to the aquatic environment. To help guide the environmental assessment and ultimate management of such releases, Canada's Metal Mining Effluent Regulations (MMERs) set authorized discharge limits for most of the more frequently encountered COPCs. However, there are a number of elements commonly present at detectable concentrations in mine effluents in Canada, including selenium and molybdenum, not currently addressed by MMERs. For these two elements, criteria and guidelines have been established for the protection of aquatic life (e.g., Canadian Council of Ministers of the Environment; CCME; CCME, 2002 and 2007) and toxicity values for aquatic and terrestrial organisms. Similarly, limits for selenium and molybdenum are also absent in the U.S. regulations for mine effluent (40 CFR Part 44, sub-parts A-K). In absence of nation-wide regulatory limits for these elements, mines across Canada need to identify effluent limits that are appropriate for their operation and the local and regional environments.

Most release limits, including those set in the MMERs, are based on an understanding of environmental concentrations of COPCs that might cause some measurable degree of environmental impact. That is, the limits are risk-based. The risks from COPC are generally considered in terms of the likelihood and magnitude of effect on receptors. The ecological risk assessment (ERA) process compares quantitative levels of exposure against established toxic thresholds for the specified COPC. Accepted best practice usually involves tiered applications of the ERA process, with each successive tier marked by decreasing conservatism and increased site-specificity. A key tenet of this process is that any COPC that does not pose unacceptable risks in the first and most protective tier of ERA need not be considered any further. Those COPCs that *are* identified in the first tier ERA as having some potential to cause environmental effects are then re-assessed with an increased level of realism. The process continues until a reasonable level of both realism and conservatism are achieved. If expected COPC exposure exceeds a level that suggests the local environment may be adversely affected, then management may be required.

In any effort to determine the release limits for selenium, molybdenum, or other COPC at a given site, an understanding of the risks of impact(s) is required.

1.2 Challenges with Selenium and Molybdenum

Environmental management of COPCs in aqueous waste streams typically places some reliance on risk-based limits and/or guidelines that are established to protect aquatic life, as would be expected. Guidelines for both effluent quality and environmental quality in the receiving environment are typically protective of drinking water quality and/or aquatic life (e.g., CCME 2002). Although the aquatic ecosystem is frequently the immediate receiving environment for mine effluents and drainage, there are some scenarios where certain

COPCs in the *aquatic* environment may necessitate further consideration of risks to receptors that are classed as *terrestrial*. In this context, the priority elements identified in a recent review of potential COPCs from neutral mine drainage included molybdenum and selenium (MEND Report 10.1, 2004). This is exemplified by current concerns for selenium toxicity in fish and molybdenum toxicity in mammals, particularly ruminants that digest their food in two steps and include domestic cattle and wild buffalo (MEND 2004).

Regulatory limits that protect both aquatic and terrestrial biota need to reflect the risk-based approach for both receptor groups. At present, the assessments of risks of selenium and molybdenum to terrestrial receptors are founded on benchmarks that are not as widely accepted as those established for the protection of aquatic life. One of the key recommendations made in the 2004 MEND report was to complete a review of terrestrial toxicity related to molybdenum and selenium. Others were to provide guidance for environmental assessments and evaluate potential liabilities for application of toxicity benchmarks or regulatory limits.

The challenge with selenium and molybdenum is to adequately quantify both exposure and toxic thresholds for use in site-specific risk assessment (SSRA), particularly in the progression toward more realistic and local tiers of the assessment. This challenge arises from several attributes of selenium and molybdenum that interact to determine their environmental fate and impact. Among these characteristics are:

- Selenium and molybdenum occur as neutral or negatively charged ions under oxidizing conditions. This presents effluent treatment challenges, requiring processes that are atypical of those applied to most metals that exist in a positively charged state. It also leads to unique patterns of environmental behaviour of these elements that may shift significantly depending on redox conditions.
- Partly due to redox sensitivity, and the characteristics of the local receiving environment, selenium and molybdenum may exist in one of several forms in the environment, and the form may differ from one environmental medium to another.
- The environmental partitioning and toxic potential of selenium and molybdenum depend in part on their chemical form, and also on the class of organism in question, with some instances where toxic effects might be expected at naturally occurring levels of either element.
- Both selenium and molybdenum are essential elements in animal nutrition, and thus subject to unique biochemical and physiological regulatory processes in the body. Molybdenum is also an essential element for plants (while selenium is not).
- The margin between the dietary levels of selenium and molybdenum considered necessary for nutritional sufficiency and that are potentially toxic is relatively low.
- Selenium is readily metabolically transformed and transported into reproductive tissues and developing offspring in animals, and as a result, there is a potential for effects on reproductive success, that may have population-level implications.

In combination, these and other biological and physical factors can combine to yield markedly different degrees of exposure and very different expectations of effect, depending on the specific receiving environment in question.

As noted, under certain conditions the environmental dynamics of selenium and molybdenum in aquatic ecosystems may be such that their presence in water at a given concentration may pose a greater risk to *terrestrial* organisms, residing in or near the water, than to resident *aquatic* biota. For selenium, there are well-studied occurrences of impacts to terrestrial animals resulting from food web association with contaminated waters. These impacts arise through complex processes in multiple media, and involve multiple species (plants, fish, invertebrates). Thus, possible effects could influence multiple organisms (fish, birds, reptiles; e.g., Barceloux, 1999a). For molybdenum, the concerns of possible effects of aquatic discharges on sensitive species of terrestrial animals do not have comparable case histories. Indeed, the history and the toxicology of molybdenum are the basis for a more narrow concern than for selenium, with the exposure of sensitive herbivores (esp. ungulates with rumen) to molybdenum being the key concern (e.g., Eisler, 1989).

1.3 Current Objectives

This report contains a review and summary of the general characteristics of selenium and molybdenum, including typical source characteristics, environmental fate and transport, and potential health effects.

Information generated from the review of literature for selenium and molybdenum is then integrated with mine-related release scenarios for locations with differing geology that are located across Canada. These scenarios then act to frame the need to complete assessments of selenium and molybdenum releases within a SSRA framework to provide a broad understanding of the potential impacts to the local and far-field environment associated with a mine. For example, environments with naturally high concentrations of selenium or molybdenum will require different considerations for risk assessment compared with others where these elements exist at naturally low concentrations. This process is done by considering available information with a focus on sensitive aquatic or terrestrial biota as potentially limiting receptors in the ERA process. Key to this effort is defining the range of toxic concentrations and effects of selenium and molybdenum to such receptors. Also important is the development of a thorough understanding of how the toxicity data should be considered in the site-specific application of the ERA process.

The overall objective is to provide recommendations for a site-specific process of identifying and managing environmental risks associated with releases of selenium and molybdenum to the aquatic environment. The depth and scope of discussion is greater for selenium than for molybdenum, commensurate with the nature of the current ecological concerns.

In recent years, both elements of interest, particularly selenium, have been the subject of much research and discussion. Many comprehensive reviews and compilations have been published by public and private agencies. These reviews frequently consider broad

geographical themes and are not necessarily relevant to activities at mine sites. It is not the intent of this review to add to this volume of information. It is also not the intent to resolve current scientific debates and existing uncertainties as to toxic mechanisms or defensibility of water quality guidelines. Rather this review is intended to draw upon the broader understanding, limited or not, of some of the key factors that determine the likelihood of occurrence of ecological impacts of selenium and molybdenum stemming from activities at mine sites (esp. effluent, milling, mining). That understanding is used as the basis for recommendations regarding the management criteria, processes for limiting releases, and the assessment of risk to environments.

2.0 GENERAL STATUS

Both selenium and molybdenum are naturally-occurring semi-metallic elements that are widely distributed in the earth's crust. Both elements are essential trace elements for most animals, plants, and microorganisms, such as bacteria. As expected with essential trace elements, high concentrations can have negative or toxic consequences for sensitive species. For this reason, guidelines for environmental concentrations have been developed and applied across different environments (Eisler, 1985, 1989; Nordberg *et al*., 2000).

Because selenium and molybdenum share chemical properties, with other elements in the environment, the fate and toxicity of both selenium and molybdenum can be modified through interactions with other elements. Frequently, the interactions between selenium or molybdenum and other elements lead to environmental antagonism, and this process reduces the actual compared with apparent toxicity of these elements to sensitive receptor species. For selenium, elements that have similar properties include arsenic and mercury, among others. For example, high concentrations of arsenic can inhibit selenium uptake. In addition, sulfate shares several properties (e.g., stereochemistry) with both selenium (as selenate) and molybdenum (as molybdate) and can reduce their uptake. Collectively, environmental antagonism due to these similarities represents a simple explanation why high concentrations of either arsenic or sulfate reduce the toxicity of selenium and molybdenum. In addition, the enzyme kinetics in organisms also acts to determine the relative concentrations taken up in an environment (Barceloux, 1999a; Marino *et al*., 2003). In combination, fluctuations in the ambient environment, including seasonal cycles, and metabolism synergistically shape the toxicity of selenium and molybdenum to exposed species. These processes represent another reason to consider selenium and molybdenum simultaneously in this review.

The environmental concentrations of selenium and molybdenum can be modified also by agricultural activities. A recent review showed how selenium and molybdenum originating from agroecosystems are released to the environment (He *et al*., 2005). This recent review identified that adsorption-desorption, complexation, and precipitation-dissolution were the most important processes shaping accumulation and bioavailability in soils. The role of irrigation water and fertilizer application in agriculture was also considered. It is important to note that He *et al*. (2005) confirmed these processes associated with agriculture can lead to deficiency and toxicity due to modified concentration of elements. Thus, it is essential to resolve other sources of selenium and molybdenum that may occur near mine sites.

Atmospheric dry and wet deposition of selenium and molybdenum in Canada occurs and is monitored by Environment Canada (e.g., Glooschenko and Arafat 1988). This route has been identified as a minor source of these elements to terrestrial and aquatic habitats. However, some anecdotal evidence exists that indicates the ash from forest fires can lead to locally high concentrations of both selenium and molybdenum in depositional areas of lakes and this process would be consistent with the observed change in the water chemistry of lakes exposed to experimental and natural fires (e.g., Allen *et al*., 2003).

Identification of such effects will be dependent on local processes, however, such as the rates of flushing of the lake, precipitation patterns, and surface runoff from burned land.

2.1 Selenium

The distribution and major environmental characteristics of selenium have been well-researched and are documented in a number of comprehensive overviews. For the purpose of this report, the following major sources of general selenium characteristics were reviewed:

- U.S. Environmental Protection Agency, Integrated Risk Information System (IRIS) database (2005a);
- U.S. Agency for Toxic Substances and Disease Registry (ATSDR) – Selenium Toxicology Profile (ATSDR, 2003); and
- World Health Organization (WHO), Environmental Health Criterion for Selenium (WHO, 1986).

These information sources and numerous topical journal articles (e.g., Barceloux 1999a) have served as the primary basis of the following discussions of key aspects of selenium. This discussion focuses generally on North America and in particular on Canada, although some reference to other locales is also used to provide context.

2.1.1 Sources

Selenium is a naturally-occurring semi-metallic element that is widely distributed in the earth's crust. It is rarely found in pure form, and is usually complexed with other substances, such as sulfide minerals, silver, copper, lead, and nickel minerals. Selenium is found in geological materials at concentrations that are variable but generally low in comparison to most elements associated with base metal mining. In addition, selenium also readily accumulates in the residues from the production of sulfuric acid.

Selenium is used in many products, including: semiconductors, photo cells, an additive to rubber and stainless steel. The largest use of selenium is likely in the glass industry, to remove colour in glass and to create ruby-tinted glass and enamel (Barceloux, 1999a, USGS, 2006, also see www.minerals.usgs.gov).

The main anthropogenic sources of world-wide selenium release to the environment include the mining and processing of commodities such as copper, lead, zinc, phosphate, and uranium (WHO, 1986). In Canada, selenium is often associated with ores of copper, lead, nickel and uranium. Concentrations of selenium in metal ores are generally too low to be used for primary extraction, and most selenium is generated as a byproduct of processing of other ores (e.g., copper). Copper smelting was previously identified as the largest source of selenium in Canada, and the mining of selenium is a minor source (CCME, 1987).

In terms of world production of selenium, for the period of 2000 to 2004, the top five producers (in order of highest to lowest by average weight) were: Japan, Canada, Belgium, Germany, and Chile. However the United States withholds production data on selenium for proprietary reasons (USGS, 2006, also see www.minerals.usgs.gov). It is important to note that production in a country does not equate directly with export to the world market. For example, China has rapidly increased imports of selenium, particularly for the manufacture of consumer goods, and this is credited with the recent rise in the commodity price for this element over the last few years (USGS, 2006).

Because the commodity prices of metals, including copper and zinc, have also increased during recent years (USGS, 2006), it is likely the incidental release of selenium will be concomitant with higher production volumes at these mine sites. Thus low commodity prices tends to lower production and peripheral releases of selenium to the environment whereas higher commodity prices leads to greater production and releases of selenium.

Other major sources of selenium releases to the environment occur from the mining, refining, and the use of hydrocarbons, such as coal and petroleum. Specifically, coal contains variable amounts of both organic and inorganic selenium. On a global basis, the average selenium content in coals varies from brown coals with 1.0 ± 0.15 ppm to hard coals with 1.6 ± 0.1 ppm (Yudovich and Ketris, 2006). On an ash basis, the selenium content dramatically increases, from 7.6 ± 06 ppm in brown coals to 9.9 ± 0.7 ppm in hard coals (Yudovich and Ketris, 2006). The combustion of coal yields selenium to the environment, adsorbed to particulates in exhaust gases, and to coal ash. For example, high concentrations of selenium in the air of China were attributed to burning of hard coal (Xie *et al*., 2006). Similarly, the mining of coal has been associated with increased releases of selenium primarily through runoff of disturbed lands (e.g., Eisler, 1985).

The refining of petroleum products, such as crude oil, also releases selenium. However, most of this selenium can be efficiently removed through treatment circuits (e.g., ferric chloride precipitation) and this technology is in wide-scale use (e.g., Twidwell *et al*., 2000). Further, the petroleum industry actively developed methods to reduce selenium releases in their effluent and exhaust gases several decades ago (e.g., Eisler 1985).

2.1.2 Typical Environmental Concentrations

Originating from diffuse natural (soil, bedrock) or anthropogenic sources, selenium can be found at variable levels throughout the world in air, soil, and water, and also in the tissues of plants and animals. The concentrations of selenium in these various media depend on the characteristics of the local geological materials, and also on the potential presence and influence of anthropogenic sources, such as base metal mines, agriculture operations, and other industrial operations that may function as sources of selenium release (e.g. industrial processes using coal). In many regions, natural geophysical and biological processes are probably dominant determinants of the status of selenium in the environment. Natural sources can vary significantly from region to region, and their possible confounding

influence must be taken into account in any SSRA of the effects of industrial activities on selenium in the environment.

Igneous rocks typically contain relatively low levels of selenium (typically less than 1 mg/kg), and similar levels probably occur in metamorphic rocks. Sedimentary rocks (sandstone, limestone, phosphorite, and shales) may contain selenium at concentrations exceeding 100 mg/kg. This likely reflects the propensity for selenium to accumulate in sediments (discussed in Section 2.2), the precursor of sedimentary rock.

In absence of significant anthropogenic sources or deposition of ash from forest fires, the selenium content of surface soil is largely driven by the selenium content in the parent material. Seleniferous soils (occurring throughout the western US, extending into Alberta, Manitoba, and Saskatchewan) can contain selenium up to 100 mg/kg (Logan *et al*., 1987). The highest known naturally occurring concentration of selenium in soil is in the order of 8,000 mg/kg, but selenium concentrations under natural conditions are typically less than 1 mg/kg. The levels of selenium in the soils within a defined watershed can be the primary determinant of selenium levels in the water. Surface waters can also be affected directly by selenium found in effluents from mining, industrial, or agriculture operations. It has also been concluded that concentrations of selenium in surface water can be directly and measurably influenced through the deposition of selenium in the ambient atmosphere (U.S. DOI, 1998; Hren and Feltz, 1998; Barceloux, 1999a; Lemly, 2004a,b,c).

Select habitats, particularly soils, are considered as deficient in selenium. Soils that are deficient in selenium are typically located in montane areas, as in western China, other locales of Asia, and on tropical islands (Pipken *et al*., 2004). However, such habitats do not usually show elevated concentrations in receiving waters (e.g., Lemly, 2004c).

Concentrations of selenium in air and water are typically very low compared to most other elements (i.e., less than 0.01 µg/m^3 in air and <10 µg/L in water; WHO, 1986). For example, Fournier *et al*. (2005) report typical concentrations in unpolluted freshwaters as ~0.2 µg/L. Irrigation activities for agriculture can act to increase selenium loading to sediments and soils (Hren and Feltz, 1998). In a Canada-wide survey of surface waters, selenium concentrations were reported to range from <0.1 µg/L to 40 µg/L (Health Canada, 1992). In British Columbia, the concentration of total selenium in rivers and streams is typically less than 1 µg/L; however, concentrations as high as ~200 µg/L were measured, for example, downstream of coal mines (Nagpal *et al*., 1995; Nagpal and Howell, 2001).

World-wide, metal mine waste-waters and effluents from a number of non-ferrous industries were reported to contain selenium in the range of 14 to 56 µg/L (ATSDR, 2003). A review of reported conditions at select mines in Canada revealed typical concentrations of selenium in the range of 5 to 110 µg/L prior to treatment and release in mine waters and processing effluents from uranium, copper, gold and coal mines (MEND, 2004).

In the United States, the range of selenium concentrations in freshwater that is considered to represent background (i.e., unpolluted) conditions was identified as 0.1 to 0.4 µg/L (U.S.

DOI, 1998) yet higher concentrations exist in some western watersheds (Eisler, 1985; Lemly, 2004c). The tendency for selenium to accumulate in sediments is reflected in the background levels, which range from 200 to 2,000 µg/kg (dry weight, dw), for freshwater sediments in the United States (U.S. DOI, 1998). For 390 uncontaminated lakes in 13 regions of British Columbia (Rieberger, 1992), a much higher range of background concentrations of selenium in sediment was observed and can be inferred to represent a direct relationship with local sediment characteristics. Further resolution of the role of sediment content and uptake of selenium is warranted. Regional means varied from <10,000 (method detection limit) to 20,000 ug/kg (dw) with the highest for any lake being ~85,000 ug/kg (dw).

A review of typical selenium levels naturally occurring in human food sources shows that levels are variable but typically <1.5 mg/kg (fresh weight, fw) in most animal and plant products (WHO, 1986). In general, selenium levels are reported as highest in organ meats and seafood (up to 1.5 mg/kg fw), somewhat lower in cereals (up to 0.8 mg/kg fw or higher), lower still in non-organ meats (up to 0.4 mg/kg fw), and typically lowest in fruits and vegetables (typically <0.1 mg/kg fw). Absolute levels of selenium in food products tend to reflect ambient levels of selenium in the region of food production. In Canada, dietary analysis revealed concentrations of selenium as follows; up to 1.3 mg/kg in cereals, and means of 0.03 mg/kg in meats and 0.05 mg/kg in vegetables (Health Canada, 1992). A survey of Australian foods (ANZFA, 2002; also see http://www.foodstandards.gov.au) revealed similar trends, with the highest levels of selenium found in organ meats (~1 to 2 mg/kg fw) and fish and seafood (~0.3 to 1 mg/kg fw), with low levels (<0.05 mg/kg fw) typically found in fruit and vegetables. The relative differences among major food types (fish, animal, plant) are reflective of typical tendencies for selenium accumulation in certain biological tissues (ANZFA, 2002). In general, the observed levels in the major groupings of human foods suggest magnification of selenium in fish flesh and in the organs of mammals relative to plants (leafy, fruit, nuts).

2.2 Molybdenum

The distribution and major environmental characteristics of molybdenum, like selenium, have been documented in a number of comprehensive reviews. For the purpose of this report, the following major sources of general molybdenum characteristics included:

- U.S. Environmental Protection Agency, Integrated Risk Information System (IRIS) database (2005a);
- Canadian Water Quality Guidelines for the Protection of Aquatic Life: Molybdenum (1999);
- U.S. Department of Interior (U.S. DOI 1998); and
- World Health Organization (WHO), Review of Molybdenum in Drinking Water (WHO, 1996).

These information sources and numerous topical journal articles (e.g., Barceloux 1999b) were used as the primary basis of the following discussions of key aspects of molybdenum. This discussion focuses generally on North America and in particular on Canada, although some reference to other locales is also included to provide context.

2.2.1 Sources

Molybdenum is a naturally-occurring semi-metallic element that is widely distributed in the earth's crust. Molybdenum is frequently present in the geologic environment as the primary metal sulfide, usually molybdenite (MoS_2), in porphyry molybdenum deposits (e.g., Morford and Emerson, 1999). There are also secondary forms of molybdenum, usually complexed as metal-molybdates, in deposits dominated by copper, uranium, and zinc. Molybdenum, like selenium, is commonly found in rocks and soil at concentrations that are variable but also low in comparison with most other elements typically associated with metal mining. Interestingly, about 2/3 of the worldwide molybdenum reserves are found along the Western Cordillera. Although some mines in this region focus primarily on molybdenum, three quarters of global production comes as a byproduct with the production of other metals, especially copper. Low concentrations of molybdenum also occur in coal and petrochemical products, and the mining, refining, and use of these products lead to releases of molybdenum to the environment (e.g., Eisler, 1989).

Molybdenum is used in many industrial applications. It is an essential alloy in both iron and manganese steel, as a catalyst in petroleum refining, as a lubricant, a colour pigment, and as an additive to fertilizer. In addition, metallic molybdenum is used in electrodes, heating elements, and electronic components (Stokinger, 1981; Barceloux, 1999b).

In terms of world production, for the period of 2000 to 2004, the top five producers of molybdenum (in order of highest to lowest by average weight) were: the United States, Chile, China, Peru, and Canada (USGS, 2006). In Canada, all major producers of molybdenum are located in British Columbia and shipments from Canada for 2000-2004 have shown an increasing trend (Figure 2.1), likely due to increased prices for this commodity. Most of this production is sold as a molybdenum-trioxide concentrate (also refer to www.mmsd1.mms.nrcan.gc.ca under mineral statistics for additional details).

In the last few years, China has severely reduced their molybdenum exports. This reduction is partially credited with the recent rise in the commodity price on the world market. The reduction in exports in China is a direct consequence of the closure of about 90% of the small molybdenum mines due to profitability issues and a lack of adequate electricity at these mostly rural sites (USGS, 2006: also refer to www.minerals.usgs.gov). The reduction in Chinese production and exports of molybdenum essentially guarantees the commodity price will remain elevated for the foreseeable future. This increased value of molybdenum will lead to minimally a maintenance of current production and likely to additional mine development. For example, the MAX Molybdenum Mine, located south of Revelstoke, British Columbia, received fast-track approval and opened on 29 October 2007.

A second mine in development, located away from the Western Cordillera, is the Preissac Molybdenum Mine, near Cadillac, Québec.

2.2.2 Typical Environmental Concentrations

Molybdenum originates from natural origins (soil, bedrock) and anthropogenic activities. These sources result in highly variable environmental concentrations throughout the world in air, soil, and between marine and freshwaters. This variability leads to differences in the concentration of molybdenum observed in the tissues of plants and animals that use these habitats. The concentrations of molybdenum in these various media and organisms are therefore strongly shaped by the local geological materials. Another potential influence is anthropogenic activities, such as base metal mines, and other operations (e.g. farming with fertilizer), that may function as sources of molybdenum releases to the environment. In many regions, variable natural geophysical and biological processes are probably dominant determinants of the status of molybdenum in the environment. Natural sources can vary significantly from region to region, and their possible confounding influence must be taken into account in any SSRA of the effects of industrial activities on molybdenum in the aquatic and terrestrial environments. However, the typical range of molybdenum in soil is from 1 to 2 mg/kg while molybdenum deficient soils are associated with concentrations less than 0.2 mg/kg in North America (Barceloux, 1999b).

Long-distance transport of molybdenum has not been identified in recent or historical studies. For example, long-term monitoring and sediment analysis has shown that atmospheric deposition of wet and dry molybdenum can be considered as a minor contributor to aquatic and terrestrial habitats in different regions of Canada (Barrie 1988, Gélinas and Schmit 1998, Gélinas *et al*., 2000). This trend indicates that the primary sources of molybdenum in an environment are likely primarily due to local processes.

The concentrations of molybdenum in natural freshwater sources in Canada have been observed to range from below the methods detection limit (i.e., 0.1 µg/L) to 500 µg/L (MOEE, 1994). By contrast, the waters of the Laurentian Great Lakes were observed to range from 0.15 to 2.8 µg/L (Rossman and Barres, 1988). Chappell (1975) indicated that in areas disturbed by human activities, the surface water concentration of molybdenum had a mean of about 70 µg/L. In a survey of 15 major river basins in the United States, molybdenum was detectable in 32.7% of surface water samples, with a mean of 60 µg/L, and a range of 2 to 1500 µg/L (NAS, 1977). These concentrations in lakes and rivers are considered to represent the typical natural range of molybdenum for surface waters (WHO, 1996). The molybdenum in treated municipal sewage sludge averages about 15 mg/kg and ranges from 1 to 40 mg/kg (Barceloux, 1999b). Thus, sources of molybdenum from treated sewage effluents must be separated from loadings from mine sites to fully assess environmental concentrations and appropriately frame the SSRA.

Groundwater concentrations of molybdenum vary by location and associated geology. In Canada, molybdenum concentrations in groundwater range from below methods detection limit to greater than 1,000 µg/L. A survey of groundwater from different watersheds in the

USA during the 1940s identified molybdenum concentrations that ranged from below the method detection limit to a high of 270 µg/L (Kehoe *et al*., 1944). These concentrations are consistent with more recent groundwater surveys in the USA, except for areas near molybdenum mines that show significantly higher concentrations (Barceloux, 1999b). For example, groundwater near the Molycorp Molybdenum mine in New Mexico, USA, shows concentrations that are frequently greater than 2000 µg/L (ATSDR, 2005).

Concentrations of molybdenum from Canada in treated mine waste waters and processing effluents released to surface waters varies from site to site. Generally, observations of molybdenum concentrations from copper, molybdenum and uranium mines in Canada have been reported as being in the range of 1,000 to 10,000 µg/L (MEND, 2004).

A review of typical molybdenum levels naturally occurring in human food sources shows that surface plants like leafy vegetables and cauliflower contain relatively high concentrations compared with rooted plants like potato tubers (Eisler, 1989; Gupta, 1997). The actual concentration of molybdenum in plants depends on site-specific factors shaping the soil, including: organic matter, mineral content (sulfur, phosphorus, manganese). However, some plants are hyperaccumulators of molybdenum and can show very high tissue concentrations. Such plants have been used for remediation of high molybdenum concentrations in soil (Retana *et al*., 1993; Gupta, 1997; Barceloux, 1999b).

The environmental concentrations of molybdenum are routinely monitored in aquatic and terrestrial environments of many countries. This list includes Australia, Belarus, China, European Union, Peru, and USA. This monitoring is due to reviews completed primarily by the WHO that identified molybdenum as hazards in drinking water (e.g., WHO, 2006).

3.0 ENVIRONMENTAL FATE AND TRANSPORT

3.1 Selenium

Selenium that is released from either natural sources (mobilization of geological materials) or industrial sources (e.g. smelting, mining) will readily undergo transport and subsequent partitioning to various media in the receiving environment. Selenium does not break down in the environment, but its chemical form can change. It may also be subject to differential partitioning to various media, and is widely recognized as being bioavailable and readily assimilated into the food web.

The environmental fate of selenium is governed by complex interactions between chemical and physiological processes. The specific conditions encountered in a given environment will play a significant role in determining the ultimate fate of selenium releases, and also the potential environmental impact.

3.1.1 Chemical Forms and Speciation

In the water, sediment or soil of most natural environments, selenium exists primarily in one of several inorganic forms: selenate (SeO_4^{-2}), selenite (SeO_3^{-2}), elemental selenium (Se^0), or selenide (Se^{-2}). Selenium commonly complexes with oxygen and exists in an anionic or negatively charged species (primarily selenite and selenate) under most environmentally-relevant scenarios. In well-oxygenated waters, selenate is stable and the most common form under circum-neutral to alkaline pH conditions. Selenite will form in less oxygenated waters, and is slightly less soluble and more reactive than selenate. Selenium can also be found in the form of various selenium-sulfur compounds occurring in environments containing reduced sulfur species. Under markedly acidic and reducing conditions, elemental selenium (insoluble and inert) is likely to form, and with further reduction, selenides can form. Selenides can be the precursor to organic selenides (e.g. volatile methylated selenides and very soluble seleno-amino acids), and inorganic metal selenides, that are relatively insoluble. Selenite and selenate tend to be the dominant forms of soluble selenium found in the water column in natural freshwater environments. However, these forms can be subject to various physical or biological processes that result in a change in selenium speciation and/or partitioning to other media (e.g., Pipken *et al*., 2004).

The methylation of selenium acts to increase the bioavailability and therefore the toxicity to aquatic species, and this has been known for decades (e.g., Chau *et al*., 1976). A recent review separated the toxicity and biogeochemistry of selenium species between flowing (lotic) and standing (lentic) fresh waters (Simmons and Wallschläger, 2005). In addition, the accumulation patterns for selenium from water to sediment to insects has been quantified for lentic and lotic habitats in Utah, USA (Hillwalker *et al*., 2006). These insects showed consistent selenium tissue concentrations within species. Insects in the lentic habitat had higher concentrations of selenium compared with the lotic habitat. Interestingly, selenium dynamics has not been extensively studied in marine environments.

The various forms of selenium can also be transformed by microorganisms or macro-organisms according to physiological processes. In water, macrophytes and other plants (algae, phytoplankton) can readily take up selenite and selenate from the water column and incorporate selenium in the tissue as selenomethionine. Growth of plants, followed by harvest has been used as a remediation strategy for selenium-contaminated surface waters (e.g., Carvalho and Martin, 2001; also see Section 5.0). Anaerobic microbial reduction of selenate and selenite to insoluble elemental selenium can represent an important mechanism for immobilizing and removing selenium from the water column (Chau *et al*., 1976; Lemly, 2004c). Zhang and Frankenberger (2005) described an economical method for the removal of selenium from surface waters involving microbial reduction coupled with treatment with organic carbon. Such a process may represent a feasible method for large-scale treatment of selenium-contaminated water.

As noted, methylated selenides may be formed under reducing conditions, mostly via microbial activity in sediments, likely as a protective detoxification mechanism. Forms include dimethyl selenide, dimethyl diselenide, methane selanone, methane selenol, and dimethyl selenyl sulfide. These chemical species all tend to be short-lived in the aquatic environment as a result of volatilization losses to the atmosphere. While this type of loss can account for perhaps 20% or more of the selenium load at a site (Gao and Tanji, 1995; Azaizeh *et al*., 1997), these forms have not been well studied, and they generally are not considered in SSRA for selenium releases. Regardless of potential environmental compartments or residence times, these methylated forms tend to be quite bioavailable and may facilitate transfer of selenium to sediment-dwelling biota (e.g., Lemly, 2004c). Then the biota conveys the selenium to the higher trophic levels.

Organic selenides along with selenium-amino-acids and selenium-proteins, produced by biological reduction of selenite, usually occur at considerably lower concentrations in water than the inorganic selenium species. In the well-studied case of the Kesterson Reservoir, six or more forms of dissolved organic selenium were found in the water column (U.S. DOI, 1998). However, Fan *et al*. (2002b) reported that selenomethionine was the dominant form of soluble selenium in a drainage basin due to biotransformation in the water column.

Each of the selenium compounds that might occur in the aquatic environment exhibits its own chemical and biochemical behavior, mobility, and toxicity. The environmental form and fate of selenium is governed by interactions involving biological, chemical, and physical processes. In trying to forecast the potential effects of selenium on vertebrate animals, the form of selenium in diet can be determined with some confidence. However, the amount of selenium in the diet will be dependent on multiple interactions between speciation and environmental partitioning and so it is difficult to quantitatively predict. Thus, the biological, chemical, and physical compartments need to be considered explicitly.

For a given mine-related release, it would be a key first step to understand the particular chemical form(s) of selenium in the discharge. The next step would be to resolve the relative contribution from the mine from other sources (e.g., agriculture, domestic sewage,

ash from forest fires). In order to understand the potential for ecological impacts, it would also be necessary to gain the best understanding of the key characteristics of the receiving environment (e.g., water volume, pH, salinity) that will determine the fate and partitioning of the selenium following release. This process is explained in detail in Section 6.

3.1.2 Partitioning in Water and Sediment

Almost all of the selenium load that initially enters aquatic ecosystems in dissolved form will, over time, either adsorb to particulate matter and/or be taken up by various biota (plankton, aquatic plants, invertebrates, fish), becoming available across the food web.

Surveys conducted at Canadian mines (ESG, 1999) identified that downstream concentrations in sediment of several elements and COPCs, including selenium, are elevated relative to baseline levels and also frequently exceed environmental quality guidelines. Often, the water-to-sediment partitioning of these elements improves the quality of overlying water and reduces the potential for environmental impacts to biota in the water column. However, the partitioning of selenium to sediments can also be a key initial step leading to accumulation in the food web and exposure of organisms of higher trophic status (e.g., Malisa, 2001). This phenomenon and its toxicological implications has been the focus of much recent research, as discussed in detail in Section 4.

The form and fate of selenium in sediments is dependent on site-specific conditions. Where phytoplankton and algae are abundant in the water column, selenium may be taken up directly from the water to the plant tissues and subsequently deposited to sediments as these plants die off. This process can significantly increase the selenium in the surface layer of organic detritus. Suspended particulates (organic or inorganic) can also simply adsorb dissolved selenium from the water column and deposit to bottom sediments. These processes, combined or in isolation, can lead to a significant rate of delivery of selenium from the water column to bottom sediment layers. In a study to assess the potential effectiveness of treatment wetlands for removing selenium from agricultural drainage water, Gao *et al*. (2003) found that wetlands could remove in the range of 48 to 76% (mass) of the selenium load, entering the water column primarily in the form of selenate. The selenium was sequestered primarily into the surface sediments of the wetland. This is indicative of the extent that selenium can be initially sequestered in sediments, especially in shallow, slow moving waters with high rates of primary production and/or high levels of suspended solids. Over the long term, the continual sedimentation processes can sequester the majority of the cumulative load (Simmons and Wallschläger, 2005). It is frequently reported that as much as 90% or more of the total selenium mass in the aquatic environment will exist in sediments (U.S. DOI, 1998, Hren and Feltz, 1998; Simmons and Wallschläger, 2005). Similar processes will be evident for selenium that arises from agroecosystems (He *et al*., 2005); irrigation waters can transport selenium across large distances and watershed boundaries (e.g., U.S. DOI 1998).

In terms of their adsorption to sediments, selenium is considered to be a redox-sensitive element (e.g., U.S. EPA, 1999). Under reducing conditions encountered in anaerobic sediments, selenium tends to be present as selenite, which is substantially less soluble than selenate. The less soluble selenite will exhibit a greater partitioning from water to sediment relative to selenate. For stronger reducing conditions, selenides may form, rendering the selenium even less soluble and more sediment-bound. It has been reported (e.g., Peters *et al*., 1997) that bioturbation of anoxic sediments by benthic invertebrates may result in oxidation of the selenium in reduced form and transform it to a more soluble and bioavailable species (i.e., selenate; also see review by Hillwalker *et al*., 2006).

The degree of partitioning and strength of selenium binding to sediments is also in part dependent on other attributes of the sediment, such as pH, and the proportion of clay, iron, and organic matter content in the matrix. In general, the proportion of selenium that is found in water-soluble forms (and thus bioavailable) increases as pH rises and decreases with organic matter content and total iron content of soils and sediments (e.g., Simmons and Wallschläger, 2005). Indeed, waters with high organic content and neutral pH will have less soluble selenium than water with basic pH and a paucity of organic content.

Various factors act in combination to determine the overall rate of sediment partitioning. The combined effect of all factors can be expressed in terms of the water-sediment partitioning coefficient (Kd*)*. Default Kd values for numerous elements have been defined by a number of parties. For example, a selenium Kd of 3,000 L/kg (dw) has been assigned as a conservative default by the International Atomic Energy Agency (IAEA) for marine sediments (IAEA, 2004). Sheppard and Thibault (1990) report a geometric mean Kd for selenium (unspecified form) of 1,800 L/kg (dw) in organic soil. By convention, organic soil Kd values are often adopted as representative of sediments in freshwater lakes. In modeling selenium impacts in the Delta Bay estuary in California, the USGS (2004) assumed a Kd of 3,000 L/kg (dw) for the mixed sediments in this shallow bay. In the USGS (2004) report, a review of data for other sites identified Kd values ranging widely from 300 to 40,000, with most values under 5,000. In reviewing a number of reports regarding selenium sediment partitioning in various types of waterbodies, Luoma and Presser (2000) also report a wide variability in Kd values, ranging from 300 to 20,000 L/kg (dw). If a quantitative estimate of selenium partitioning to sediments is required for an assessment of potential ecological risk, a Kd of 10,000 L/kg (dw) could be used as a conservative generic value. However, there can be large variability in the portioning of selenium to sediments, and site-specific values are recommended for use wherever possible. Such an approach will improve the realism of any SSRA.

An important aspect of the partitioning of selenium to bottom sediments is the role of the detrital layer. In systems with high productivity and available organic matter, the behaviour of selenium is such that it is preferentially partitioned to the thin layer of organic material at the sediment-water interface. This is exemplified by the high-productivity case of the Kesterson Reservoir, where whole-sediment selenium concentrations ranged from 5 to 10 mg/kg (dw), but concentrations in the detrital layer were in the range of 40 to 130 mg/kg

(dw) (Luoma and Presser, 2004). The organically-enriched detrital layer is a biological resource, and as a result, the selenium in this layer has a high potential for entry into the food web. Several authors have suggested that the amount of organic matter in sediments is a key factor in determining the extent that selenium may impact biota at all trophic levels. The presence of periphyton on bottom substrates can also readily facilitate the transfer of selenium from sediments into the food web.

Selenium exhibits a strong tendency to partition to sediments in the water column. [This trend also partially explains why soluble selenium is low in water with high dissolved organic content (e.g., Eisler 1985).] The degree of partitioning and the ultimate potential for the sediment-bound selenium to become a significant source for wildlife exposure through the food web depends on a number of factors. In general, the rate of water-to-sediment partitioning of selenium is likely to be highest in shallow, slow moving waters with high nutrient content and productivity in the water column, and with anaerobic sediments (Chau *et al*., 1976; Simmons and Wallschläger, 2005). The greater the level of biological activity in the water column (algae, phytoplankton) and in the sediments (microbes, invertebrates, macrophytes) the more likely that selenium in sediments will be taken up by primary producers and become available for uptake into the food web (e.g., Lemly, 2004c).

Despite the general tendencies noted above, selenium's complex biogeochemical behaviour is such that for any given release of selenium to water, it is extremely difficult to reliably predict the amount or form of selenium that will remain in the water column or partition to sediments. It is equally as difficult to subsequently predict the fate of selenium that does initially partition to the sediments (e.g., Simmons and Wallschläger, 2005). One consequence of this pattern of variable environmental partitioning is represented by environmental regulators identifying highly conservative regulatory guidelines for selenium (see Section 4 for further details and applications in case studies, noted in Section 5).

In soil, selenite usually is quickly bound to iron oxides and becomes unavailable for uptake by plants (Logan *et al*., 1987). Selenite and selenate are both strongly adsorbed by the iron and aluminum oxides, but both phosphate and sulfate effectively compete with selenite and selenate for these sorption sites (Langmuir *et al*., 2004). Specifically, elevated sulfate or phosphate concentrations may lower the rate of partitioning of selenium to soil components. Because the sulfate and phosphate concentrations vary among locations, it is not feasible to identify a generic relationship for the inhibition of selenium partitioning to soil, given the other modifying factors (e.g., pH) that could play a role with this process. A similar soil partitioning process was noted for a tracer study that used radioactive selenium (i.e., ^{79}Se; Ashworth and Shaw, 2006). This latter study was interesting in that it quantified the movement of radioactive selenium across compartments. Further study of the movement of selenium in different terrestrial environments is likely warranted. Such a study could also be structured to represent different precipitation regimes, as the movement of water through soil acts to influence the form of selenium and destination (e.g., what proportion will move to surface waters or remain bound to the sediment). However, He *et al*. (2005) have attempted to resolve facets of this relationship, but further study would be useful.

3.1.3 Uptake and Partitioning in Biota

Selenium that is present in physical media (sediment, water, soil) can become available for uptake by resident plants and animals. Selenium has received wide recognition as a potentially important COPC and bioaccumulative compound, especially when present in sediments (Hren and Feltz, 1998; U.S. EPA, 2000b; Lemly, 2004b,c; Simmons and Wallschläger, 2005). Existence of high background concentrations of selenium in small portions of some watersheds (e.g., rock formations, lakes) can complicate assessment of uptake patterns of selenium in downstream surface waters. In addition, processes occurring at lower trophic levels (e.g. uptake by micro-organisms, plants) have a potentially great influence on the degree of the total selenium exposure of higher trophic level animals. Similarly, the integration of selenium in animal tissues and depuration in faeces, although minor processes, complicates the assessment of potential risk from exposure. The multi-faceted nature of this process justifies the use of SSRAs and consideration of multiple receptor species representing different components of the food web.

Studies have resolved the key factors that shape the uptake of selenium by different biota (e.g., Simmons and Wallschläger, 2005). These factors determine the degree that selenium becomes subject to plant and animal uptake and accumulation, and includes:

- the concentration and chemical form(s) of selenium in the ambient environment;
- the medium(a) that the biota reside and the corresponding exposure route(s) (e.g., water, soil, sediment and/or foods);
- the chemical and other characteristics of those media (e.g., dissolved oxygen content and hardness of water, pH, redox state of water, soil and sediments, salinity of water or soil, and ambient temperature);
- the presence of other chemicals (e.g., arsenic, iron, sulfate, phosphate, mercury, cadmium) that can reduce or enhance the bioavailability of selenium;
- the species of plant or animal, and their specific characteristics, especially the dietary components and feeding habits of fish and animals; and
- the period of exposure.

Available evidence suggests that some degree of bioaccumulation of selenium will occur in most aquatic and terrestrial ecosystems (e.g., Lemly, 2004c). Studies have shown levels in biological tissues may become substantially elevated compared with ambient media. The overall understanding of the distribution of selenium in biota yields these general trends:

- marine organisms generally contain higher selenium residues than their freshwater and terrestrial counterparts;
- selenium tends to concentrate in the organs of vertebrates, especially the liver and kidneys, and to a lesser extent in reproductive tissues (including eggs);

- selenium concentrations in animal tissues tend to increase with time of exposure and age of organism; and
- most research indicates that the majority of selenium taken up by organisms of higher trophic status in the foodchain (most fish, birds and mammals) originates through food-chain exposure rather than directly from ambient media.

Even in aquatic environments containing only low, naturally occurring levels of selenium (~0.1 to 0.4 µg/L), bio-uptake can lead to relatively high levels in biological tissues (e.g., Barceloux, 1999a). According to a review by the U.S. DOI (1998), in uncontaminated environments, selenium occurs at concentrations in tissues that are typically several orders of magnitude higher than concentrations in the water column (Table 3.1). This implies a dose-response that can be represented as a linear relationship (Figure 3.1).

This phenomenon of biological uptake of an element from the surrounding environment is described as biomagnification (strictly reflecting only direct uptake from water) or bioaccumulation (reflecting the combined uptake along all pathways of exposure). Thus, bioconcentration factors (BCFs) are typically derived from laboratory-controlled studies, while bioaccumulation factors (BAFs) can be derived from lab experiments but more often are empirically derived from field observations. It is important to note that biomagnification refers to the increase of compounds or elements across three trophic levels in ecosystems (e.g., water to plants to invertebrates or water to invertebrates to fish; Rand *et al*., 1995).

Use of BCFs and BAFs for Assessments Involving Selenium

Case studies and evidence from reviews in British Columbia have led to the identification of selenium BCFs and BAFs that have been used to develop a water quality guideline (Nagpal and Howell, 2001; Table 3.2). Similarly, the recently revised U.S. EPA water quality criterion for selenium to protect aquatic life also reflects reviews of varied case studies (U.S. EPA, 2004a). From these reviews and other literature (IAEA, 1994, 2004; U.S. DOI, 1998; Karlsson *et al*., 2002), the following general conclusions can be drawn regarding the uptake of selenium into the tissues of aquatic biota:

- for a specified exposure level and organism, BCFs tend to be lowest for selenate, in the order of 5 to 10 times higher for selenite, and 5 to 10 times higher again for selenomethionine;
- for soluble forms of selenium commonly encountered in the water column (selenite and selenate), uptake by invertebrates and fish occurs primarily through the food web (bioaccumulation) rather than from the water column (biomagnification);
- selenium uptake and concentrations in invertebrates are in the same range as concentrations in fish tissues;
- selenium tends to accumulate in the tissues of birds more so than mammals; and

- in birds, mammals, and fish, the concentration of selenium in liver is generally about two to three times higher than that in muscle identifying tissue-specific BAFs.

Organisms may exhibit compensatory change in their uptake physiology of selenium under exposure to element concentrations that exceed nutritional requirements, as would be the case in instances of environmental contamination. Subsequently, the rate of uptake could decrease with an increase in exposure concentration or changes in the overall nutritional status of the organism in question. Numerous studies have shown that absolute values of BAFs and BCFs tend to decrease as the concentration of selenium in the ambient media or in food sources increases (e.g., Fan *et al*., 2002a,b; U.S. EPA, 2004a).

Thus, BCFs and BAFs are not inherent properties of any element, including selenium and molybdenum. The magnitude of these factors reflects the response of biological systems (plants and animals) to many integrated processes. Bioavailability is a product of complex interactions between physical media, biota, and the element in question.

Overall, BCFs and BAFs can be critically important in the risk assessment process. The toxic potential of any COPC, including selenium, is largely dependent on the degree that it can be transferred from the ambient environment and/or diet to tissues and cells where toxic action can occur. Many exposure or toxicity thresholds now recommended for use in the assessment of impacts of selenium on ecological receptors are presented as tissue concentrations of selenium, including the draft freshwater fish tissue criterion of 7.91µg/g (dw) for chronic exposure recently proposed by the U.S. EPA (2004a).

Overall, biological uptake is a process that is intricately associated with sediment and water selenium dynamics, and of equal complexity (e.g., Malisa, 2001). As with sediment and water, it is difficult to reliably predict the amount of selenium that will be found in various biological tissues at any concentration of selenium in the ambient environment (Adams *et al*., 1998; Brix *et al*., 2004; Lemly 2004c; Luoma and Rainbow 2005; Carmichael and Chapman, 2006). Table 3.3 lists generically applicable BCFs/BAFs, and conservative default values that can be used in estimating exposure in the SSRA process. However, where possible, site-specific BCFs/BAFs should be used to assess the potential impacts of a release of selenium to the aquatic environment.

It is prudent to note there are scientific limitations with the generic use of BCFs or BAFs for risk assessment. This subject has been considered extensively (e.g., McGeer *et al*. 2003) and is particularly relevant to essential metals and metalloids (U.S. EPA, 2004a, 2005b). For essential metals and metalloids, the toxicity is proportional to the metabolically-available concentration and not to the total accumulated content (Barceloux, 1999a; Rainbow, 2002). As a result, the U.S. EPA's Risk Assessment Forum is evaluating other bioaccumulation models (empirical and mechanistic) for use in risk assessment, with reports expected in 2007 (aquatic) and 2008 (terrestrial) (Sappington *et al*. 2006). Also, the

U.S. EPA (2004a) draft criterion for selenium contains no mention of BCFs or BAFs anywhere except in the Appendix C of the document.

For a better understanding of the selenium available at a site, if feasible, it is useful to estimate the Total Maximum Daily Load limits (TMDLs). This estimation can use the U.S. EPA's regulatory framework, as explained elsewhere (e.g., Lemly, 2001a, b, 2004b). It requires the identification of sources of selenium at a site.

The following subsections review some key aspects of the accumulation and transformation of selenium by the major classes of biota.

Plants

Selenium is readily taken up by all forms of plant life, and typically translocated to all tissues (roots, stems, leaves, fruit, etc.). Uptake of selenium into plants is influenced by several factors, including plant species, form and ambient concentration of selenium, soil/sediment pH, clay content, and the abundance of other ions (Mg, Fe, Al) in the growing medium. In general, many of the factors that affect the degree to which selenium bonds to sediment or soil solids inherently affect plant uptake (e.g., Hartikainen, 2005; Clemens, 2006).

Selenite is identified as the primary form that is taken up by simple plant life (phytoplankton, micro-algae). By contrast, higher plants (both terrestrial and aquatic) typically absorb selenium primarily in the selenate form. Selenate is actively absorbed by higher plants because of similar stereochemistry as phosphate. For this reason, phosphate interacts with selenium availability. The presence of high levels of soluble phosphate can interfere with the uptake of selenate from soil or sediment (Wan *et al*., 1988; Barceloux, 1999a).

Sulfate also competes with selenate for uptake by plants, and high ambient sulfate levels may limit initial uptake into tissues. However, some hyperaccumulator species will still absorb selenium as selenate, and accumulate concentrations approaching 700 mg/kg (dw), even in the presence of relatively elevated sulfate (e.g., Retana *et al*., 1993).

In a study of selenium fate and transport at a uranium mine site (Sharmasarkar and Vance, 2002), selenium accumulation in different plant types ranged from 11 to 1,800 mg/kg (dw). This is consistent with previous studies where selenium was found to accumulate to concentrations exceeding 1,000 mg/kg. Availability of selenium for plant uptake was affected by the degree of soil binding, shaped by other factors like the degree of organic carbon present in soil. Similar uptake dynamics are expected in aquatic systems, with the rate of selenium uptake governed in part by the degree of binding to sediments.

Greenhouse experiments have indicated that several food crops are capable of accumulating selenium to concentrations that may be harmful to animals (Wan *et al*., 1988). In the case of selenium, and also molybdenum, uptake to plant tissues is generally not sufficient to cause toxicity to the plant directly, but has secondarily led to toxicities to animals consuming the enriched tissue (Foy *al*., 1978; McGrath *et al*., 1995; Bañuelos *et*

al., 1996). Terrestrial plants, particularly members of the mustard family, have been shown in various studies (e.g., Banuelos, 2002) to be able to accumulate tissue selenium concentrations exceeding several thousand mg/kg, and not be adversely affected by these levels. Generally, selenium hyperaccumulators are often able to attain concentrations of 1,000's of mg/kg (dw) in different soil environments (Mikkelsen *et al*., 1988; Barceloux, 1999a). These studies have also shown that selenium is translocated from roots to above-ground tissues. However, accumulation into edible portions of food crops has been reported as low in comparison with other plant tissues (Wan *et al*., 1988).

In general, absorbed selenium is reduced and complexed into seleno-amino acids in certain plant tissues (Wan *et al*., 1988; FAO and WHO 2001). Selenomethionine is the common form of selenium present in plant tissues. In many hyper-accumulating plant species, less selenium is incorporated into proteins (selenomethionine) than in non-accumulator species (Brown and Shrift, 1981; Barceloux, 1999a). Selenomethionine has been found to be more bioavailable and more persistent in the tissues of animals (that cannot produce selenomethionine), and more likely to induce toxic effects than selenium in inorganic forms (e.g. selenite). Thus, plants can play an important role in determining the selenium status (deficient, sufficient, toxic) of animals through food web linkages (e.g., Lemly, 2004c).

Plants themselves do not appear to be particularly sensitive to toxicological effects of selenium. Relatively minor effects (e.g., 10% growth reduction) on terrestrial plants do not typically occur until plant tissue concentrations of selenium are in the range of 10 to 40 mg/kg (dw) (MacNicol and Beckett, 1985). Some plants show hypertolerance to selenium, and this partially represents long-term evolutionary selective pressures on these plant species in selenium-rich environments (e.g., Clemens, 2006). The U.S. EPA (2004a) reviewed the toxicity of selenium to aquatic plants, and this revealed little evidence of negative consequences from exposure to selenium. Also, studies with micro-algae that assessed exposure to selenite indicated that significant toxic effects do not occur until concentrations in the water column approach the mg per litre level (Morlon *et al*., 2005). These studies identified that the accumulation of selenite persisted up to these levels in the water column.

Overall, plant life appears likely to be able to survive in environments with relatively high selenium and facilitate the exposure of plant-eating wildlife to selenium from soil or sediments. Available information indicates the ingestion of aquatic plants by herbivorous wildlife represents a route of concern. For example, Nagpal and Howell (2001) reported a range of BCFs for aquatic macrophytes of 30 to ~15,000 L/kg (dw), suggesting a potential for high tissue concentrations similar to those observed for terrestrial plants. Certain plant species are recognized as high accumulators of selenium, including relatively common cattails (*Typha* sp.; Carvalho and Martin, 2001).

Fish

It is largely accepted that the uptake of selenium from the environment by fish is mediated primarily through food. For example, Buhl and Hamilton (2000) conducted a study where fish were exposed to various concentrations of selenium in both their diet and water. It was found that selenium concentrations in muscle were strongly correlated with the dietary concentration but not with waterborne selenium. In this study, it was found that the concentrations in fish tissue were lower than in food by a factor of ~0.2 to 0.6. With food being a key route of exposure, uptake by fish can be termed as bioaccumulation in most cases, and the composition of diet can play a role in the degree of selenium exposure of both fish and the animals that feed on them.

In a comprehensive review of selenium concentrations in the Colorado River basin (King *et al*., 2003), no clear relationship(s) were found between the concentration of selenium in sediment and the concentration of selenium in fish tissues. The authors suggest that other factors were important in determining the concentration of selenium in fish, and in fish-eating birds within this river environment. These factors (e.g., flow rates, physico-chemical character of the local environment) affect the speciation, solubility, and bioavailability of selenium through the food web, and need to be considered in any forms of environmental assessment of selenium.

As noted in Section 4.0, it is important to also consider the role of seasonality in temperate habitats as a factor that adds complexity to the dynamics of selenium in aquatic ecosystems.

Default selenium BCFs/BAFs have been defined by various agencies for the purpose of assessing exposure and risk to human health (see Tables 3.2 and 3.3). In establishing the water quality criterion for protection of human health, the U.S. EPA (2002) assumes a selenium BCF of only 4.8 L/kg (ww) to account for exposure through fish ingestion. The IJC (1982) reported that BCFs for selenium in fish ranged from 8 to 78 L/kg (ww), and selenium in the order of 5 to 10 µg/L in aquatic ecosystems could lead to food web contamination and acute effects on predatory fish. Fish BCFs have also been defined for the assessment of radionuclide exposure of humans as a result of fish ingestion. The BCFs for this purpose are derived from, and are applicable to, the stable element analogues. The selenium BCF for freshwater fish established by the IAEA (1994) and the NCRP (1996) is 200 L/kg (ww), or approximately 800 L/kg (dw). These guidelines assume the water content in fish flesh at 75% although this content is known to vary among individual fish and among fish species (e.g., Evans, 1993). Given this latter consideration, the U.S. EPA has identified guidelines for 75-80% water content (Figure 3.2). In marine environments, the selenium BCFs are higher, and range from ~16,000 L/kg (dw) to 40,000 L/kg (dw) (NCRP, 1996; IAEA, 2004).

The Ministry of Land and Water Protection (MWLAP) in British Columbia reviewed case studies and established a selenium water guideline for the protection of wildlife with an aquatic association for the province (Nagpal and Howell, 2001). Food web transfer was

considered in the development of this guideline, and bioaccumulation of selenium in fish was quantitatively summarized. The documented BCFs/BAFs for fish covered three orders of magnitude, ranging from 5 to >5,000 L/kg (dw).

The BCF/BAF data reviewed by MWLAP suggest that rates of bio-uptake are dependent on species of biota, local environment, and the form of selenium. Generally, BCFs/BAFS tended to be lowest for selenate and highest for organic forms (i.e., selenomethionine). As generally expected, the magnitude of BCF/BAF tended to be lower when the ambient concentration was higher. However, relatively high uptake was recorded under conditions of relatively large ambient selenium concentrations. For example, in a study of fish residing in a reservoir affected by coal fly-ash, selenium concentrations in the tissues of largemouth bass were ~4,000 times greater than the concentration in the contaminated water (i.e., ~10 µg/L) (Lemly, 1985).

The draft U.S. EPA aquatic life criterion for selenium (U.S. EPA, 2004a) used a review of recent case studies to frame their revised BCFs and BAFs. Lab-derived BCFs for selenium as selenite or selenate, at 10 µg/L or more in water, ranged from 2 to 470 L/kg (dw). Field-derived BAFs for undifferentiated total selenium ranged from 273 L/kg (dw) to over 6,500 L/kg (dw). Within this range, cases with the concentration in the water column of >10 µg/L produced an average BAF of just under 500 L/kg (dw), while water concentrations ranging from 0.33 to 2.5 µg/L resulted in an average BAF of ~4,600 L/kg (dw). In total, the data considered by the U.S. EPA and other data from various independent studies clearly suggest that the rate of uptake of selenium into fish tissues tends to be inversely correlated with the concentration in the water column.

Studies have revealed that releases of selenium to the water can affect both fish and piscivorous wildlife in very different ecosystems (e.g., Nobbs *et al*., 1997; Barceloux, 1999a; Lemly, 2004c). Predictive assessments can be used to assess the potential for impacts of a release of selenium to water to these wildlife species through estimates of the concentration of selenium in fish tissues. Such an analysis can use BCFs or, more preferably, BAFS, to estimate selenium concentration in fish tissue. Table 3.2 provides a summary of generic BCFs that can be considered for such a purpose. However, it is well understood that BCFs/BAFs can exhibit great variability depending on a number of factors, chief among them being the ambient concentration. Even in cases where site-specific BCFs/BAFs are available for use, if they have been determined in advance of a proposed effluent discharge, care must be taken in extrapolating these values to higher ambient concentrations of selenium that may arise as a result of some future discharge scenario.

Aquatic Invertebrates

The accumulation of selenium by invertebrates, especially those associated with sediments, can be a key link leading to food web exposure of higher trophic animals. Aquatic invertebrates are themselves initially exposed to selenium via one of three pathways:

- direct uptake of dissolved selenium in the water column or in sediment porewater;
- ingestion of primary producers (algae, plankton, macrophytes) that have taken up selenium from ambient media; or
- direct uptake of selenium in particulate form (organic or mineral) via filter-feeding or detrital scavenging.

Varies studies have assessed the uptake of selenium by invertebrates. According to Luoma and Presser (2000), uptake through food is the most important contributor to selenium body burdens of aquatic invertebrates, and direct uptake from water is typically a minor contributor. In the review of accumulation rates of invertebrates, Nagpal and Howell (2001) identify high variability in BCFs, ranging from 71 to ~4,000 L/kg (dw). Similarly, the U.S. EPA (2004a) reports lab-based BCFs for invertebrate ranging from 91 to 1440 L/kg (dw), and a BAF range of 969 to 31,800 L/kg (dw) from field assessments. In that range, there is a very strong inverse correlation of BAF values with the ambient concentrations of selenium. This relationship is represented as Figure 3.3.

Additional study of the uptake of selenium by invertebrates may be warranted. For example, Fan *et al*. (2002a) reported a mean selenium BCF of 2,665 L/kg (dw) and this is within the cited range identified by Nagpal and Howell (2001). However, the sample variation noted in Fan *et al*. (2002a) was high (standard deviation of 4,389) although the water and invertebrate samples were collected concurrently in very similar water bodies.

Variability in uptake rates of selenium by invertebrates may be a result of several factors, as noted previously. In a study of bioaccumulation of selenium by bivalve filter feeders, Fournier *et al*. (2005) found that the degree of selenium uptake was dependent on the form (selenite, selenate, or selenomethionine) of dissolved selenium in the water column. In the laboratory, selenomethionine was reported to be the most bioavailable form. The uptake of selenate was slightly greater than that of selenite, but the rate of selenomethionine uptake was in the order of 10 times greater than that of either of the inorganic forms. A key finding in this investigation was that the ultimate rate of uptake of selenium by these invertebrates appeared to be consistent for all forms when the addition of selenium to experimental trials was through selenium-exposed algae as an intermediate step. In this study, it appeared that selenium, regardless of the form introduced to the water column, was converted to a common form by algae. This demonstrates the importance of selenium speciation, and the importance of primary transformation in determining the bioavailability and food web partitioning patterns. These observations are consistent with the noted patterns observed for aquatic insects considered by Hillwalker *et al*. (2006).

Reducing conditions in sediments can lead to the formation of elemental selenium, and the mass of elemental selenium in sediments can account for up to 90% of the total selenium balance in an aquatic environment (Simmons and Wallschläger, 2005). Selenium in this form is generally considered to have a relatively low bioavailability, and thus a low likelihood of inducing ecological effects (e.g., Lemly, 2004c). However, some studies have shown that invertebrates can actually assimilate elemental selenium in sediments. Schlekat *et al*. (2004) identified uptake of elemental and particle bound selenium by filter feeding bivalves, albeit at relatively low rates of uptake (assimilation efficiencies of 20% or less). Thus, for selenium uptake by invertebrates, the following general pattern exists:

selenomethione > selenate > selenite >elemental selenium

The feeding strategies and life cycles of invertebrates as a group are highly diverse. The degree of uptake of selenium in the aquatic environment could vary significantly from one species or feeding strategy to another as a result of differences in food types and feeding locations. In most cases, benthic invertebrates are considered as a uniform collective group in assessments of contaminant impacts in aquatic environments and ERAs. It is generally assumed that the role of invertebrates in food web transfer is common for pelagic (free swimming), infaunal (residing in the sediment) and epibenthic (living on the surface of the bottom sediment layer) invertebrates. However, there is evidence from some studies that the specific feeding habits of invertebrates can significantly affect metal uptake. For example, Desy *et al*. (2002) found the uptake of cadmium by invertebrates feeding on aquatic macrophytes was proportional to the concentration in the plant tissues and was not related to ambient concentrations in sediment and water. In another study, Peterson *et al*. (2002) noted that the body burdens of selenium and other elements in aquatic invertebrates can also be strongly influenced by precipitation patterns, with high precipitation yielding lower body burdens of metals and vice versa. In such cases, assumed BCF relationships between the invertebrate and either the sediment or the water column would be imprecise.

Overall, it is apparent that the rate of uptake of selenium by invertebrates is governed by a complex interaction of various factors. The interaction between sediment and selenium concentration determines bioavailability across seasons and sites (e.g., Malisa 2001). As a result, there is very high variability in the potential degree of selenium uptake into the food web through these organisms. Table 3.2 presents generic default values of selenium BCFs/BAFs for invertebrates, and other major classes of aquatic biota, that could be used for risk assessment purposes, but the use of site-specific BAFs (as opposed to BCFs) is highly recommended.

Birds and Mammals

Food is widely recognized as the main route of selenium exposure for air-breathing vertebrates, including birds, mammals, and also most reptiles and amphibians (Barceloux, 1999a; Newman *et al*., 2004). Accordingly, the amount of selenium taken into the tissues of these animals is primarily dependent on diet composition.

Laboratory studies were used to resolve the effects of selenium on mammals. Classic experiments with rats that were fed selenite and selenate exhaled a volatile selenium-containing compound identified as $(CH_3)_2Se$ (McConnell and Portman, 1952). Thus the toxicity of methylated selenium species has been generally understood since the 1950s.

From the study of human nutrition, it appears that the absorption of selenium from the digestive tract is not subject to homeostatic regulation. The rate-limiting step shaping the overall availability of dietary selenium may not be initial absorption, but rather its conversion within tissues to its metabolically active forms (FAO and WHO, 2001; Hartikainen, 2005).

Dietary uptake efficiency of various selenium compounds by animals is reported to range from about 44% to 95% of the amount ingested, depending on a number of factors, including the form of selenium (Opresko, 1993a). For most forms (selenite, selenate, selenomethionine) the uptake rates are typically very high (80 to 90% or more). Uptake rates tend to be lower under long term exposure to selenium in food, as opposed to short duration pulse exposure. Natural conditions would be reflective of the former circumstance. Similar rates of gastrointestinal uptake are reported in major reviews of selenium from a human nutrition perspective (WHO, 1986; Barceloux, 1999a; FAO and WHO, 2001). These reviews also reveal that selenium in fish tissues tends to be considerably less available than selenium in other food types, frequently by as much as half.

The form of selenium in the food ingested at higher trophic levels depends in part on the composition of the diet of the particular organism in question. The two most common forms of selenium that can be found in the diet of higher trophic status animals are selenomethionine (mainly from plants tissues) and selenocysteine (mainly from animal tissues). Studies of human nutrition have also shown that feeding of selenium bound in organic form results in higher tissue and blood-serum levels than does feeding of animals with selenium as inorganic selenate or selenite. There is also experimental evidence that selenomethionine is more readily absorbed during food digestion than is selenocysteine, (WHO, 1986; FAO and WHO, 2001; Hartikainen, 2005).

Ultimately, dietary composition will determine both the form and amount of selenium taken into the gastrointestinal tract and thus available for uptake. The preferred food and also the feeding habits, such as the specific habitats that an animal forages, can play a role in the total dietary selenium exposure. The influence of feeding habits (food types and feeding habitats) could be very significant. Such studies indicate that terrestrial species are at less risk than aquatic or semi-aquatic species. This view identifies the need to consider water-associated birds (including waterfowl, fish-eating birds etc.) separately from terrestrial birds and mammals.

Water birds

Other studies have used lab studies to resolve the different responses across bird species. For example, a bird muscle BAF of ~10 L/kg (dw) has been reported in a review of selenium data (Nagpal and Howell, 2001). In a study of selenium uptake and effects on

reproduction of water birds (Hoffman *et al*., 1998; Hoffman 2002), BAFs derived from the measured concentrations in bird eggs and ambient water were from 1,000 to 1,500 L/kg (dw) for selenium in water of less than 10 µg/L. In highly contaminated water (i.e., 190 µg/L), the BAF was noted as about ten-fold lower (i.e. ~100 to 150 L/kg dw).

Field studies have identified similar patterns between marine and freshwater environments in terms of the bioaccumulation of selenium. For example, Luoma and Presser (2002) compiled measures of selenium in the livers of 11 species of birds residing in a contaminated marine bay, taken over a four-year period. The range of concentrations of selenium in liver tissues across birds was very large (i.e., 3.8 to 134 µg/g dw). A key factor in this variability was suggested to be diet, with shallow-water feeders (shore birds, wading birds, and certain dabbling ducks) with liver selenium mostly <10 µg/g (dw), piscivore (e.g., cormorant, *Phalacrocorax* spp.) at ~19 µg/g, and surf scoters (*Melanitta perspicillata*), specialist feeders on mollusks and bivalves, having by far the highest liver selenium levels (134 µg/g dw). Also, scaup (*Aythya* spp.) are known to feed extensively on mollusks and bivalves, and showed the second highest concentrations of selenium in liver tissues (36.4 µg/g dw). Wayland *et al.* (2005) reported evidence of variable accumulation of selenium in female marine ducks, with concentrations of selenium being related to body weight, reproductive stage (pre-nest, nesting, no nest) corresponding to differences across the tissues of the body (e.g., muscle, liver, kidney). Similarly, Martinez (1994) identified differences in selenium uptake in birds from the same river waters. Specifically, selenium body burdens of several species of aquatic birds in the Colorado River basin varied by diet, with the herbivorous species with significantly lower tissue selenium levels than the birds feeding on invertebrates and fish. Thus, selenium concentrations in water birds will intrinsically reflect life history differences (e.g., age, diet, sex) and habitat use.

Patterns of uptake of selenium not only vary across species with different feeding habits but also across ecosystems in water birds. For example, Outridge *et al.* (1999) reviewed the potential hazards of environmental selenium exposure to water birds across ecosystems in Canada. This review identified differences in selenium uptake across these ecosystems and also considered the consequences of selenium exposure during migrations; soil and sediment differences were identified as a key factor shaping selenium uptake in migrating bird species. The migration history can act as an important influence on the selenium exposure and accumulation mechanism, and should be considered if feasible.

Custer and Custer (2000) completed a field study of trace element levels in waterfowl and zebra mussels (*Dreissena polymorpha*) in the lower Great Lakes. The study revealed selenium concentrations in liver tissue of the birds ranged from ~20 to 40 µg/g (dw) and did not differ among the four species that were studied. This liver tissue concentration contrasted with the mean concentration of 4.7 µg/g (dw) of selenium in the soft tissues of the mussels. Overall, this relationship indicates a BAF (from diet) of 10 to 20 to the livers of these birds that rely largely on these now common invertebrates as a food source.

Another common expression for the relationship between the amount of any element in diet and the amount that gets incorporated into animal tissues is the ingestion transfer factor (TF_{ing}) (mg/kg in tissue per mg/day ingested, simplified to day/kg). There are several sources of ingestion transfer factors that are used in the predictive assessment of human exposure to stable element and radioisotope contaminants in the environment through food products (IAEA, 1994; Staven *et al*., 2003) and are available for SSRAs. Development of transfer factors is reflective of extensive controlled feeding studies of domestic livestock, and by extension, very little comparable data exists for wildlife species. Still, these ingestion transfer factors can be coarsely applied to estimate selenium uptake by animals in natural settings although these ignore some physiological considerations (e.g., reproductive condition, seasonal feeding and temperature regimes).

Synthesis studies indicate the ingestion transfer factors vary among wildlife species. Generally, the rate of ingestion transfer is much higher for birds than it is for mammals. For example, selenium ingestion transfer factors for avian livestock are in the order of 10 day/kg (ww), whereas transfer factors for large mammalian livestock (e.g. beef cattle) are much lower (~0.01 day/kg ww). This is primarily a function of food intake rates of the animals in question, relative to body weight, but also reflects physiological differences (e.g., moisture content) to some extent. The net effect in applying these transfer factors, adjusting for body weight, is that the tissue concentration of selenium would be in the order of 10 times higher in a given bird than in a large mammal receiving the same diet.

Overall, food web structure and specific feeding habits of birds and animals may play an important role in determining their selenium status (deficient, sufficient, toxic). There is a complex interaction of factors that ultimately determine the amount of selenium entering via food that ends up in various animal tissues where it may have toxic implications. Broad application of generic conservative transfer factors (BAF, TF_{ing}) may significantly over-estimate foodchain exposure and associated impacts. Additional information on the role of food web structure influencing the consequences of selenium is included in the Section 4.0 (Toxicity) and 5.0 (Environmental Impacts).

Biomagnification

Biomagnification refers to the increase in tissue concentrations of a given contaminant with successive links along the food web (e.g., Rand *et al*., 1995). For elements that biomagnify, animals at highest trophic levels will have the highest tissue concentrations of those elements, and thus be more likely to suffer from toxic effects.

While some evidence indicates that selenium might biomagnify in aquatic organisms under natural conditions (e.g., ATSDR, 2003), there is overall uncertainty regarding the capacity for selenium to biomagnify. In humans, and likely in animals, selenium absorption is not affected by body selenium status. In studies completed to understand human nutritional processes (experimental animal studies and direct measurements of selenium absorption in humans), it was determined that selenium compounds can be readily absorbed in the

intestinal tract and there appears to be no obvious physiological feedback controls to govern the rate of absorption. Therefore excess uptake may occur. However, the excretion of dietary selenium is considered to be rapid for most animals. It is generally understood that rapid excretion greatly lowers the likelihood of detrimental biomagnification.

This understanding of biomagnification reflects different field studies. For example, Peterson and Nebeker (1992) reported a BAF of 2,600 L/kg for small forage fish and 6,800 L/kg for large predatory fish in the same environment. Similarly, Lemly (1985) reported BAFs of 485 and 1,690 L/kg for forage fish and predatory fish, respectively, in the same habitat. In both cases, there is an approximate 3-fold increase in BAF values between the successive trophic levels, implying biomagnification. Lemly (1997) reported that biomagnification of selenium usually ranged from 2 to 6 times between the primary producers (algae and plants) and the lower consumers (invertebrates and forage fish). Studies of slimy sculpin (*Cottus cognatus*) across habitats in British Columbia identified differences in tissue levels of selenium in fish from reference areas with contrasting geology (i.e., within and outside the coal zone) and indicates the presence of site-specific rates of biomagnification (Carmichael and Chapman, 2006). In a study of selenium levels in the eggs of several species of vertebrates residing in or near a contaminated waterbody affected by coal combustion, (Hopkins *et al*., 2000) the mean selenium concentrations (7.3 to 7.64 µg/g dw) measured for alligators, a top predator, were low relative to selenium concentrations (10 to 37 ug/g dw) measured in several prey items (frogs, fish, turtles). This suggests an absence of biomagnification of selenium in this case. Similarly, Hopkins *et al*. (2005) report that the concentrations of selenium in various tissues of lizards (~9 to 14 ug/g dw) was actually slightly less than the concentration in the artificially controlled insect diet (~15 µg/g). In a related study, Hopkins *et al*. (2006) reported evidence for biomagnification of selenium in a toad species that correlated with increased prevalence of larval deformities and reduced survivability compared with a reference population.

In general, the organo-selenium forms that are most abundant in biological tissues (e.g. selenomethinine) are not considered to be subject to significant biomagnification (McGeer *et al*., 2003). Overall, there is no significant evidence that selenium biomagnification occurs continuously across the food web. Further study of this facet of environmental dynamics of selenium is likely warranted.

In summary, the uptake of selenium by organisms can be characterized as follows:

- plants and algae can readily take up selenium in various forms from the ambient environment, and transform it into biologically available forms;
- initial uptake by these primary producers is the key step leading to uptake by organisms of higher trophic status; and
- following initial entry into the food web, there is no significant or widespread evidence for biomagnification of selenium at higher trophic levels.

3.2 Molybdenum

Molybdenum that is released from either natural sources (mobilization from geological materials) or industrial sources (e.g. smelting, mining, fertilizer) will readily undergo transport and subsequent partitioning to various media in the receiving environment (e.g., Barceloux, 1999b). Molybdenum, like selenium, does not break down in the environment, but its chemical form can change. This element, like selenium, may also be subject to differential partitioning to various media, and is widely recognized as bioavailable and readily assimilated into the food web. This integration in the food chain is partially a consequence of molybdenum being an essential element required by microorganisms, plants, and animals, and due to the high water solubility of many molybdenum-containing compounds (e.g., Eisler 1989).

The environmental fate of molybdenum is governed by complex interactions between chemical and physiological processes. The specific conditions encountered in a given environment will play a significant role in determining the ultimate fate of molybdenum releases, and also the potential environmental impacts. These processes will also be influenced by seasonal (e.g., weather) and geographical (e.g., latitude) considerations. For example, southern and northern locales will differ, like coastal and inland locales, and such spatial considerations need to be included in SSRAs for molybdenum.

3.2.1 Chemical Forms and Speciation

Molybdenum can exist in five oxidative states; the most common state in nature is the +6 ion. As noted previously, molybdenum typically complexes with other metal species and oxygen. In water, the chemical form and availability of molybdenum varies with mixing patterns, pH, oxidation rates of organic sediments, reduction potential of sediments, and sedimentation patterns (Driscoll *et al*., 1994). For example, in circumneutral water that contains oxygen concentrations of at least 3 ppm, the common form will be the stable molybdate anion (MoO_4^{-2}). At low pH (3-5), molybdate frequently shifts to hydrogen molybdate ($HMoO_4^{-1}$) (Crusius *et al*., 1996). In this lower pH range, molybdenum is commonly adsorbed to sediment particles composed of clay or other oxic minerals (Goldberg and Forster, 1998). The molybdate anion can be reduced to molybdenum disulfide or molybdenite (MoS_2) in low redox environments. In solutions with moderate to high concentrations of molybdate, the formation of different complex polymolybdate compounds is common. The major metallic molybdenum species include molybdenite (the common mineral mined to recover molybdenum) and ferrimolybdenite ($Fe_2[MoO_4]_3$) while the minor ones include powellite ($CaMoO_4$) and wulfenite ($PbMoO_4 \cdot 8H_2O$) (Crusius *et al*., 1996). Overall, partitioning and accumulation of molybdenum is favoured in sediments with higher iron, calcium, and organic matter content, and under low redox conditions and pH of 3 to 5 (Fox and Doner, 2002).

Atmospheric transport of molybdenum has been documented previously. For example, the large majority of molybdenum observed in rain collected in Japan was present as soluble molybdate. Interestly, all of these rain samples also contained molybdenum bound to iron-containing particulate matter (Kawakubo *et al*., 2001). It is also likely that variable quantities of molybdenum are deposited across habitats (e.g., lakes) that receive ash from forest fires, given that other trace elements accumulate through this mechanism (Carignan *et al*., 2000; Allen *et al*., 2003). It is not readily apparent if mobile molybdenum is also transported through ash from volcanoes. These alternate sources need to be included in risk assessments of molybdenum across watersheds.

The abundance of molybdenum in the ocean suggests the biological reactivity and toxicity is low (e.g., Collier, 1985). This low reactivity of molybdenum, as molybdate in sea water, is due to chemical antagonism or interference with sulfate anions. Specifically, the similarity in effective size and stereochemistry between sulfate and molybdate make it difficult for enzymes to preferentially uptake molybdate unless high specificity exists for this compound (e.g., Howarth and Cole, 1985). The chemical form of molybdenum changes with salinity concentrations and this can shape microorganism and phytoplankton production rates (Prange and Kremling, 1985; Howarth, 1988).

3.2.2 Partitioning in Water and Sediment

The majority of the molybdenum load that initially enters aquatic ecosystems in dissolved form or bound to particulates will, over time, either adsorb to particulate matter and/or be absorbed by biota of various forms (plankton, aquatic plants, invertebrates, fish), and becomes available to the entire food web (Driscoll *et al*., 1994; Crusius *et al*., 1996; Barceloux, 1999b). It is this partitioning to biota that contributes to the need to complete site-specific assessments of molybdenum dynamics. Generally, the molybdenum concentration in the water column is directly correlated with the concentration in the sediment. Thus, any change in the molybdenum concentration in the water will be reflected by the sediment. For example, as the concentration of molybdenum in the water decreases, a corresponding decrease will occur in the sediment. However these changes in the sediment concentration of molybdenum will not be instantaneous with changes in the water but occur over a period of months (Howarth and Cole, 1985). The concentration of molybdenum in sediments is shaped by site-specific factors like flow rate, the characteristics of sediment, and the frequency of disturbance of sediments, and other factors (e.g., atmospheric deposition). Interestingly, the molybdenum concentration in sediments can vary significantly over short distances and this also points to the need for site-specific studies (Barceloux, 1999b).

3.2.3 Uptake and Partitioning in Biota

Molybdenum that is present in physical media (soil, sediment, water) can become available for uptake by resident microorganisms, plants, and animals. Generally, water-soluble molybdenum forms can be readily taken up by biota in the environment whereas forms like

molybdenum disulfide is not readily available. Studies have shown this uptake occurs across cellular membranes and can be due to simple exposure to water or through consumption of sediment particles that contain molybdenum-containing compounds. The solubility of these compounds also permits active excretion by some organisms. For example, in fishes and mammals, the excretion of molybdenum readily occurs due to the action of the kidney, and the presence of high levels of sulfates and/or copper in the diet will enhance this excretory process (Karnacky, 1997; Barceloux, 1999b). By contrast, aquatic and terrestrial plants grown in media with high concentrations of molybdenum can show correspondingly high concentrations in the plant tissues.

Plants

Molybdenum is an essential element required by microorganisms and plants. The key role of molybdenum in plants is as a component of enzymes responsible for the production of phytohormones, for nitrogen assimilation, and protein catabolism (e.g., Mendel and Schwartz, 1999). The high solubility of many molybdenum species in water allows it to readily accumulate in all plant tissues (roots, stems, leaves, fruit). However, the sorption of molybdenum to soil or sediment particles strongly affects molybdenum uptake rates by plants. This sorption process is affected by several factors, including pH, ion content of the soil, etc. (Barceloux, 1999b). Thus, accumulation of molybdenum in plants is determined by the contribution from both the water and sediment/soil compartments (e.g., Mendel and Schwartz 1999). It has also been observed that cultivated vegetation shows higher molybdenum levels than natural vegetation grown in similar soils (e.g., Chappell and Peterson, 1976). Laboratory studies revealed a high clay content of soil resulted in lower sorption of molybdenum and greater plant uptake of molybdenum (Eisler, 1989).

In Canada, molybdenum levels in soil vary across small and large scales and are influenced by local and geological factors. For example, soils in of British Columbia are frequently high in molybdenum compared with the other western provinces like Alberta and Saskatchewan. These vagaries in molybdenum content of soil result in differences in the molybdenum content in the resident plants. Estimates of spatial variability of molybdenum content of sediment/soil represent useful information for any SSRA.

In forest habitats of Sweden, Tyler (2005) quantified the molybdenum in the soil, in five species of fungi, and in the leaves of common beech (*Fagus sylvatica*) through the growing season. This study identified how molybdenum concentrations in the soil were unrelated to the concentration in the fungi, suggesting no strong biomagnification pattern for these decomposers. In contrast, the molybdenum in the beech leaves increased through the growing season and had accumulated to about 25% of the concentration in soil by autumn.

Aquatic plants show patterns of molybdenum uptake similar to terrestrial species. For example, the growth of phytoplankton in both freshwater and marine habitats is directly linked to the concentration of molybdenum in the water column. Thus, blooms of phytoplankton assimilate molybdenum, and when these microscopic plants die, the

molybdenum settles out of the water column into the sediments (Howarth and Cole, 1985; Marino *et al*., 2003). In wetland habitats, Kufel (1991) assessed two plants, common reed (*Phragmites australis*) and cattail (*Typha augustifolia*), for uptake dynamics of molybdenum. That study showed molybdenum accumulation in both species with biomagnification patterns that were not correlated with concentrations in the environment, as the plants accumulated higher concentrations than the concentration in the water or sediments. Tissue analyses of both reed and cattail also identified that molybdenum accumulated in the shoots during the growing season and then was likely translocated to the roots and other underground tissues in autumn. Habitats with poor drainage, like swamps, typically have reducing conditions in the water and this often lead to excessive molybdenum in the associated foliage (e.g., Chappell and Peterson, 1976). Cultivation of plants that show hyper accumulation of molybdenum, followed by harvest, represents a viable method for remediation of molybdenum concentrations at some sites.

Biomagnification

The noted studies collectively indicate that most aquatic and terrestrial plants readily bioaccumulate molybdenum, in both wet and dry conditions, in natural and managed environments. An exception to this pattern was cultivated corn (*Zea mays*) grown on soils high in molybdenum, due to biosolids application. This corn showed zero accumulation of molybdenum; no explanation for this observation was offered (O'Connor *et al*., 2001a, b). The pattern of molybdenum bioaccumulation in plants explains why plant tissue concentrations have been observed to exceed 1000 mg/kg when grown in soils with high molybdenum. The transfer factors for marine and freshwater plants range from 100 to 1000, respectively. For example, Sheppard and Thibault (1990) reported a geometric mean Kd for molybdenum ranging from 10 L/kg for sandy soil to 125 L/kg for loam soil. The value for organic soil was intermediate (i.e., 25 L/kg) to the sandy and loam soils. By convention, the sediment Kd values can be approximated by using the organic soil Kd, or by multiplying soil Kd values by a factor of 10 for the appropriate texture type.

Aquatic Invertebrates

The accumulation of molybdenum by invertebrates, especially those associated with sediments, has not been well studied, and deserves further investigation. Peterson *et al*. (2002) assessed the molybdenum concentrations in benthic invertebrates from a stream associated with a former uranium mine in Utah. This study in Utah revealed the tissue from homogenized benthic invertebrates contained residues of molybdenum that ranged from 2-4 times the background, indicating low BCFs. It would have been instructive if the invertebrates were not homogenized. However, these tissue residues were highest at the sampling site closest to the mine. An increase in tissue concentrations between the first and second years of the study were attributed to decreased precipitation during the second year. This study also indicated no negative consequences of the molybdenum exposure on the benthic invertebrates in this stream. A comparable study also quantified the molybdenum concentrations in samples of tissues from homogenized benthic invertebrates

for a stream in New Mexico that receives episodic discharges from a molybdenum mine (Lynch *et al*., 1988). The findings from New Mexico were similar to the results in Utah, as homogenized tissue samples of invertebrates showed low BCFs (Lynch *et al*., 1988). Generally, aquatic invertebrates are themselves initially exposed to molybdenum via three mechanisms. These are:

- direct uptake of dissolved molybdenum from the water column or sediment porewater,
- ingestion of primary producers (algae, plankton, macrophytes), or
- direct uptake of molybdenum in particulate form.

The importance of each mechanism will vary across habitats and seasons. For example, the biomass of plants like macrophytes in lakes during winter will be low compared with the summer (e.g., Eisler, 1989).

Fish

It is largely accepted that the accumulation of molybdenum from the environment by fish occurs primarily through food and water via the gastrointestinal tract while exposure through the gill membranes represents a minor route. In fish, the accumulation of molybdenum occurs throughout the body and concentration varies among tissues, with the highest observed in the organs (e.g., liver, kidney, spleen). A general BCF for fish flesh ranged from 8-45 (dw), and was observed for different species found in freshwater and brackish habitats (Karlsson *et al*., 2002). The removal of excessive molybdenum from the blood via the kidney represents the primary route of excretion and a simple explanation for these relatively low BCFs (Karnacky, 1997).

Birds and Mammals

The uptake of molybdenum in birds and mammals occurs primarily through water and diet in the gastrointestinal tract. Specifically, water-soluble forms of molybdenum are readily absorbed but insoluble forms are not. Following absorption, molybdenum is distributed throughout the body, via the blood, with the highest levels generally found in organs (liver, kidneys, spleen; Opresko, 1993b; Karlsson *et al*., 2002). Like fish, the kidney of mammals and birds readily removes molybdenum from the blood. This excretory pathway efficiently excretes molybdenum when present in high concentrations in the blood. However, the unique digestive systems of ruminants (i.e., ungulates that digest their food with a multi-chambered rumen) of domestic (e.g., cattle and sheep) and wild (e.g., moose and deer) origins does not efficiently excrete molybdenum. The ruminants use a two-stage digestive process that involves eating plants and then regurgitating and chewing a semi-digested form called cud. This digestive strategy makes these species far more susceptible to the toxic effects of molybdenum than other animals (Chappell and Peterson, 1976; U.S. DOI, 1998). Extensive studies of birds and mammals have been completed within the context of understanding diet requirements (Barceloux, 1999b).

Specifically, for ruminants, the concern with molybdenum stems from interactions with copper while food is being digested in the multi-chambered rumen. In the rumen, the thiomolybdates transform to insoluble copper thiomolybdate complexes. This chemical reaction occurs in the unique chemical environment of the rumen and, most importantly, before the excessive molybdenum can be removed from the blood by the kidneys. These copper thiomolybdate complexes become insoluble and this can cause deficiency in copper. This phenomenon has been documented in domestic and wild ruminants, and is referred to as 'molybdenosis' (Chappell and Peterson, 1976; U.S. DOI, 1998; Barceloux, 1999b). Generally, domestic ruminants can show symptoms of molybdenosis when feed contains as little as 2 mg/kg molybdenum while other domestic animals can tolerate feed with molybdenum at concentrations of 1,000 mg/kg. In contrast, wild ruminants do not show such simple exposure:dose relationships because the diet is more variable and can compensate for copper lost to copper thiomolybdate complexes (U.S. DOI, 1998; Barceloux, 1999b; refer to the section 4 (Toxicity) for additional details). By contrast, similar concerns do not exist for those ungulates that do not posses a multi-chambered rumen because they are not susceptible to thiomolybdate transformation.

Mammals like grazing livestock (e.g., sheep) in western habitats of North America have been identified as at risk from molybdenosis. For example, this includes the consumption of molybdenum in water and forage grasses by cattle in southern Manitoba and British Columbia in Canada (e.g., MEND, 2004; also see www.geoscape.nrcan.gc.ca for details on local sites) and the western states of the USA, where molybdenum in soil and plants is naturally high (U.S. DOI, 1998; Barceloux, 1999b; CCME, 2002). Livestock that consume plants with high molybdenum have been observed to suffer copper deficiency and develop molybdenosis (Chappell and Peterson, 1976; U.S. DOI, 1998). To prevent this disease in livestock, copper supplements are provided in the diet, to offset losses due to the formation of copper thiomolybdate complexes (e.g., Chappell and Peterson, 1976). The uptake of molybdenum can also occur through the water consumed by the livestock, as some locales show high water-borne concentrations. In addition, irrigation water used for some plants in these western locales can also act as a significant source of molybdenum in the diet (Chappell and Peterson, 1976; Hren and Feltz, 1998).

Several pathways exist for the accumulation of molybdenum by wild birds and mammals. One common route for these species is through the water to aquatic plant (e.g., macrophyte) to herbivore route for species like duck. A second pathway is through ingestion of water and sediment directly, also typical for species like moose. Other animals that consume aquatic plants, like muskrat, may also be exposed to elevated molybdenum uptake. Additional information is included in section 4 (Toxicity). Because roaming species like duck, muskrat, and moose are not dependent on commercial or artificial food sources, they can be used as representative environmental monitors of ambient environmental molybdenum concentrations. These species can provide focus for SSRAs.

In the last decade, the existence of molybdenosis has been documented in moose from Europe (e.g., Sweden; Frank, 1998) and North America (e.g., Minnesota, USA; Custer *et*

al., 2004). The moose considered in these studies all showed clinical signs of deficiency in copper that was likely caused by elevated consumption of plant material high in molybdenum. Interestingly, the moose afflicted with molybdenosis were found in both natural (bog, forest) and agricultural (farm fields) areas (Frank *et al*., 2000b; Custer *et al*., 2004). In Sweden, Frank (1998) reported that the liming of lakes in response to acid precipitation led to an increase in the accumulation rate of molybdenum in the aquatic vegetation consumed by moose. Thus, the lower pH of the limed lakes modified the partitioning pattern of molybdenum and led to greater bioavailability to herbivores. In contrast, moose sampled in the Yukon, Canada, have not shown any clinical signs of copper deficiency or molybdenosis, likely owing the naturally low molybdenum concentrations in the soils of that region (Gamberg *et al*., 2005). The comparison of moose between Sweden and Yukon is interesting in that the degree of anthropogenic disturbance differs but many other factors are similar. The pattern of increased molybdenum uptake rates in aquatic plants in limed lakes is analogous to the increase in uptake of molybdenum by ryegrass grown on soil that received the addition of lime and fertilizer (Eisler, 1989). Observation of susceptibility of moose to molybdenum has led to the development of a national monitoring program for molybdenum in Sweden (Frank, 2004).

Differences in the molybdenum tissue concentrations were observed for omnivorous and herbivorous dabbling ducks and omnivorous and carnivorous diving ducks sampled in Japan (Mochizuki *et al*., 2002). Specifically, the dabbling ducks showed kidney and liver concentrations of molybdenum greater than 30 µg/g compared with the diving ducks with less than 11 µg/g of molybdenum in the kidney and liver. In addition, the concentration of molybdenum in all ducks correlated closely with the copper concentration. These toxicity pathways for various domestic and wild species led to the careful development of water quality guidelines in Canada for molybdenum in surface water, drinking water, and water used in agriculture (e.g., CCME, 2002; also see www.ccme.ca).

4.0 TOXICITY

Measures of toxicity, ultimately the primary consideration in the determination of regulatory guidelines or limits and the basis for benchmarks applied to environmental assessments, are of four major types, as follows:

- the concentration of the COPC (i.e., external exposure) that constitutes the lethal dose (internal exposure, LD) in x% of the test organisms;
- the LD can also be represented as the critical body residue associated with a response such as growth depression or death;
- the effect concentration (EC) of the COPC resulting in a specified level of sub-lethal *effect* (e.g. a percentage reduction in reproduction) in x% of the test organisms;
- the Lowest Observed Adverse Effect Level (LOAEL), that is the lowest of the series of actual test doses that does cause a statistically significant adverse effect; and
- the No Observed Adverse Effect Level (NOAEL), which is the highest COPC dose in an actual test series that does not cause a statistically-significant adverse effect.

Estimated No Effect Value (ENEV)

While the LD-x, EC-x and LOAEL values are based on measured effects, NOAEL values have a less definitive meaning because “no effects” can be observed at many concentrations less than the LOAEL value, for example. For this reason, NOAEL values are estimated by either careful testing with small increments of dose or they are estimated by application of a safety factor to a known effects level. In theory, the true threshold for the particular adverse effect lies between the NOAEL and the LOAEL (Rand *et al*., 1995).

A related theme is that the use of LOAEL and NOAEL for setting limits requires caution. Such caution is a reflection that this process is extending the findings (e.g., LOAEL) from a laboratory to a field situation, and may also include the use of an application factor. The suitability of application factors to fully represent dose-response relationships requires careful consideration (e.g., CCME, 2002). This extension of a LOAEL with an application factor can result in incorrect conclusions about concentrations that may be expected to result in no effects. For example, site-specific biological or physical features may reduce the ability of the laboratory study to accurately represent the behaviour of the COPC in the environment. Similarly, biological or physical features of an environment may inhibit the bioavailability of a COPC and result in the LOAEL to be viewed as overly protective.

Each of these endpoints can be considered as the basis for either the assessment of site-specific risks or in establishing broadly protective limits. The most protective approach in either case is to use the NOAEL. For regulatory guidelines or criteria, the NOAEL is often

adjusted downward through the application of a safety factor to ensure species that are possibly more sensitive than the test organism(s) are protected. Hence, LOAELs can be used in a similar manner, with higher safety factors typically used. Also, EC-x values for appropriate endpoints can also be used, typically with an x value of 10 or 20. Such application of EC-x typically considers reproductive endpoints with the rationale that protecting 80-90% of the species or population from some degree of reproductive impairment will protect the populations as a whole. The LD-x values can be considered in a similar manner, typically when sub-lethal data are unavailable or inconclusive. For LD-x values, safety factors are usually applied, and values of x, again, are usually 20 or less.

In the assessment of potential ecological impacts, effects on reproduction are very important to consider as they may have significant population-relevance. As a general rule, levels of contaminant exposure that could seriously affect survival rates of adults are much higher than levels that might impair some aspects of reproduction. Frequently, the most vulnerable life stages of species are the youngest, sometimes also including the egg stage (e.g., Rand *et al*., 1995). Also, exposure at the lower levels may not have immediate impacts on existing sexually mature specimens, but could have adverse effects on the long-term viability of the population of a given species. For species of special status (rare, threatened, endangered) the implications are potentially critical. For this reason, an understanding of potential reproductive effects of a COPC is a key consideration in establishing guidelines for environmental protection (Josephy, 2006). Such concerns have motivated the consideration of entire life cycles for species and industrial products across habitats. For example, the U.S. EPA promotes life cycle assessment to more fully assess potential human and ecological effects stemming from environmental releases (U.S. EPA, 2006; also see www.epa.gov/ORD/NRMRL/lcaccess/ for additional information).

Behaviour of selenium and molybdenum in the environment is consistent with most trace elements. That is, those trace elements identified as potentially toxic substances interact with several other chemicals that act as either synergistic or antagonistic factors (e.g., Rand *et al*., 1995). In this case, synergistic behaviour refers to enhancement of the toxic effect and antagonistic refers to a counter effect to the reported exposure pathways and toxicity.

4.1 Selenium

4.1.1 General Aspects

Selenium has been well studied from the perspective of its potential to have harmful effects on the environment and human health (WHO, 1986; FAO and WHO 2001; ATSDR, 2003). The mechanism by which selenium exerts toxic effects is unknown, but it is likely due to the similarity with sulfur, and that selenium can substitute for sulfur during the formation of proteins. For this reason, the rate of bodily incorporation of selenium is relatively high in cells and tissues where protein formation occurs at relatively high rates, such as reproductive tissues. In general, the levels of selenium that might cause harmful effects are relatively low, to the point where even naturally occurring concentrations of selenium may

approach levels of concern (e.g., Lemly, 2004c). It is also evident that naturally occurring selenium can lead to patterns of adaptation and/or tolerance (e.g., Kennedy *et al*., 2000). Thus, the separation of natural from anthropogenic sources is a key step in any site assessment (e.g., Nordbeg *et al*., 2000). As noted, it is useful to also estimate the TMDL at a site, if data exist (after Lemly, 2001a).

Selenium is, however, also an essential trace element to animal life, being an integral part of enzymes and other proteins that are crucial for metabolic processes. The difference between selenium doses that are considered essential and those that are possibly toxic is very narrow. For example, the NRC (1976) suggested that the generic range between sufficiency and toxicity for selenium is in the order of 100-fold (i.e., 0.05 mg/kg to 5 mg/kg) for different terrestrial animal species, based on laboratory studies. Demayo *et al*. (1979) estimated that there is only a 50-fold safety margin between recommended and toxic dietary concentrations of selenium for animals. In a review of dietary selenium status of various forms of livestock, McDowell and Conrad (1979) reported the dietary concentrations of 0.1 mg/kg (dw) selenium are generally sufficient, while the toxic threshold is in the range of 5 to 10 mg/kg (dw) for most common livestock. The current understanding of human nutritional requirements also suggests a relatively narrow gap between adequate levels of selenium and those that may be toxic. For example, the U.S. FDA has established a recommended daily allowance of 55 µg/day selenium for nutritional sufficiency in humans. The upper tolerable daily intake is 400 µg/day, less than 10 times the sufficiency dose. Similarly, Health Canada (1992) has adopted the same recommendations. In contrast, Maier *et al*. (1987) suggest that the margin between sufficient and toxic levels for aquatic life is approximately 10-fold.

There are several key factors that may determine the status (deficient, sufficient, toxic) of environmental concentrations of selenium with respect to animal life. These include:

- chemical form – the form of selenium (selinite, selenate, selenomethionine, etc.) appears to play a key role in its toxicity (e.g., Simmons and Wallschläger, 2005);
- availability – degree of binding and transformation with sediments, soils, and rocks and this may change from one season to the next (e.g., Malisa 2001);
- importance of seasonal variability to the toxicity of selenium. For example, selenium can be mobilized from the tissues of species such as fish during periods of starvation that typically occur during the temperate winter (e.g., Lemly, 1993a,b, 1997)
- antagonistic substances in the environment – for example, arsenic and sulfate in water or soil can offset effects of certain forms of selenium (non-organic forms for arsenic, and primarily selenate for sulfate), but not all forms (e.g., Eisler, 1985; Josephy, 2006);
- nutritional factors – the likelihood of selenium effects is reduced with high dietary status of certain proteins, minerals, and also vitamin E (e.g., WHO, 1986);

- adaptation - several studies have shown that the rate of tissue incorporation of selenium, and associated toxicological symptoms, can decrease in animals with an exposure history (including generational history; ATSDR, 2003); and
- tolerance – recent studies have pointed to the view that animals with an exposure history are actually showing acclimation and adaptation. It is highly probable that acclimation is associated with metabolic costs but it is not readily apparent if adaptation comes with a quantifiable cost (Chapman, 2007).

Overall, the capacity for environmental selenium to induce toxic effects in any given exposure scenario is a function of interactions involving multiple determinants. Nonetheless, the current state of understanding of selenium toxicity still enables the establishment of general toxic thresholds for major taxonomic groupings of biota. These complex interactions necessarily require integrated site assessments to facilitate consideration of all available information (McDonald and Chapman, 2007).

4.1.2 Aquatic Biota

The toxicity of selenium to fish has been well-researched and there is a general regulatory consensus as to what levels of exposure may cause adverse effects directly to aquatic biota. A brief synopsis of selenium toxicity to aquatic biota is provided herein for consideration in determining environmental management criteria. As stated previously, there is a broad contention that concentrations of selenium in the aquatic environment may, under some circumstances, present a potential risk to terrestrial biota while not exceeding toxic thresholds for either aquatic plants or animals.

A review of acute data (LC50) available from the U.S. EPA ECOTOX database (see Table 4.1), shows that there is considerable variability in the toxicity of selenium among various species that have been studied. This data suggests that acutely lethal effects typically occurs when selenium concentrations in water approach 500 µg/L (0.5 mg/L) or more. An important consideration is that the data for amphilibians is very limited in terms of studies on species and across habitats, but generally suggests that they may be more sensitive to selenium than fish or invertebrates.

The British Columbia aquatic life guideline for selenium was based in part on the review by Nagpal and Howell (2001) of the results of laboratory and field toxicity studies of a range of invertebrates and fish in both marine and freshwater environments. The acute and chronic values considered in that review are presented (Table 4.2, Nagpal and Howell, 2001). This synthesis allows the following general points of understanding of selenium toxicity to aquatic biota to be noted:

- For all major types of aquatic biota, both the acute and chronic toxicity thresholds tended to vary over a range of two orders of magnitude or more.
- Studies of amphibians and reptiles seem to be limited and so the estimated toxicity thresholds should be viewed with caution.

- For fish, the acutely toxic concentrations (LC50) of selenium were typically in the range of 1,000 to 100,000 µg/L (1 to 100 mg/L).
- Chronic toxicity thresholds for fish were about 10-fold lower than acute, typically in the range of 100 to 10,000 µg/L, with a lowest chronic value of 5 µg/L (0.005 mg/L).
- Invertebrates appeared to be slightly more sensitive than fish to the effects of selenium. The ranges of both acute and chronic values overlapped largely with those of fish, but were shifted slightly downward.
- The lowest chronic value for invertebrates was 2 µg/L (0.002 mg/L).

In the recent update of the water quality criterion for protection of aquatic life, the U.S. EPA (2004a) conducted a comprehensive review of available selenium toxicity data for aquatic biota, both freshwater and marine. The review process initially identified data for both invertebrate and fish test organisms that were deemed suitable for derivation of toxicity criteria and benchmarks. The evaluation of acute data distinguished between selenite and selenate. From the selected data for exposure, major derivations included:

- SMAV species mean acute values, representing the mean of acute values (mostly LC50 values) for each species sufficiently represented in the data; and
- final acute values – derived from the most sensitive 5th percentile genus.

In the consideration of chronic data, it was recognized that food is the primary pathway of exposure, and that it is most effective to establish criteria that are based on tissue concentrations of total selenium rather than ambient media concentrations of various selenium species. Data sets in which both effect measure and tissue concentrations were adequately reported were considered in criterion development. For the suitable data sets, genus mean chronic values and a final chronic value were presented. These were reflective of EC20 values, primarily for growth related endpoints. The chronic values were corrected to 100 mg/L sulfate, since selenium toxicity has been shown to have a significant negative correlation with sulfate concentrations in the water column. The major values for acute and chronic exposure reported by the U.S. EPA (2004a) are summarized in Table 4.3. From the U.S EPA review and conclusions, several broad aspects of selenium toxicity to aquatic biota can be identified, as follows:

- under acute exposure conditions, the data suggest that selenite is more toxic to freshwater aquatic biota than selenate;
- the level of selenium in water that is acutely toxic to aquatic biota is highly variable and depends on the forms of selenium and species of biota;
- the acute toxicity data considered by the U.S. EPA suggests a greater variability in the sensitivity of invertebrates compared to that of fish;

- resolution of toxicity responses of amphibians and reptiles relative to fish and invertebrates requires further study; and
- marine organisms appear to be more sensitive to selenium toxicity than freshwater biota.

Overall, the toxic effects of waterborne selenium to aquatic biota vary significantly depending on the species and the characteristics of the receiving environment. As a general rule, the toxic potential does not appear to be substantially affected by the form of selenium in the water column. From the available data, lowest chronic values range from 2 to 5 µg/L (0.002 to 0.005 mg/L), and this would represent the threshold levels at which there would be concerns for possible impacts on aquatic biota in the receiving environment. However, the toxicological data suggest that measurable effects might not be seen at concentrations considerably higher than this, depending on the aquatic species assemblage and other site-specific conditions. There is less variability in the fish-tissue threshold that have been proposed by the U.S. EPA (U.S. EPA, 2004a), as the uncertainty in the degree of uptake from ambient water is factored out. The tissue thresholds reported by the EPA in support of the criterion value (7.91 µg/g dw) suggest that effects on fish could occur when fish tissue concentrations reach the range of ~10 to 20 µg/g (dw).

Freshwater fish ecology and physiology are known to determine the possible consequences of selenium exposure (e.g., Brix *et al*., 2000). Specifically, contrasting fish ecology, like feeding habits and migration patterns can lead to differences in the concentration of microelements like selenium in tissues for different species from the same habitat (Evans, 1993; Naiman and Latterell, 2005). Indeed, species-specific habitat use represents one explanation of the differences noted for selenium concentrations between the bottom-dwelling or benthic lake whitefish (*Coregonus clupeaformis*, family Salmonidae) compared with the open-water northern pike (*Esox lucius*, family Esocidae) for fish studied in northern Saskatchewan (see Toll, 2005). That is, lake whitefish with an estimated BAF for selenium of 5944 are frequently in contact with, and consume sediment. By contrast, northern pike, with an estimated BAF for selenium of 2972 (EcoMetrix 2005a), are not usually in contact with, and rarely consume sediment (e.g., Becker, 2001). Similarly, the role of temperature on enzyme functioning, growth, and other metabolic endpoints indicates that selenium accumulation and depuration will differ between fishes located in northern compared with southern locales. Comparable influences of climate (e.g., coastal compared with mid-continent) can also influence fish ecology and physiology and should be considered during population assessments (Naiman and Latterell, 2005). Thus, fishes located in coastal Newfoundland will likely show different responses to selenium exposure compared with coastal Nunavut and central Manitoba. Taken together, such considerations identify mechanisms on why the toxic effects of selenium have been identified as inconsistent across species and habitats in North America. Similarly, these mechanisms can act to explain regional differences in terms of expected versus observed selenium concentrations in fish tissue relative to observed water concentrations.

As noted, the selenium in fish tissue can become mobilized during the winter starvation period. Similarly, exposure of fish during the winter to water with high selenium concentrations can also lead to toxicity responses that differ compared with the warmer times of the year. Such a process was previously referred to as Winter Stress Syndrome (WSS; Lemly, 1993a,b). Generally, WSS refers to mortality caused by exposure to contaminants during the winter months and attributed to metabolic functioning of the fish during winter. Such mortality can be substantial and has been observed to change population structure (e.g., dramatically reduce the density of cohorts of fish) and also strongly influence community composition. When such changes occur in aquatic ecosystems, the typical ecological interactions are not observed. Thus, it is important to consider the role of season, particularly winter, in hazard assessment (e.g., Lemly, 1997). It is interesting to note that it was primarily the identification of WSS that led the U.S. EPA to change the original (2002) draft selenium criterion from 7.91 µg/g to a value of 5.85 µg/g if fish sample assessment is not conducted in the winter and 7.91 µg/g if sampling is in the winter. Related information is presented in Section 5.0.

4.1.3 Terrestrial Biota

In the context of managing releases to the aquatic environment, the toxicity of selenium to terrestrial biota is a concern, specifically for animals with a close association with aquatic ecosystems. Based on some key case studies (e.g., Kesterson Reservoir, Lemly and Smith, 1987; also see Section 5.0), the wildlife that may be affected by selenium in the aquatic environment includes birds and, to a lesser extent, mammals, that obtain a significant fraction of their dietary intake by consuming components of aquatic food webs.

The understanding of selenium toxicity to terrestrial animals can be derived from three principal sources:

- research regarding the status of selenium as both an essential element and potential toxicant in *human* nutrition;
- information compiled in response to the long-standing recognition and concerns regarding the role of selenium in the health of domestic *livestock;* and
- more recent and focused studies regarding the possible environmental implications of selenium to *terrestrial* wildlife.

Assessment of selenium toxicity to terrestrial animals is reasonably well-developed owing to its role in livestock health. The long-understood significance of selenium poisoning of domestic livestock has lead to research on both the acute and chronic effects of selenium in laboratory animals. Additionally, human health concerns regarding long-term selenium exposure have resulted in the compilation of considerable lab animals test results. However, Chapman and Wang (2000) recognized that substantial caution is required when assessing toxicological impacts on wild animals through the use of surrogate tests conducted on domestic or laboratory animals.

Understanding of selenium toxicity to terrestrial species recognizes that the most relevant information regarding sensitivity of wildlife is obtained from direct field studies. However, the coverage of such data to date is not complete, and what is available is not without associated bias and uncertainties. For example, these studies have not fully evaluated the seasonality of exposure. The best estimates of selenium toxicity to terrestrial wildlife are likely obtained through consideration of the various sources of complementary information in a weight-of-evidence manner. This approach will readily fit within a typical ERA process.

As noted, a number of recent studies have directly investigated the potential toxic effects of exposure of wildlife species to selenium in the environment. The common consideration in most of these studies is the potential for selenium in the aquatic environment to have adverse effects on terrestrial biota. A review of field and laboratory data (Lemly and Smith, 1987) indicated that selenium at concentrations greater than 2 µg/L (0.002 mg/L) in water could undergo foodchain bioaccumulation to the point of having toxicological implications.

Another common element of these studies is the focus on tissue concentrations of selenium as the basis for determination of toxic thresholds. This approach has great merit, in that the toxicity of selenium or any other COPC in the environment is partly dependent on the amount of that COPC that is actually absorbed into the tissues where the toxic mode of action occurs. This negates some of the uncertainty associated with thresholds that consider the ambient concentration or the ingested dose of the COPC. However, in pre-emptive environmental management efforts, the tissue-based values present considerable uncertainty. This is due to the fact that it is very difficult to quantify the amount of selenium that will end up in animal tissue following any given release to the environment.

Birds

The impacts of selenium on birds have been significantly characterized in the interest of protection of avian livestock. The actual occurrence of widespread impacts is well documented, such as concurrent observations of avian livestock effects in the areas of China where impacts to human health as a result of exposure to naturally elevated levels of selenium were found to occur (WHO, 1986).

As a coarse indicator of the relative sensitivity of avian receptors to selenium, an overview of livestock trace element requirements (NRC, 1976) suggests that the selenium tolerance of chicks is higher than that of mammalian livestock. In a related study, an assessment of the effects of dietary mineral supplements on the health of chickens over a two week period was completed (Hill, 1974), and found a substantial growth reduction of chickens fed diets containing selenium at concentrations of 10 mg/kg (dw) or higher. Mortality occurred when dietary levels of selenium reached 40 mg/kg. In later studies to assess mineral toxicities, Hill (1979a,b) found significant reductions in weight-gain by birds provided with a diet containing 5 mg/kg or more selenium over a period of 4 to 5 weeks. This growth response was found to be more pronounced as the period of exposure increased (Hill, 1980). Maurice and Jensen (1979) did not report any significant effects on growth or reproductive

capacity (as indicated by egg weight) of domestic chickens exposed to a 0.1 mg/kg selenium diet over an 8-week period. This early work with domestic birds suggests that thresholds for some level of sub-lethal effect on adult birds due to dietary selenium are somewhere in the order of 1 µg/g (>0.1 but less than 5 mg/kg).

Several studies have examined the effects of dietary selenium on reproduction and development of waterfowl, such as mallards (*Anas platyrhynchos*). In a frequently cited study by Heinz and Hoffman (1987), mallards were exposed to diets containing 1 to 100 mg/kg selenium as selenite, and 10 mg/kg as selenomethionine. The 100 mg/kg selenite diet was ultimately lethal to all ducks exposed to that diet. The 25 mg/kg was lethal to one experimental animal, and otherwise resulted in reduced growth, a delay in egg laying, and reduced hatchling survival compared with the control groups. In addition, mallards fed 10 to 25 mg/kg selenite and 10 mg/kg selenomethionine produced significantly more deformed embryos than experimental controls. The 10 mg/kg selenomethionine diet produced slightly greater numbers of defects than the same dietary concentration of sodium selenite. Diets containing less than 10 mg/kg selenium, in any form, did not result in measurable effects. This would be considered the NOAEC in this study.

Based on results of the 1987 study, Heinz *et al*. (1989) conducted a follow-up study, feeding mallards 1 to 16 mg/kg selenomethionine and 16 mg/kg selenocysteine. The hens fed selenium as selenomethionine at concentrations as low as 8 mg/kg produced fewer hatchlings and had reduced survival of hatchlings. There was no significant effect on reproduction resulting from a diet of selenocysteine.

Lemly and Smith (1987) reviewed a number of studies available at the time and suggested that dietary levels of selenium as low as 3 mg/kg (dw) could have adverse effects on wildlife, as could concentrations in water in the range of 2 to 5 µg/L (0.002 to 0.005 mg/L). The latter range was based on the study by Heinz and Hoffman (1987).

In wake of the discovery of selenium-related impacts on waterfowl in agricultural drainage basins in the western U.S., considerable research has been completed to provide a quantitative understanding of the levels of selenium exposure at which measurable impacts may occur. The core focus of this research has been on the potential effects of selenium for avian health. Effects on reproductive capacity (egg number, viability), chick mortality and teratogenic effects have been the primary focus in the majority of these studies.

In a field study, an estimated NOAEL for mean selenium concentrations in egg of 0.9 to 1.45 mg/kg (dw) was derived, and this reflects certain limitations with regards to the study data (Lam *et al*., 2005). Latshaw *et al*. (2004) reported no effects on embryonic development of pheasants at 2.05 mg/kg, but a slight reduction in hatchability at 3.0 mg/kg (dw). Other studies show much higher thresholds for selenium. For example, Hoffman (2002) conducted field studies to examine the potential effects on the reproduction of two species of wading birds. The study found that mean concentrations as high as 190 µg/L (0.19 mg/L) in the water did not translate to any consistent impairment of reproduction.

Neither hatching success nor the incidence of malformations was affected by elevated selenium levels in water, despite an observed mean tissue range of 20 to 30 mg/kg for the two study species when exposed to the highest ambient selenium concentration (190 µg/L or 0.19 mg/L). While hepatic biochemical indicators and hatchling liver weight did show some evidence of toxicant-induced stress, the findings were mostly inconsistent between the two study species and did not translate into meaningful effects. Evidence exists that the LOEAL threshold may be higher in other bird species.

At present, the U.S. Fish and Wildlife Service (U.S. FWS) recommend an egg selenium threshold of 6 or 7 mg/kg (dw) to protect against adverse effects on avian populations. However, a subsequent review suggested that this threshold may not be reliable, owing to statistical limitations and confounding factors that were noted during the field study that generated the result (U.S. EPA, 2004a). In a comprehensive review of avian selenium toxicity, Adams *et al*. (2003) re-examined the field and lab data that largely served as the basis for the selenium chronic toxicity thresholds previously recommended. In that review, the 10% effect concentrations (EC_{10}) for various critical reproductive endpoints (teratogenic effects, nonviable eggs, hatchling or embryonic mortality) ranged from 12 to 37 mg/kg (dw) selenium in eggs. Adams *et al*. (2003) recommended that 12 to 14 mg/kg (dw) selenium in eggs be considered as a reliable and conservative chronic threshold. This is an area of active investigation and deserves careful consideration of the most current literature if bird species are of concern at a site.

Reptiles

In general, the uptake and transfer of selenium to developing young of oviparous vertebrates has been observed in all major taxonomic divisions (fish, reptiles, birds). This relationship varies, likely depending on the ambient environmental conditions at a given site, along with differences in physiology, ecology and life history among exposed species. Reported toxicity thresholds for selenium in eggs or hatchlings generally fall in the 5 to 15 mg/kg range. Most of the studies relate to birds, but some data are available for reptiles.

A study by Roe *et al*. (2004) reported the transfer of maternal selenium body burden to alligator eggs and hatchlings at a selenium-contaminated site. This analysis revealed both egg and hatchling selenium tissue burdens were fairly consistent, averaging around 7.5 mg/kg (dw). In that study, egg viability was reduced with elevated levels of selenium compared with reference eggs from a nearby uncontaminated site. However, selenium could not be conclusively identified as the cause. The selenium levels for this study were noted as lower than U.S. FWS thresholds for other oviparous vertebrates (i.e., birds).

A recent study of accumulation of selenium by lizards reported that body burdens at about the same level as in the primary dietary constituent (insects; Hopkins *et al*., 2005). This food web link involving selenium identifies a key pathway between plants, invertebrates, and vertebrates. The corresponding level of selenium in ovarian tissues of lizards was found to be higher than whole-body measures. At concentrations approaching 15 mg/kg

(dw), no effects on either survival or sub-lethal endpoints were observed in these lizards. This suggests that reptiles exhibit sensitivity to selenium that is comparable to birds.

Mammals

An ecotoxicological profile for selenium has been developed as part of the Oak Ridge National Laboratory Risk Assessment Information System (RAIS). The profile considers laboratory studies assessing both lethal and sub-lethal toxicity of selenium to mammals. In consideration of the acute lethal toxicity data available from RAIS, studies of various laboratory animals have identified LD50 values for selenite and selenate typically in the range of 1 to 10 mg (per kg of body weight in a single dose). It has been found that the relatively soluble forms (e.g., selenite, selenate, selenomethionine) have a relatively low LD50 (<10 mg/kg), but for organically bound and relatively insoluble forms (e.g., methylated selenides), the acute LD50 can be as much as two orders of magnitude higher. Elemental selenium is highly insoluble and is relatively non-toxic (LD50 ~7000 mg/kg).

Specific results of chronic toxicity assessments encompassed in the RAIS profile include the following:

- a chronic NOAEL of 0.61 mg/kg/d (selenite) for sub-lethal effects (growth reduction, liver impairment) in hamsters;
- a chronic NOAEL of 17.8 mg/kg/day (selenium sulfide) for sub-lethal effects (liver necrosis) in rats;
- a chronic NOAEL of 216 mg/kg/day (selenium sulfide) for sub-lethal effects (renal effects) in mice;
- a chronic NOAEL of 0.42 mg/kg/day (seleniferous wheat) for sub-lethal effects (growth reduction) in rats;
- a chronic LOAEL of 0.5 mg/kg/day (dw) in diet (selenite) for sub-lethal effects (10% reduction in body weight) in rats;
- a chronic LOAEL of 0.38 mg/kg/day (selenite) for sub-lethal effects (liver lesions) in mice;
- a chronic LOAEL of 0.56 mg/kg/day (selenite) for lethal and sub-lethal effects (growth reduction, organ damage) in mice;
- a chronic NOAEL of 0.42 mg/kg/day (selenate) for impaired reproduction in mice;
- a chronic effect level (i.e., EC-20) of 0.42 mg/kg/day (selenite) for impaired reproduction in mice. Study duration was multigenerational;
- a chronic NOAEL of 7 mg/kg/day (selenite) for reproductive effects in mice. Study duration was 7 days; and

- an upper chronic NOAEL of 4.5 µg/g (dw) (selenite) for reproductive effects in rats.

These results suggest that the threshold for mammalian selenium toxicity is in the order of 0.4 mg/kg/d. However, higher doses (>5 mg/kg/day) can be tolerated depending on the duration of exposure and the form of selenium. Selenite and organically bound selenium (e.g., seleniferous wheat) appear to exhibit relatively high toxicity to mammalian receptors, while inorganic and insoluble forms (e.g., selenium sulfide) have a much lower toxicity.

A review of selenium toxicity was conducted by Opresko (1993a) for the purpose of establishing reference exposure doses for humans. The study considered acute, sub-chronic and chronic data for both humans and animals, including several key studies on reproductive effects in animals. The human reference dose determined in this review was 0.005 mg/kg/d. This dose was determined by applying a safety factor of 3 to a NOAEL of 0.015 mg/kg/day taken from a human epidemiological study. The corresponding LOAEL was 0.23 mg/kg/day, and both the NOAEL and LOAEL assumed an average body weight of 55 kg. Owing to the high degree of similarity between human and mammalian physiology, these human exposure thresholds could be extrapolated to derive thresholds for animals.

In addition to the human data, Opresko (1993a) reviewed several studies assessing chronic toxicity of selenium, as selenite, to lab animals. Most of the studies involved single dose ingestion, and effects were reported for doses ranging from 0.173 to 0.57 mg/kg/day. One study with multiple levels of dose of selenite to mice over a two-year period revealed a NOAEL of 0.84 mg/kg/day.

In establishing a reference dose for the protection of human health, the U.S. EPA (2004b) reviewed a significant body of toxicological data for mammalian organisms exposed to selenium. From that review, several key considerations can be identified:

- the toxicity of selenium is variable and dependent on chemical form. Organically complexed selenium may have greater effects than inorganic forms (e.g., selenite);
- selenium is known to have effects on reproduction and embryonic development of birds and animals;
- the potential for adverse impacts of continuous exposure may accrue over generations; and
- NOAELs in the cited animal studies ranged from 0.04 to 0.39 mg/kg/day.

The comprehensive review of selenium toxicity data by ATSDR includes a toxicological profile for selenium (ATSDR, 2003). In that review, the range of NOAELs associated with the cited studies was 0.024 to 9.4 mg/kg/day for a variety of mammalian test organisms. This range reflects multiple forms of selenium (selenite, selenate, selenomethionine, selenecystine, and unspecified organic forms) and multiple sub-lethal endpoints under

intermediate or chronic exposure. The ATSDR review also ranks LOAELs from "serious" (typically mortality) to "less serious" (sub-lethal) effects. The LOAEL range for less serious effects under chronic or intermediate exposure duration was 0.055 to 9.4 mg/kg/day.

In revising their recommendations for toxicological exposure benchmarks for wildlife, Sample *et al*. (1996), made specific revision to the benchmark for selenium to reflect an updated understanding of its toxicological potential. The review considered six chronic studies, each with reproductive measures as the endpoint(s) of concern. All exposure was oral (dietary) and involved both organic and inorganic forms of selenium. In the ultimate determination of a toxicity benchmark for wildlife, Sample *et al*. ruled out the results of several studies as unreliable for benchmark determination. Ultimately, they identified an LOAEL of 0.33 mg/kg/d and an NOAEL of 0.2 mg/kg/d, based on the findings of Rosenfeld and Beath (1954). That study exposed the test organisms to selenium in the form of selenate. Sample *et al*. did differentiate effects related to various forms of selenium in defining the noted benchmarks, and NOAELs and LOAELs are available for various forms.

Overall, the various sources of information regarding the mammalian toxicity of selenium suggest that the toxic potential is dependent on the form of selenium and on the species of mammal, as expected. For both acute and chronic effects, there is at least a 10-fold range of variability in the various NOAELs and LOAELs reported from different sources (Josephy, 2006). The threshold dose for lethal effects appears to be ~1 mg/kg/day, while lowest chronic doses reported to induce sub-lethal effects are in the order of 0.05 mg/kg day. The lowest NOAEL and LOAEL values of relevance to mammalian exposure are 0.015 and 0.23 mg/kg/day, respectively, from a human epidemiology study. An intermediate value could be used to derive conservative dose benchmarks (body-weight adjusted) for wildlife receptors to be considered in impact and risk assessments.

4.1.4 Toxicological Interactions

The toxicity of selenium in the ambient environment may be subject to reduction or enhancement owing to interactions with other ambient substances. Existence of selenium in the environment can also alter the toxic effects of other elements. For example, selenium is toxicologically antagonistic to cadmium, mercury, silver and thallium. Arsenic and selenium also counteract the toxic effects of each other in most forms, although arsenic and selenium may act synergistically if selenium is present in certain methylated forms (e.g., Levander, 1977). In general, selenium will bond with arsenic, cadmium and mercury and limit their toxic reactivity as an evolved detoxification mechanism in animals (Gailer, 2002). There is also reciprocation, and the toxic effects of selenium may also be offset by arsenic, cadmium, mercury, and possibly manganese (Moller, 1995).

The antagonistic effect of selenium on the toxicity of mercury has been studied extensively in many species. This interaction is still an area of active research, but appears to be species dependent. Selenium apparently reacts with mercury within the organism and detoxifies mercury via the creation of an inert mercury-selenium complex or facilitates its

excretion (Hoffman, 2002). An interesting application of this phenomenon was demonstrated by adding selenium to a mercury-contaminated lake for a period of 3 years. The results were an approximate 10-fold reduction in the tissue concentrations of mercury in the resident northern pike (Paulsson and Lundberg, 1989).

In some instances the interactions of selenium with specified elements are not clear or consistent. Heinz and Hoffman (1998) completed a study to examine interactions between mercury and selenium with respect to effects on avian reproduction. In that study, they found that the addition of selenium (10 mg/kg dw as selenomethionine) to the feed largely ameliorated the symptomatic effects (leg weakness) of dietary mercury (10 mg/kg dw as methylmercury) on adult male mallards. This is consistent with the results of numerous studies that suggest an antagonistic relationship between mercury and selenium. However, in the same study, the presence of both selenium and mercury in diet had greater negative effects on reproductive endpoints (teratogenesis, percentage of eggs hatched, number of young produced) than did either selenium or mercury in isolation. The measured concentrations in the eggs of mallards exposed to the selenium-only diet was 7.6 mg/kg (dw), associated with a statistically significant reduction in the number of young. For the subject group provided with the selenium-mercury diet, the tissue concentration was slightly higher (i.e., 9.2 mg/kg) but the degree of reproductive impact was notably higher with regard to all endpoints. This study, using a form of selenium more representative of exposure that may occur in the wild, suggest that the dynamics of interaction of selenium and mercury are more complex than might be indicated by lab studies using inorganic forms (selenite or selenate).

Selenium toxicity to fish and invertebrates has also been shown to have a significant negative correlation with sulfate concentrations in the water column (U.S. EPA, 2004a). The similarity in selenium and sulfur chemistry appears to be responsible for this effect, because sulfate (SO_4^{2-}) would compete for sites and metabolic processes that could also involve selenate (SeO_4^{-}), for example. The uptake of selenium by plant life is also inhibited in the presence of ambient sulfate, lowering the potential for toxic effects on the plants and animals that eat them.

In any given scenario, the presence of other chemical substances may result in different toxicological responses than what might otherwise be expected. Thus, the short and long-term history of a site needs to be considered in any assessment (e.g., Nobbs *et al*., 1997).

4.1.5 Summary

In summary, there are several general conclusions regarding the toxicity of selenium to animal life, as follows:

- Food is the most important route of, or pathway for, selenium exposure leading to potential toxic impacts.
- The toxicity of selenium is variable and dependent on chemical form, and will be shaped by interactions with sediments, soils, plants, and animals.
- The organically-complexed forms of selenium found in food items tend to have greater toxic effects than inorganic forms.
- All classes of aquatic biota exhibit a comparable susceptibility to selenium toxicity, with LOAELs in the range of 2 to 5 µg/L (0.002 to 0.005 mg/L) in the water, but commonly much higher for many species in other exposure settings.
- Examples exist where biota has been exposed to water with 2 to 5 µg/L of selenium and no adverse effects have been observed.
- In contrast, concentrations of selenium in the water column exceeding 2 µg/L may also have adverse effects on birds and animals as a result of food web magnification, and this process reinforces the need for site-specific studies.
- The threshold dose for mammalian selenium toxicity is in the order of 0.05 mg/kg/d. However, higher doses (>5 mg/kg/day) can be tolerated depending on the duration of exposure and the form of selenium involved.
- Reproductive effects are typically limiting, especially for birds, and egg selenium concentrations have been identified as an effective measure of risk. The toxicity thresholds for selenium in eggs range from 5 to 15 mg/kg (dw).
- Evidence of differences in responses to selenium exposure between northern and southern locales exists, and identifies that temperature and seasonality should also be considered in any SSRA for selenium.
- The history of contamination at a site can complicate the expected toxicological relationships, and should be considered in any assessment.

4.2 Molybdenum

4.2.1 General Aspects

The literature contains an extensive list of references to molybdenum toxicity. There is no question that molybdenum can cause health and reproductive effects and even death in livestock and wildlife, from excessive doses, commonly referred to as molybdenosis. In contrast, there is little to no risk to aquatic organisms, such as fish, for water concentrations of several tens to hundreds of mg/L of molybdenum. The primary concern related to

molybdenum toxicity is more importantly focused on what the safe dose or appropriate benchmark for the protection of animal species should be. It is clear that some ruminants, specifically domestic livestock and wild species like moose, are more sensitive to molybdenum than other species. This discussion, therefore, considers aquatic species but focuses on semi-aquatic and terrestrial species because these latter species have a higher risk due to the uptake of molybdenum stemming from the consumption of water, sediment, soil and plant tissues that potentially contains high concentrations of molybdenum. This list includes herbivores (e.g., cow and moose), and semi-aquatic rodents (e.g., muskrat).

In the environment, as noted, molybdenum occurs as various complexes, and the degree of toxicity is dependent on the specific form that is present. The physical and chemical state of the molybdenum, route of exposure, and compounding factors such as dietary copper and sulfur levels all affect toxicity. Generally, insoluble forms like molybdenite is practically nontoxic whereas soluble molybdate can be chronically and/or acutely toxic.

4.2.2 Aquatic Biota

An evaluation of the toxicity of molybdenum to aquatic species other than fish has been limited and likely deserves further study. The results to date indicate low risk from molybdenum to aquatic invertebrates like the water flea *Daphnia magna*. For example, Diamantino *et al*. (2000) reported relatively low toxicity of molybdate to water fleas in the laboratory, with an estimated acute 48-h LC50 of 2,848 mg/L. This LC50 concentration is comparable to other previous studies with this species (e.g., 48-h LC50 of 3,220 mg/L; Kálmán, 1994). The chronic toxicity of molybdate to water fleas in this study was also low, with NOAEC and LOAEC for growth and mortality as 50 and 75 mg/L, respectively. The high concentrations needed to illicit reduced growth and mortality in the laboratory suggest little risk to water fleas from dissolved molybdenum. In a review, Eisler (1989) reported that aquatic biota generally is not very susceptible to toxic effects of molybdenum, which shows no effect on growth or survival at concentrations of less than 50 mg/L in the water column.

Davies *et al*. (2005) reviewed the studies that evaluated the toxicity of molybdenum to fish and compared them with the current CCME guideline of 73 µg/L (0.073 mg/L) molybdenum for freshwaters (see Tables 4.4, and 4.5). Davies *et al*. (2005) also attempted to reproduce the molybdenum toxicity to fish results reported by Birge (1978) and Birge *et al*. (1980) that were used to set this current CCME guideline for molybdenum. However, Davies *et al*. reported it was not possible to replicate the observations from these important early studies. Thus, Davies *et al*. recommended that the molybdenum guidelines for freshwaters in Canada be re-evaluated because the current findings show equivocal toxicity of molybdenum to fish. The observed low toxicity of molybdenum to fish led Davies *et al*. (2005:484) to state: 'Evaluating molybdenum toxicity through acute toxicity studies may not accurately estimate molybdenum toxicity in an environmental context.' These findings are congruent with other studies that identified low acute toxicity of molybdenum to fish (e.g., Hamilton and Buhl, 1990, Reid, 2002). Interestingly, Reid (2002) suggested the chronic effects of molybdenum exposure may pose a greater risk to fish. Previously the lowest EC20 for molybdenum toxicity to fish was 360 µg/L or 0.36 mg/L (Suter and Tsao, 1996).

This variability in the estimates of molybdenum toxicity offers additional evidence for the need to assess the toxicity of molybdenum to aquatic biota on a site-specific basis. In addition, this variability motivated the International Molybdenum Association to conduct detailed studies of the toxicity of molybdenum to aquatic biota, and these studies are currently in progress. For further details, refer to Appendix A.

4.2.3 Terrestrial Biota

This evaluation of the toxicity of molybdenum extends across trophic levels in ecosystems, from plants to cows to moose to muskrat. Such an approach is justified given the diverse habitat and resource use across species. Generally, molybdenum toxicity primarily affects ruminants while other terrestrial biota does not show similar symptoms.

Plants

Typical soil concentrations of molybdenum range from 1 to 2 mg/kg. However, aquatic and terrestrial plants may accumulate concentrations exceeding 1,000 mg/kg (dw) under certain conditions (as detailed in Section 3.2.3). A number of studies have shown no definitive toxic response in plants grown on soils with high molybdenum concentrations in the laboratory and the field. For example, concentrations of molybdenum as high as 26 mg/kg (dw) did not impair growth of bermudagrass and caused no toxic symptoms (Ward, 1978). In this latter study, it was reported that the concentration rate of molybdenum in bermudagrass was typically in the order of 20-25 and as high as 40 (on a dry weight basis). In a study of Ballica grasses grown on soil with high molybdenum concentrations, the resulting plant tissue concentration ranged between 5 and 10 mg/kg (as extractable soil molybdenum content (Schalscha *et al*., 1987). For ryegrass grown on soil with high molybdenum concentrations, the tissues commonly showed concentrations less than 1 mg/kg (dw) with a maximum of 5.1 mg/kg, and showed no apparent effects from this exposure. This evidence suggests that molybdenum biomagnification is not likely to be an issue for plant health but rather as an intake pathway for molybdenum to herbivores (Barceloux, 1999b; Clemens, 2006).

Efroymson *et al*. (1997) reviewed the literature to establish a conservative screening benchmark for molybdenum phytotoxicity of 2 mg/kg in soil, and 0.5 µg/ml in solution. The authors noted the contention of some researchers that phytotoxicity of molybdenum has never been documented in field settings. The implication is that the presence of elevated levels of molybdenum does not hinder plant growth and, therefore, concentrations may accumulate to where consumption of plant tissues can result in significant doses to wildlife.

Wildlife

There are three main issues with molybdenum toxicity in wildlife. First, a high dietary intake of molybdenum (as molybdate) can be toxic to mammals and is commonly, but not always, associated with copper deficiency. Second, copper supplementation in the diet can limit or

reverse the effects of molybdenum toxicity. Third, high dietary intake of sulfur can exacerbate any toxicity from molybdenum and is particularly important for ruminants.

Most mammals (pigs, rabbits, horses, humans) and birds (chickens) are monogastric with a single chamber in the stomach whereas some ruminants such as cattle, sheep and goats are polygastric and have four stomach chambers. Ruminant digestion is aided by an abundant microfauna that digest cellulose and other plant compounds in an anaerobic environment resulting in compounds that the animal is readily able to assimilate. In the reducing environment of the rumen, thiomolybdate compounds are rapidly formed (Smart *et al*. 1986; Eisler, 1989). There are four types of thiomolybdate: TM1, TM2, TM3 and TM4. These thiomolybdates may bind with copper in the stomach, and do so with increasing ability so that TM4 > TM3 >TM2 >TM1 (Osman and Sykes, 1989). The result is that copper absorption is impaired and a copper deficiency may ensue. Some thiomolybdates may be absorbed into the blood stream, particularly TM3 and may interfere with reproduction (Phillippo *et al*., 1987) or cause other effects included in the diagnosis of molybdenosis (e.g., Frank *et al*., 2002). Such a scenario is possible, as there are parts of the world where copper is naturally low in soils. In North America, this includes continental locations like northern Saskatchewan and Manitoba (Boila *et al*., 1987) and coastal locations like parts of Alaska (O'Hara *et al.,* 2003). In these habitats, the wildlife may actually be copper deficient. A deficiency is assumed when copper levels in the liver are low (< 10 ppm, as suggested by Gooneratne *et al*., 1989; Gooneratne and Christensen, 1989) though deficiency symptoms may not be observed.

There are a number of studies that have identified toxicological effects of elevated dietary intake of molybdenum in animals (Fairhall *et al.*,1945; Neilands *et al.*, 1948; Arrington and Davies, 1953; Jeter and Davis. 1954; Ostrom *et al.*, 1961; Gray and Daniel, 1954; Schroeder and Mitchener, 1971; Wide, 1984; Fungwe *et al.*, 1990). It is now known that molybdenosis may occur in animals if they digest plant tissue containing more than 10 mg/kg (dw) of molybdenum, but typical soils do not usually produce molybdenosis (Ward, 1978; Eisler, 1989). These studies indicate the effects of molybdenum exposure vary from no effects from doses as high as 80 mg/kg/day in guinea pigs to moderate to severe reproductive effects in doses as low as about 3 mg/kg/day in mice. This toxicological data suggest that although reproductive effects can be observed at dose levels of about 3 mg/kg/day, doses that are tens to hundreds of times higher are required to produce severe developmental and growth rate effects on other test animals in the laboratory. Generally, an LOAEL value of 2.6 mg/kg/day for a mouse can be used as the benchmark for molybdenum toxicity effect(s) in mammals. This benchmark should be adjusted to lower values for larger animals. For example, a benchmark of 0.3 mg/kg/day for a black bear and 0.24 mg/kg/day for a moose, reflects the mouse LOAEL (e.g., U.S. DOI 1998). Evidence for this view is provided from allometric models that link metabolism with body size and is justified from observations from ecological studies (e.g., Peters, 1991).

Eisler (1989) completed a review of different studies of animals fed diets containing various amounts of molybdenum. This review identified domestic cattle to be the most sensitive to elevated molybdenum, while deer, sheep, horses, and rabbits were much less sensitive. These studies revealed that subchronic and chronic oral exposures to molybdenum can result in gastrointestinal disturbances, growth retardation, anemia, hair discolouration, hypo-thyroidism, bone and joint deformities, sterility, liver abnormalities, and death. Diarrhea is the most typical manifestation of molybdenosis in cattle. Teratogenic effects have not been observed in mammals, but embryotoxic effects (reduced weight, reduced skeletal ossification, nerve system problems), and reduced survival of offspring were noted.

Opresko (1993b) reviewed animal toxicity data for molybdenum in support of development of human reference doses. In that review, chronic toxic effects on growth, bone formation, connective tissue disorders, and organ function were reported to occur at dietary levels of molybdenum in the order of 400 to 1,000 mg/kg (dw). Less severe effects (biomarker effects) occurred at dietary levels as low as 20 mg/kg (dw). In sheep and cattle, a condition known as "teart disease", marked by acute symptoms of weakness and diarrhea, occurs when these animals graze on plants containing high amounts of molybdenum. Longer exposure can lead to discoloration of hair, skeletal deformities, sterility due to damage of testicles, poor conception rates, and deficient lactation. The threshold for dietary molybdenum that may cause teart is about 10 mg/kg (dw). This corresponds with the reported limit for molybdenum in forage for livestock protection of 10 mg/kg (NRC, 1980).

The Opresko (1993b) review noted the lowest reported chronic dose was associated with a reduction of second generation offspring survival in rats provided with 10 µg/L (0.01 mg/L) molybdenum in drinking water, corresponding to a dose of 1.9 mg/kg/day. Sample *et al*. (1996) presented an LOAEL for mammals of 2.6 mg/kg/day derived from a study of reproductive success in mice. Specifically, the third generation of mice was impaired by this level of molybdenum exposure. A NOAEL could not be derived directly from the study data, so a safety factor of 10 was applied to derive a NOAEL of 0.26 mg/kg/d.

For risk or impact assessment purposes, values intermediate to the LOAELs and NOAELs reported by Sample *et al*. (1996) could be used as the basis for derivation of body-weight adjusted benchmark doses for representative wildlife species. Soils directly associated with molybdenum deposits, particularly in western Canada, frequently have concentrations much higher than the 1 to 2 mg/kg range typical of other areas. Similar examples of local molybdenum deposits leading to elevated soil concentrations exist elsewhere in North America, Asia, and Australia (Hollister, 1991). Hence, site-specific guidelines are used at these locations.

Studies with birds identified a limiting chronic dose of molybdenum at an LOAEL of 35.3 mg/kg/day and this dose caused embryonic failure (Sample *et al*., 1996). This translates to a NOAEL of 3.53 mg/kg/day; this threshold was derived through the use of a safety factor of 10. For avian livestock, the toxic level of molybdenum is relatively high, in the range of 200 to 500 µg/g (dw) (e.g., Eisler, 1989).

The information about copper, molybdenum and sulfur metabolism in wild terrestrial animals is limited and much is inferred from a few field observations. These studies can be used to approximate actual risk to wildlife. However, the nutritional requirements of livestock can provide some understanding regarding how molybdenum may affect animals in a natural setting. In a review of the status of various trace elements, McDowell and Conrad (1979) report dietary sufficiency levels for molybdenum of 0.5 mg/kg (dw) for cattle feed. The dietary levels of molybdenum considered to be toxic were much higher, and quite variable depending on the species of animal. For dairy cattle, the range between sufficient and toxic was only about ten-fold, with 6 mg/kg (dw) regarded as toxic. This was similar to the reported toxicity levels in feed for pigs (i.e., 5 to 20 mg/kg). In general, a low copper: molybdenum ratio in diet (i.e., <2) rather than the absolute dietary concentration of molybdenum is the primary determinant of susceptibility to molybdenosis in wildlife; effects are not expected when this ratio is near 5 (Buck, 1978; Ward, 1978; Mills and Bremner, 1980). High dietary inorganic sulfur (300 to 4,000 ppm) is believed to block the transport of molybdenum through the cell membrane, thereby reducing the intestinal absorption and renal tubular reabsorption (Fairhall *et al*., 1945; Neilands *et al.*, 1948; Vyskocil and Viau, 1999). Molybdenum does not accumulate in the body of most animals and is cleared primarily through faeces but also to varying degrees through urine when molybdenum is removed from the diet (Underwood, 1971; Vyskocil and Viau, 1999).

Evidence also exists to suggest that molybdenosis can occur as a result of exposure to aquatic sources of molybdenum in unusual circumstances. Molybdenum was identified as the likely causative agent of copper deficiency linked to a mysterious moose disease in parts of North America and Sweden. These disease outbreaks have been reported in moose from northern Minnesota (Custer, 2003), Manitoba (Gooneratne *et al*., 1989; Gooneratne and Christensen, 1989), and Saskatchewan (Thomas, 1996). These areas are also known to have soil that is deficient in copper (Smart *et al*. 1986; Boila *et al.,* 1987; Eisler 1989). In step with these observations, low levels of copper have been reported in the livers of cattle in northern Saskatchewan (Gooneratne *et al*., 1989; Gooneratne and Christensen, 1989). In Sweden, the discovery of molybdenosis was coincident with the widespread liming of lakes conducted to counteract the effects of acid precipitation (Frank, 1998; Frank *et al*., 2000a-c; Frank *et al*., 2002; Kapustka *et al*., 2003). Key considerations in Sweden include the extensive nature of the liming efforts across adjacent watersheds and subsequent mobilization of molybdenum in adjacent habitats. This circumstance would lead to fairly high exposure rates throughout the large feeding range of moose. It is important to note that for cases of molybdenum exposure confined to nearshore areas of single water bodies, exposure of sensitive large herbivores would be considerably lower. In addition, it is likely that naturally low copper and trace mineral levels are normal in some geographic areas and there may be adaptation by wild ruminants to these concentrations and cause comparable patterns in moose. Understanding the exposure pathways and risk from molybdenum to ruminants like moose requires a detailed consideration of the interaction between the animal and environment. One way to convey these relationships is to consider a hypothetical risk assessment for moose located near a mine in Canada.

Hypothetical risk to Moose from Molybdenum Exposure due to Mine Operations

Moose have two main sources of nutrition, a combination of leaves and twigs, frequently referred to as browse and aquatic plants (referred to as macrophytes) when available (e.g., LeResche and Davis, 1973). Generally, macrophytes only represent about 3% of the total diet, on a weight basis, given the short period each year they are available. Thus, for a mine in northern Canada, a realistic upper bound effluent condition for molybdenum is about 6 mg/L, and downstream, the steady-state molybdenum concentration in a typical lake would be 1.66 mg/L. The molybdenum discharged from a mine may be taken up by aquatic macrophytes within the waters and by riparian plants growing on and near the waters edge. Vegetation that may accumulate elevated levels of molybdenum includes species such as water lily (*Nuphar* spp.), pondweeds (*Potomogeton* spp.), and riparian species like, cattails (*Typha* spp.), sedges (*Carex* spp.), willows (*Salix* spp.) and alder (*Alnus* sp.). Aquatic macrophytes will biomagnify molybdenum from the water, but given the short growing season (two to three months) it is unlikely that they will accumulate the high levels found in terrestrial species grown on soil with high molybdenum levels (Taylor and McKee, 2000).

The typical diet of moose can be used to estimate exposure to molybdenum in the field (Taylor and McKee 2000, 2003). Research suggests that a typical BCF of molybdenum in macrophytes would lead to a plant tissue concentration of about 332 mg/kg. In contrast, the terrestrial browse will only contain background molybdenum concentrations. This contribution of molybdenum from browse corresponds to a concentration that is much less than 1 mg/kg, or effectively zero. Thus, a weighted average molybdenum in the diet can be calculated as (6.6 kg x 0 mg/kg + 0.22 kg x 332 mg/kg)/6.82 kg = 10.7 mg/kg. This average diet represents a molybdenum value that is very similar to the recommended critical threshold of 10 mg/kg identified for domestic cattle feed (dw; O'Connor *et al.*, 2001a).

To understand risk to moose from molybdenum, the exposure period needs to be known. For example, to ingest a significant quantity of molybdenum from a water body like a lake, a moose has to occupy the local area, the muskeg and/or lake, for an extended period of time (e.g., summer). A typical scenario of assessment for a moose would assume that an exposed animal would use the muskeg for 5% of an entire year, a local lake for 20% of the year for a total of 25% of total feeding time. This habitat use estimate is conservative, recognizing that a single moose will typically range over 25 to 100 km^2 in ecosystems typical of the boreal forest. This approach over-accounts for any focal foraging in an area to obtain essential diet constituents (e.g., to seek additional salts, such as sodium chloride). Thus, the exposure to molybdenum is limited in spatial extent, because the vegetation (aquatic or riparian plants) that contains elevated molybdenum from a mine will only be those plants in direct contact with water with elevated concentrations. Further, as soon as the intake of feed and water containing elevated molybdenum ceases, the molybdenum excess in the animal diet diminishes (e.g., excreted in urine) and any short-term effects are reversed, as shown by studies that either cease feeding with molybdenum-spiked feed or add copper supplements where copper deficiency was the main manifestation of

molybdenum toxicity in test animals (e.g., reviewed by Eisler, 1989). Such an assessment would predict no significant adverse effect to moose via molybdenum release from a mine.

Mitigating Site-Specific Circumstances

There are several site-specific conditions and issues that will strongly alleviate the potential for molybdenosis in moose. First, the primary pathways for molybdenum to moose and other ruminants are through the ingestion of macrophytes and water (and indirectly, sediment) from habitats (muskeg, water) with high molybdenum concentrations. In temperate zones, these pathways will be completely blocked during most winter months and molybdenum intake will be negligible for these time periods. It is also likely that aquatic plants, like water lilies, will not develop sufficiently for consumption until the mid or end of June each year and the plants will senesce (die-back) by the end of August. This means that the aquatic plants (plus sediment) that will represent 90% of molybdenum intake for an exposed moose will be available for consumption over about 60 to 80 days per year, and water from the muskeg / lake that represents the other 10% of molybdenum intake will be available for only about 150 days per year. This is a very different type of exposure than that investigated for domestic cattle that graze on fresh vegetation for 120 to 150 days per year, and ingest sun-cured, cut grass or hay from the same region over winter.

Sulfur uptake in macrophytes may also be a concern if the sulfur content of the aquatic plant exceeds 3.5 g/kg (or 3,500 mg/kg) in combination with elevated dietary intake of molybdenum, particularly for ruminants (Smart *et al*. 1986; Boila *et al.*, 1987; Eisler, 1989). In general, a BCF for sulfur in macrophytes of 100 L/kg was previously noted (NRC, 1983). Biomagnification of sulfur by macrophytes could therefore result in a range of sulfur content: (50 mg/L x 100 L/kg or) 5,000 mg/kg (or 5 g/kg). However, this is the additional sulfur contributed by sulfate in water to macrophytes, and should be weight-adjusted to the diet composition of the moose (e.g., majority of browse and minority of macrophytes). This additional sulfate would contribute an average of 160 mg/kg to the total diet, and represent only a small incremental increase over the natural background levels of sulfur of 1 to 2 g/kg in browse (e.g., NRC, 1976).

4.2.4 Toxicological Interactions

Nutritional studies have identified that copper, manganese and iron can act as antagonists to molybdenum toxicity (Eisler, 1989; Barceloux, 1999b; EVM, 2000). The challenge with interactions is that they are difficult to quantify in natural settings. Generally, interactions are not included in assessment of environmental effects because of the problem with quantification, but are considered on a site-specific basis. These analyses require information on the site (volume of terrestrial, aquatic compartments, effluent attributes, and other ancillary factors (e.g., pH of the aquatic compartments).

Sulfur interaction in molybdenum toxicity is complex. Several studies have shown that adding sulfate to the dietary intake in chicks, rabbits and rats with high molybdenum intake rates can be protective and will decrease the effect of toxicity (Mills and Davis, 1987). Such

studies led to the speculation that sulfate promotes excretion in urine and reduces molybdenum retention in tissues. Contrary evidence was found in some studies with rats that were fed diets with 800 mg/kg of molybdenum. Before high levels of sulfur were added to the diets, the rats had only marginal copper deficiencies. When sulfur was added to the diet in high doses (9,400 mg/kg as L-cystine) there was a profound increase in anemia, diarrhea and mortality (Mills and Davis, 1987). However, dissolved sulfate in water has been concluded to be synergistic by limiting weight gain in cattle at concentrations above 1,500 mg/L (NRC, 1980). Other studies showed increased dietary intake of either sulfate (6,400 or 27,000 mg/kg) or methionine (10,000 mg/kg) alleviated the toxic effects of intake of molybdenum (800 mg/kg) until the copper intake was decreased from greater than 3 to less than 1 mg/kg of copper in the rat diet. Boila *et al*. (1987) in a survey of legumes and grasses in Manitoba noted that cattle and lactating dairy cows should have a dietary allowance for sulfur of 2,000 and 1000 mg/kg, respectively with maximum intakes of 4,000 and 3,500 mg/kg respectively. The positive effect on copper status on dairy cows was noted when sulfate in drinking water was lowered from 500 mg-S/L (or 1500 mg-SO_4/L) to 42 mg-S/L (or 126 mg-SO_4/L) (Smart *et al*., 1986; Eisler 1989). It is evident that the form of sulfur may play a role in the molybdenum-copper-sulfur interactions and dietary copper levels (e.g., Barceloux, 1999b).

4.3. Site Remediation of Selenium and Molybdenum with Plant Growth

Observation of the accumulation of selenium and molybdenum by plants led to the suggestion that this mechanism may represent a possible route for bioremediation of sites with high molybdenum concentrations in the soil. Studies to date have confirmed that plants grown on soil high in either selenium or molybdenum can actively lower the soil concentration through translocation to the plant tissue (Eisler, 1989; U.S. DOI, 1998; Lemly, 2004c). This process involves growing the plant on the site with high selenium or molybdenum and then removing the plant tissues from the location. One approach involves growing annuals or perennials and then removing them after each growing season. A second approach concerns the creation of wetlands and then allowing the growth of these semi-aquatic plants prior to harvest. A third strategy involves the promotion of soil microorganisms that enhance volatilization. Such remediation activities have been completed at mine sites across North America. Similarly, these strategies have been applied to reduce selenium and molybdenum concentrations on agricultural lands. These efforts have been generally regarded as economical and successful.

Studies involving selenium remediation have primarily focused on the use of selenium-accumulator plants, usually wetland species. These plants act to reduce concentrations of selenium in soil and water. This strategy results in lower transfer rates of selenium from soil to water and from lakes to downstream waters. The chemistry of this process has been well described (e.g., Zhang and Moore, 1996). These investigations initially involved small-scale studies, and now extend over large areas and different environments (Bailey *et al*., 1995; Bañuelos and Meek, 1990; Bañuelos *et al*. 1993, 1996, 1997; Hansen *et al*., 1998; Bañuelos, 2002; Lin and Terry, 2003; Ashworth and Shaw, 2006). One well documented example involves the watershed associated with Kesterson Reservoir in California. The remediation activities at this reservoir reflected an attempt to ameliorate the selenium

impacts on fish and associated wildlife (reviewed by Wu, 2005). Kubachka *et al*. (2007) recently reported that awareness of the metabolic pathways of selenium in the terrestrial plant Indian mustard (*Brassica juncea*) can be used to enhance phytoremediation results.

Studies involving the remediation of molybdenum have been diverse. For example, bermudagrass (*Cynodon dactylon*) grown for a period of nine weeks removed 3.6 to 10.6% of the total mass of molybdenum in soil (concentrations as high as 26 mg/kg) (Ward, 1978). Studies have also revealed that site remediation through plant growth can be enhanced with the addition of different media to the soil (Barceloux, 1999b; Gaskin *et al*., 2003). For example, the addition of lime and fertilizer acted to enhance molybdenum uptake by ryegrass on coal ash-soil mixtures (U.S. DOI, 1998). Other studies have added phosphorus to reservoirs to encourage phytoplankton growth to reduce total water-borne molybdenum concentration. These latter methods encourage the growth of microbes and phytoplankton to remediate large water bodies such as reservoirs (for an example see: http://www.microbialtech.com/water.html).

An alternate remediation approach involves growing woody plants like trees and immobilizing the molybdenum and selenium in the wood. Current studies in the central USA showed that blight-resistant American chestnut (*Castanea dentate*) is well suited for this purpose. Recent studies of this nature have been completed on soils with high molybdenum and selenium concentrations stemming from coal mining. This programme is referred to as the 'The American Chestnut Mined Land Reclamation Project' with current efforts in Kentucky and other sites in development (see The American Chestnut Foundation, www.acf.org for additional details).

5.0 ENVIRONMENTAL IMPACT CONSIDERATIONS

Most regulatory limits have been established to control levels of COPCs in discharges or in the receiving environment. These limits were typically a result of ERA exercises and are based on the risks associated with exposure to the COPC. Generally, these assessments of risk are highly conservative and reflect efforts to identify a concentration of a given COPC that will have absolutely no expectation of any adverse effect. The assessment typically considers the most sensitive endpoint (e.g., species, life stage) under the conditions that maximize the likelihood of effects occurring. In many cases, an additional safety factor is applied to account for any possible uncertainties and to ensure absolute environmental protection. Accordingly, the regulatory limits can be taken as indicative of the level of exposure that does not warrant any concern. For example, environmental concentrations below guidelines reveal little need for concern of effects while concentrations above guidelines reveal a need to carefully assess a site and does not necessarily identify that effects will be evident at a site.

In assessing the impacts that may occur due to exposure at any specific location, the nature of the guidelines for COPC should be understood to determine what adjustment may be warranted to provide a reasonable benchmark for that site. Identification of potential modifying factors at a location needs to be considered in this analysis. These factors may change across seasons. For example, does the lake or river that receives effluent show large seasonal fluctuations in volume? If so, then the low volume seasons must be considered within the assessment for shifting dilution ratios. A corollary that also needs to be considered is: do seasonally high flows or floods displace water to riparian zones? If so, then this distribution of effluent needs to be assessed for risk to sensitive endpoints.

Direct assessments of receiving environments actually influenced by anthropogenic releases of selenium or molybdenum can also provide an indication of the levels of exposure that environmental impacts may be expected. This assessment would be independent of the existing guidelines or limits. Understanding the relationship between actual exposure and effects in sensitive endpoints provides key information that can be considered in the development of site-specific benchmarks or guidelines for use at other locations with similar habitat and other physical features.

5.1 Selenium

5.1.1 Existing Guidelines

In recognizing the potential effects of selenium throughout the environment on a variety of life forms, numerous regulatory criteria have been established to protect against the occurrence of such adverse effects. Numerous agencies have also developed threshold levels that are intended as general limits of exposure beyond which impacts may occur. Table 5.1 summarizes some of the regulatory guidelines and exposure thresholds.

The Canadian environmental quality guidelines for selenium in water (CCME, 2002) are as follows:

- 1 µg/L for aquatic life protection;
- 20 -50 µg/L for irrigation (to protect livestock feeding on irrigated feed crops); and
- 50 µg/L for livestock watering.

The aquatic life guideline is based on toxicity data for the most sensitive species of plants and animals found in Canadian waters, and intended to protect all species of aquatic biota 100% of the time. The guideline was developed with the consideration of results from numerous acute and chronic toxicity tests of selenite and selenate, and recognizes that selenemethionine is about 10 times more toxic than either of these inorganic forms (for additional information, refer to CCME, 1987). The guideline ultimately recognizes that waterborne concentration limits for elements like selenium are limited in their effectiveness. This guideline was based on field-level evidence of acute lethal effects through food web exposure for predatory fish in waters containing 5 to 10 µg/L (0.005 to 0.01 mg/L). A safety factor of 5 was applied to ensure protection of all species in all waters.

The CCME guideline for irrigation water (i.e., 20 µg/L for continuous use, and 50 µg/L for intermittent use) is intended to protect livestock that might be exposed to irrigated feed. It is based on a threshold for dietary-related selenium toxicity of 5 µg/g. However, at the time of development of the guideline (i.e., mid-1980s), the rationale noted that there was no evidence of toxic response to selenium in natural waters in Canada.

British Columbia has also developed water quality guidelines for the protection of aquatic biota and wildlife against potentially toxic levels of exposure to selenium (Nagpal and Howell, 2001). The aquatic life guideline is 2 µg/L (or 0.002 mg/L) for both freshwater and marine ecosystems. This guideline was developed through review of an extensive body of data from a variety of toxicity assessment studies. Ultimately, this guideline was determined on the basis of the lowest NOAEL reported for ecologically relevant effects (i.e., 10 µg/L or 0.010 mg/L). Similar to the CCME guideline, a safety factor of 5 was applied to generate a guideline that is considered to confer the broadest level of protection across species and habitats. The British Columbia Guideline document (Nagpal and Howell, 2001) makes note of the fact that the potential toxicity of selenium can vary greatly for a number of reasons, primarily factors that affect bioavailability. Accordingly, a site-specific guideline, if appropriately developed, is considered more appropriate than the generic one.

To protect wildlife, the 2001 British Columbia Guidelines recommend that the maximum concentration of total selenium in water should not exceed 4 µg/L (0.004 mg/L). This guideline was based on field studies that indicated this concentration of selenium in water will protect between 90 and 95% of waterfowl (identified as the most sensitive terrestrial wildlife receptor) from reproductive impairment (the most sensitive known endpoint, evaluated by the concentration of selenium in the eggs). The proposed guideline assumes

that the exposure pathways required for significant selenium transfer to waterfowl, via food and water, are sufficiently protective. The guideline is the average of two key values; 1) the water concentration that gives rise to egg selenium concentrations that are equivalent to background [i.e., 2.3 µg/L (0.0023 mg/L) in water that leads to 3 µg/g dw in eggs, Ohlendorf *et al*., 1990; Skorupa and Ohlendorf, 1991] and, 2) the selenium concentration that will not impact 90% of avian species (i.e., 6.8 mg/L or lower in the water column, from Adams *et al*., 1998). This determination also recognizes that the food of most waterfowl stems from water, so analysis of only water concentrations for guideline development is appropriate.

The chronic water quality criterion for selenium currently in effect in the U.S.A. is 5 µg/L (0.005 mg/L), set by the U.S. EPA in 1987. Since that time, substantial evidence has been documented for adverse effects of selenium on aquatic ecosystems at concentrations below 5 µg/L, primarily as a result of high rates of bioaccumulation that can occur in specific environments. This prompted a reexamination of the criterion, and an issuance of a new draft criterion in 2004 based on whole body tissue concentrations in fish (U.S. EPA, 2004a). The 2004 draft chronic criterion for selenium is a concentration 7.91 µg/g (dw) in whole-body fish tissue. This value reflects the lowest reported LC20 for whole body fish tissue.

The U.S. EPA (2004a) acknowledges that any given fish community may contain species with different sensitivities to selenium than those considered in their criterion development. Such sensitivities would be due to behavioural, habitat, or physiological factors. Consequently, focal site-specific studies could be used to modify the criterion.

The Government of Canada has identified the maximum acceptable selenium in soils. That is, the guideline identified for maximum cumulative addition of selenium to soil is 2.8 kg/ha and the corresponding maximum concentration in soil is 14 mg/kg (dw). These apply to the following media that may be applied to soils: processed sewage waste, compost, and related products (e.g., sewage-based products, fertilizer, supplements; also see www.inspection.gc.ca/ for additional details). These guidelines include products such as manure that has been composted, industrial sewage products, and waste tankage from municipal sources, from garbage, and other sources.

Overall, the guidelines and thresholds can be used as a basis for setting discharge limits or assessing the potential for impacts in environments that are exposed to such limits. However, as evident from the basis used for the derivation of these values, they are highly protective. The presence of selenium at levels that are higher than prescribed does not mean that impacts will necessarily occur. This is recognized in the various regulatory frameworks through the acknowledgement of the suitability of site-specific limits. There are many documented cases that illustrate the level of selenium exposure that may cause adverse effects is dependent on site-specific conditions.

5.1.2 Case Studies

Much of the information that serves as the basis of understanding of the toxic effects of selenium is from laboratory studies that use surrogate species to assess risk to wild species. One such example is the use of domestic birds to assess waterfowl. Other studies have only considered one trace element (i.e., variable selenium concentrations) at a time and are not fully realistic in terms of environmental exposure and risk. One study used mallard (*Anas platyrhynchos*) exposed to relatively high levels of single trace elements (e.g., Heinz and Hoffman, 1987, 1998). The degree that the findings from surrogate species or exposure to just selenium or molybdenum can be extrapolated to wild animals is uncertain, because:

- experimental species (typically domestic or lab strain animals) may differ in their sensitivities to a contaminant;
- unlike the conditions established in most lab studies, animals in the wild are exposed simultaneously to varying levels of multiple substances, many of which may reduce or increase the toxic effects of the element of interest;
- non-chemical stressors (extreme heat, low food) often encountered in the natural environment, and not wholly considered in lab studies, can induce significant physiological stress on wild animals and exacerbate effects of contaminants; and
- the importance of low water temperature (e.g., autumn, winter, spring in Canada) also requires consideration. As noted, the exposure to contaminants during the winter months can also exacerbate effects and is attributed to metabolic processes.

It is important to examine trace element concentrations while simultaneously examining the health under natural conditions to minimize the risk of incorrectly extrapolating the results of laboratory-based studies to wild animals. Examination of real-world cases serves this purpose. Case study examination provides an understanding of net effect of interactions of the major factors that play a role in determining the potential toxic implications of selenium in the aquatic environment. For this general purpose, several case studies of contaminated sites and different land use activities are examined herein. However, it has proved somewhat challenging to obtain detailed site histories, and this detracts from the potential benefits of this section.

Use of Coal and Selenium Waste: A Brief Overview

The presence and potential impacts of high levels of selenium in various waste streams associated with large-scale use of coal, particularly fly ash settling pond overflows, has been the focus of considerable investigation over the last decade. In these cases, selenium concentrations have ranged as high as 1,000 µg/L (or 1 mg/L). In 1978 and 1979, discharges from fly ash settling ponds resulted in very high selenium concentrations (e.g.,

2,200 to 2,700 µg/L) in two reservoirs in east Texas, which in turn caused significant fish mortalities (Garrett and Inman, 1984; Cherry *et al*., 2000). Selenium body burdens from bioaccumulation in fish were very low, ranging from 2.0 to 9.1 µg/g (dw). Other elements (e.g. arsenic and mercury) were also found to be elevated in fish tissues, but selenium was deemed to be the primary causative agent for the observed mortality. The fish kills and also the impairment of reproduction of certain species had long term implications, causing a shift in fish community composition, favouring planktivorous over piscivorous species.

Selenium Waste from Coal Ash: Two Examples

Studies of fly ash settling pond discharges into a reservoir in North Carolina (Belews Lake), determined that concentrations of selenium of ~10 µg/L resulted in the loss of 80% (16 of 20) of the resident fish species over a few years of exposure. Further, two of the four remaining species were rendered sterile, and their elimination would occur if conditions remained the same (Table 5.3; Cumbie and Van Horne, 1978; Lemly, 1985, 1987). The fish species that accumulated the highest levels of tissue selenium were the piscivorous and insectivorous species, and these feeding groups also comprised the largest proportion of the species eliminated from the reservoir. Planktivores and bottom feeding omnivores had lower levels of tissue selenium and were represented by the few fish species that were not extirpated. Information on the selenium distribution in the Belews Lake ecosystem is presented (Table 5.3). This distribution reveals the relationship between water, sediment, and different biological receptors for the site.

In consideration of the evidence available from the study of waters affected by coal fly ash, Lemly (1993a, 1997) concluded that selenium concentrations of 2 µg/L (or 0.002 mg/L) in the water column represent a long-term hazard to fish due to the high rates of bioaccumulation of selenium under the conditions encountered in reservoir systems. It should be noted that the conditions encompassed in these studies are somewhat extreme, and selenium dynamics in other environments may differ. The prediction from this work was that if the selenium load to the Belews Lake was reduced, the populations of organisms in the ecosystem would likely respond in a positive manner. Indeed, the 30 years of monitoring information available to date reveal that declines in the selenium concentrations of the water were commensurate with declines of the selenium in the sediment. Such declines of selenium in the water and sediment compartments were then reflected as lower tissue concentrations in the resident fish. The current status of these fish populations indicates recovery and presence of persistent populations. Interestingly, these fish still show whole body selenium concentrations that are about 2x the draft U.S. EPA criterion value (Barwick and Harrell,1997; Finley and Garrett, 2007). Such observations from this long-term study site provide direct evidence of: 1) proportional declines of the sediment load of selenium with concentrations in the water, 2) relatively rapid responses of the fauna of the lake to declines in the selenium load in the sediment, 3) persistence of fish populations within a regime of elevated selenium concentrations in tissues, and 4) long-term monitoring provides important resolution of the short-term and long-term responses of species to water-borne selenium.

Another case of resident fish exposed to high concentrations of selenium from coal fly ash that showed self sustaining populations exists in Stingy Run, Ohio (Reash *et al*., 2006). This stream receives effluent that contains treated fly ash and has resulted in high tissue concentrations of selenium in the resident bluegill (*Lepomis macrochirus*) and bullhead minnow (*Pimephales vigilx*). Although the selenium tissue concentrations in these fish are 2 -3 times higher than the proposed U.S. EPA (2004a) toxic thresholds for selenium, there is only limited evidence of biological deformities (e.g., Lohner *et al*., 2001) and the populations are considered abundant and stable. The tissue patterns in these fish are concordant with high tissue concentrations in a resident invertebrate, a caddis fly (Trichoptera), consumed by these fish. Thus, the expected consequences of high selenium concentrations on this ecosystem have not been observed to date in the monitoring activities. Reash *et al*. (2006) suggested that the interaction between other metals present in the ecosystem, like arsenic, copper, and zinc is likely limiting the potentially toxic effects of the selenium. These authors identify that these results confirm the need for site-specific analysis of selenium exposure, and that general caution should be used when contrasting observations from field situations for selenium concentrations in fish tissues with the proposed toxic thresholds for selenium.

Selenium in Ecosystem Components Downstream of Coal Mines

McDonald and Strosher (1998) examined the distribution of selenium in the ecosystems located downstream of coal mining activity in the Elk River Valley, British Columbia. Measures of selenium in water, sediment, and biota were compiled for habitats exposed to drainage from coal mine activities, and also for reference habitats not affected by the mining (see Table 5.2). Review of the site data will reveal the drainage from coal mine activities appears to have resulted in selenium concentrations in the water in the range of ~7 to 10 µg/L (or 0.007 to 0.010 mg/L), compared with concentrations of 0.1 to 0.2 µg/L (or 0.0001 to 0.0002 mg/L) in the reference habitats. However, in this fast-flowing river, the concentrations of selenium in sediments, algae, insects, and fish tissues in exposed habitats increased only modestly (two to five times) relative to reference habitats. It should be noted that fish tissues in both the exposed and reference environments contained selenium at levels that exceed some suggested thresholds for toxic effect.

Exposure of Avian Wildlife to Selenium Downstream of Coal Mines

Harding *et al*. (2005) completed field study of the effects of elevated levels of environmental selenium in American dippers (*Cinclus mexicanus*) and spotted sandpiper (*Actitis macularia*) from nine lotic (creek and river) habitats in the vicinity of coal mines in the Elk River Valley of British Columbia. For American dippers, this study revealed mean egg selenium concentrations in the range of 7.3 to 8.4 µg/g (dw) for reference and exposed habitats, respectively. For spotted sandpiper, the mean egg selenium concentrations ranged from 3.8 to 7.3 µg/g (dw) for reference and exposed habitats, respectively. These concentrations in eggs are below most levels suggested as toxic thresholds (e.g., Eisler 1989). The corresponding mean water-borne selenium in exposed habitats ranged from

8.1 to 34.2 µg/L and was 0.6 to 1.4 µg/L in reference habitats. It is notable that the water concentrations of selenium in habitats exposed to selenium from coal mines are higher than those observed in the Belews Lake case study. These selenium concentrations at exposed habitats are also well above current water quality guidelines that have been established to protect wildlife, including waterbirds (i.e., 2 µg/L in British Columbia and the federal 1 µg/L). The authors attribute the observation of typical egg hatchability and high overall waterbird productivity to the low transformation rates of selenium in these lotic habitats. It was also noted the theoretical models over-predicted the egg concentrations of selenium, based on the observed water concentrations of selenium. This case study illustrates that bioaccumulation can be a highly site-specific process, and that animal tissues and populations can remain unaffected, despite high ambient selenium levels (due to anthropogenic and natural sources), which exceed government guidelines.

Environmental Releases of Selenium from the Petrochemical Industry

For activities related to the use of coal, it is appropriate to briefly consider the petrochemical industry. The refining of petroleum and petroleum products leads to the release of selenium to the environment. For example, a large oil refinery on the St. Lawrence River in Montreal reported a processing rate > 15,000 m^3/d of crude oil. At this site, the total selenium released to the river during 1992 averaged about 110 g/d in 12,000 m^3/d of wastewater (MSSC, 1996). Past release rates of selenium were reported as significantly higher for this refinery. In recent years, efforts have been focused on reduction of selenium in wastewater from the petrochemical industry. The long-term contribution of selenium to the environment from such activities is not known, as the analytical methods to quantify selenium in petrochemicals were recently developed (e.g., Walker *et al*. 1976). In addition, studies have indicated the natural bacterium *Thauera selanatis* as well suited for the remediation of petrochemical industry wastewater with selenite (Lawson and Macy 1995).

Consequences of Agricultural Runoff through Seleniferous Soils to a Reservoir

The identification of problems at the Kesterson Reservoir in California in 1983 has been regarded as the landmark case of widespread adverse ecological effects associated with the translocation of selenium in an aquatic environment (e.g., Eisler, 1985). This reservoir collected the runoff from large areas of irrigated agricultural land in a region of naturally seleniferous soils. The reservoir received substantial loads of selenium, primarily as selenate, along with nutrients, pesticides, and other substances that could play a role in the occurrence of ecological impacts (as reviewed in Table 5.2). The net effect was significant. It is suspected that many species of fish were extirpated from the reservoir, and that the reproductive impacts on aquatic bird species were widespread and significant.

The Kesterson discovery led to significant research, and an understanding of the potential for selenium-related impacts to biota as a result of food web accumulation. This research helped clarify the biogeochemical cycling of selenium, and also the potential implications to

wildlife. Research at the Kesterson Reservoir by the USGS showed that selenium entering the waterways as selenite could be 100% assimilated into an invertebrate (i.e., clam) tissue if phytoplankton acted as a food source. This could result in food web exposure of wildlife (especially birds) to toxic levels of selenium, even if initial levels in the water were acceptable from the perspective of protecting aquatic biota [i.e., tissue levels in birds of 10 µg/g with initial water column concentrations of <0.2 µg/L) (or <0.0002 mg/L]. In response to this unique case, much research has been undertaken to understand the toxic implications of selenium in the environment. These studies have shown that when habitat conditions differ from those encountered in the Kesterson reservoir case, selenium impacts might be much different (e.g., U.S. DOI, 1998).

Partly in response to the Kesterson discovery, the U.S. Fish and Wildlife service conducted monitoring of selenium in the environment at numerous locations that receive agricultural run-off. For example, Table 5.3 includes a summary of the findings of one such monitoring program at sites in Wyoming (Dickerson and Ramirez, 1997). The table demonstrates an example wherein ambient levels exceeded suggested thresholds, but bioaccumulation did not result in significant elevation of selenium in biological tissues. Overall, the sites considered in Table 5.2 reveal that the uptake and BAFs for selenium vary significantly and this provides additional evidence for the need to complete site-specific assessments.

Exposure of Avian Wildlife in Arctic Canada to Selenium

A long-term study of contaminant levels in wildlife in the Canadian Arctic (INAC, 2003) found that mean levels of selenium in the livers of several species of waterfowl were above the threshold considered as indicators of possible reproductive impairment (i.e. >9 mg/kg ww, or ~40 mg/kg dw). Concurrent observations of avian health did not indicate that the health or reproductive capacity of these bird species had been impaired. Another study of levels of metals in wildlife in the Canadian Arctic (Fisk *et al*., 2005) found some instances where the selenium tissue levels in sea ducks in pristine environments were above established tissue-based toxicity thresholds. Similarly, Braune and Malone (2006) reported that selenium levels in the livers of bird species sampled in the Arctic were approaching levels of concern. However, there was no evidence of any adverse effects with these occurrences of elevated tissue selenium. These cases show that selenium may accumulate in animal tissues above the established thresholds without obvious impact, even in environments without major anthropogenic sources. Further studies of avian wildlife is warranted in the high north, to better understand the bioaccumulation dynamics of selenium, as it is feasible the effects may have been overlooked in these studies.

Uranium Mine in Utah, USA

A study of environmental conditions in a stream near a uranium mill site in Utah found that levels of both molybdenum and selenium were elevated in the receiving environment relative to a reference environment (Peterson *et al*., 2002). Selenium was found to be present in the tissues of benthic invertebrates at a concentration that was greater than the

defined dietary benchmark (i.e. a NOAEL of 0.66 µg/g ww) for an avian receptor (i.e., swallow, *Hirundo rustica*). However, the assessment completed in this study determined that there were no significant risks associated with the exposure pathway.

Metabolism as an Indirect Route for Selenium Toxicity

Alternate pathways for selenium toxicity to aquatic species were identified (U.S. DOI 1998). For example, Palace *et al.* (2004) reported a pathway for toxicity of selenium stemming from the metabolism of selenomethione in rainbow trout (*Oncorhyncus mykiss*) embryos in the laboratory. As noted in Section 4.0, the stress in these rainbow trout is due to methioninase enzyme activity in the embryo that removes methylsenol from L-selenomethionine. The process causes oxidative stress through the creation of superoxide radicals that lead to tissue lesions frequently observed in birds and fish exposed to selenomethione. Vidal *et al*. (2005) reported comparable results for rainbow trout fed a concentration gradient of selenomethionine. For this latter study, loss of body weight and reduced growth rate were also documented. This relatively simple mechanism acts to explain the presence of lesions across diverse taxa and habitats. Interestingly, recent studies, yet to be formally published, have revealed an inability to reproduce these latter findings involving growth depression of rainbow trout at low concentrations of selenium (P. Chapman, Golder Associates, pers. comm.).

Distribution of Selenium in Northern Saskatchewan

Compilations of data regarding selenium distribution in the aquatic environments of northern Saskatchewan were completed recently (EcoMetrix, 2005a; Toll, 2005). These analyses identified natural gradients in selenium concentrations across different lakes that can be attributed to the unique geology of northern Saskatchewan, particularly the expansive Athabasca Sandstone region. This geology has been well described by the Geological Survey of Canada (refer to www. http://gsc.nrcan.gc.ca/; Figure 5.1). The existence of a natural range of selenium concentrations across lakes in this region represents an opportunity for learning (Carpenter, 1990) about the consequence(s) of variable concentrations of this element on these ecosystems. Specifically, an analysis of the selenium in water from different lakes compared with the selenium in the tissue of resident fish represents an opportunity to resolve the background relationship(s) that may exist for selenium bioaccumulation and exposure for these habitats. In addition, the analysis of these lakes and corresponding fish populations is predicated on the observation the populations are regarded as healthy and self sustaining. Resident fish, like lake whitefish and northern pike, represent species with contrasting ecology and habitat use. If a simple dose-response relationship exists for these lakes and fish populations, a linear relationship should exist between the concentration of selenium in water and fish tissue. However, if this relationship is not observed, the differences can likely be attributed to species-dependent processes rather than geographic or other factors.

The data set for lakes in northern Saskatchewan in the Athabasca Sandstone region show a range of selenium in water of 0.1 to 3.0 µg/L. Analyses done in these lakes over the last decade have identified many selenium concentrations in water that were at or near the method analytical detection, currently at 0.1 µg/L but previously at 0.5 and 1.0 µg/L. For these lakes, the lake whitefish and northern pike tissue concentrations of selenium ranged from 1.0 to 10.9 mg/kg dry weight with an analytical detection limit at 0.5 mg/kg (Fitzgerald *et al*., 2006; Figure 5.2). Review of this relationship shows that no linear pattern(s) are evident, although the random scatter for both species suggests differences in accumulation of selenium between the species. Thus, strong evidence exists to indicate species-dependent accumulation and depuration of selenium in northern Saskatchewan lakes that are dependent on selenium concentrations in water above a minimum threshold value (Fitzgerald *et al*., 2006). Although this evidence is strong, additional investigations of this relationship are warranted for other species and locales.

Synthesis of information from the fish populations in lakes with a natural range of water selenium concentrations can be used to understand the response of fish to effluent from a milling operation in northern Saskatchewan, in comparison to a reference lake in the Athabasca Sandstone region. For the mill site, the selenium concentrations observed in a downstream lake varied from a low near 0.001 µg/L in the 1980s to a high of about 0.02 µg/L in1997, then declined to about 0.002 during 2004 (EcoMetrix 2005a, Figure 5.3). Through this time period, the selenium in northern pike tissues in the downstream lake peaked in 1998 and declined to a value comparable to fish from a reference population in 2004 (Figure 5.4). This monitoring data set suggested that low concentrations of selenium in water can lead to elevated concentrations of selenium in fish tissue. The data suggested, and the modelling confirmed, that selenium concentrations as low as 0.3 to 0.5 µg/L in water can induce concentrations in fish tissue of about 7.9 µg/g (dw), the U.S. EPA (draft) guideline value (EcoMetrix, 2005a; Fitzgerald *et al*., 2006). In addition, the data reveal that the process of depuration of selenium from fish tissue, at least for northern pike, can occur at a relatively rapid rate when the concentration of selenium in water declines.

This decline in selenium in the northern pike tissue in the downstream lake corresponded to a reduction in the water-borne concentrations of selenium due to recent changes in activities at the milling operation (EcoMetrix, 2005a). The observation of a rapid transition of selenium tissue concentrations in northern pike from elevated levels to values similar to reference lakes confirms that physiological factors can likely influence the potential body burdens over short-term periods through the reduction of selenoproteins in tissue concentrations. It is important to identify that these wild fish populations from northern Saskatchewan do not overtly show any pattern of deformities stemming from elevated selenium concentrations. The monitoring of this downstream lake identified no clear teratogenic effects in the fish related to selenium tissue content. An important facet of the noted study is that the age and sex of the fish was not included in the analysis, so the role of age and maternal burden of selenium needs to be considered further in future studies.

Detailed laboratory studies were completed recently in northern Saskatchewan that provide some resolution of the dose-response relationships between selenium in water and fish tissue concentrations noted for this northern ecosystem. These studies provide resolution among the role of the effects of habitat, maternal influence and water-borne selenium on the fish (De Rosemond *et al*., 2005; Muscatello *et al*., 2006; Bennett and Janz, 2007). Further, they help clarify the survival rates of embryos, the severity of larval deformities, and the consequences on fry and young-of-the-year (YOY). Only the most relevant study will be considered here in detail. Specifically, Muscatello *et al*. (2006) used a laboratory study with a design that simultaneously considered the maternal influence on embryos and subsequent exposure to a gradient of water-borne selenium to resolve an effects threshold for fish in lakes downstream of a milling operation. This design represented a two-way (crossover) analysis of variance (ANOVA) and successfully resolved toxicity endpoints for embryos and larvae of northern pike. Trace metal analyses of the eggs revealed selenium was the only substance that was elevated for fish downstream of the mine relative to reference fish. This analysis identified an increase of larval deformities (skeletal, craniofacial and fin abnormalities) and edema that was linked to the selenium load of the females. That is, selenium loads in female muscle tissue of 16.58 and 38.27 µg/g dry weight (dw) were associated with 31.28 and 48.23 µg/g egg dw, respectively, and resulted in an increased rate of deformities and edema in larvae. The effects threshold for a 20% increase in larval deformities and edema above background was 21.54 µg/g dw in muscle and 33.55 µg/g in eggs. This study design also resolved a minimal role for the gradient of selenium in the water downstream of the mine directly on the developing embryos in terms of survival rates and incidence of deformities and edema. This study by Muscatello *et al*. (2006) indicates that the habitat used by fish in northern habitats, such as pike, will impart a selenium load to muscle tissue and to developing eggs, and this can lead to increased rates of larval deformities and edema if a threshold concentration is exceeded. The previous observations noted by EcoMetrix (2005a) indicate that rapid depuration of this selenium is possible over short time periods in northern fish species. Thus, it is important to assess the selenium signature in the muscle and eggs of the female fish just prior to spawning activity in order to fully resolve the response(s) that may occur following selenium exposure.

Observations of selenium in different compartments of lake ecosystems downstream of uranium operations in northern Saskatchewan, as reported in the routine monitoring studies (e.g., EcoMetrix, 2005a, Toll 2005), illustrate several key processes of distribution and partitioning. First, there can be considerable regional variability in how selenium behaves in lakes that differ in physical characteristics. Second, selenium can be present in the water column in very low concentrations and show differences between lotic and lentic habitats. These water concentrations lead to concentrations in associated media, such as sediment, that are well in excess of suggested thresholds (e.g., selenium in sediments at ~20 µg/g in two lakes). Third, the time trends for selenium in water and sediment have identified relatively rapid changes over time, consistent with the loadings in effluents. Fourth, clear species-dependent accumulation patterns and BAFs for selenium are evident in the plants and vertebrate species considered. However, comprehensive risk assessments conducted for two operations (EcoMetrix 2005a, 2005b), based on toxicological profiles and effects

monitoring data, suggest that there are no adverse effects occurring as a result of increased concentrations of selenium available to the aquatic receptors even though fish tissue concentrations are elevated.

Studies in the Elk River Valley, British Columbia

As noted, land disturbance in the Elk River Valley such as coal mining were observed to elevate the natural concentrations of selenium in the water of this watershed (e.g., Chapman, 2005). Past quantitative studies in the Elk River Valley involving selenium in fish tissue identified a gradient in selenium concentration for surface waters. This gradient is particularly evident in northern ecosystems (such as northern Saskatchewan) and across British Columbia in particular (Frankenberger and Engberg, 1998; Kennedy *et al*., 2000; SGS, 2003; De Rosemond *et al*., 2005; Simmons and Wallschläger, 2005; Tri-Star, 2005; Golder, 2006; Bennett and Janz, 2007). By contrast, different patterns of selenium accumulation are observed in more southern ecosystems such as those in the southwestern U.S.A. (e.g., Lemly, 2002). The existence of a relatively large gradient of selenium concentrations in surface waters over relatively small areas represents a natural experiment and opportunity for learning (e.g., Carpenter 1990).

One route forward to understand the role of a natural gradient of selenium across aquatic ecosystems is to use a general hypothesis to explain the dose-response relationship for resident fish. The null hypothesis that can be used to frame a study is that a typical (i.e., linear) dose-response relationship would be observed between selenium water concentrations in reference lakes, with a range of concentrations of selenium in water and fish tissues (e.g., Toll *et al*., 2005). Sometimes, the lower end of such a relationship shows a plateau that may be due, in part, to the presence of the selenoproteins (selenomethione) in the tissues independent of low selenium concentration of the water. The general relationship and explanatory hypothesis for selenium exposure and resulting concentration of selenium in tissues as a linear process was presented (Figure 3.1). Such a relationship was expected to be observed for the fish tissue concentrations associated with a natural gradient of selenium concentration in reference lakes located across the Athabasca basin of northern Saskatchewan, as observed in other southern locales (Lemly, 1997, 2002; Toll, 2005). As noted, these fish did not show the expected linear dose-response relationship (Toll, 2005). These unexpected dose-response relationships indicated the concentrations of selenium were similar for the species, and identified that this relationship of accumulation is not necessarily dependent on the life history (feeding, movement, physiology) in this northern ecosystem (Naiman and Latterell, 2005).

Elk Valley Studies: Recent Investigations

The nature of the observed responses in the fish of Elk Valley may be similar to fish from northern Saskatchewan, and linked to habitat conditions. Such views stem directly from detailed studies of this habitat (e.g., Golder, 2007). The identification of the response of fish to both local land use and watershed-level processes has been a common occurrence

(Naiman and Latterell 2005). A suite of studies reported that the selenium tissue concentrations of WCT does not necessarily reflect the changes in water-borne selenium compared with reference conditions, and with some baseline studies associated with recently modified habitats (EVS, 2005; Golder, 2006; Golder, 2007). The lack of a clear dose-response between selenium in biological tissues from birds and fish and increased selenium water concentrations at different sites in the Elk Valley is analogous to the studies in northern Saskatchewan (Figure 2.4). This observation in the Elk Valley possibly suggests a scenario involving the rapid depuration of selenoproteins, as noted in northern Saskatchewan (Figure 2.5). Fish sampling of wild westslope cutthroat trout (WCT; *Oncorhynchus clarki lewisi*) and longnose sucker (*Catostomous catostomous*) in different habitats of the Elk Valley have yielded a paucity of deformed fish, and this is also similar to northern Saskatchewan.

Other studies in the Elk Valley considered the role of water-borne selenium on the presence of deformities in larval fish, in a similar manner as the studies in northern Saskatchewan. Previously, Kennedy *et al*. (2000) reported no significant increase in larval deformities in WCT at egg selenium concentrations ranging from 8.7 to 81.3 µg/g dw (mean 21.2 µg/g). In more recent work with WCT, Rudolph *et al*. (2007) reported non-viability of eggs with selenium concentrations >86.3 µg/g dry weight, and successful fertilization and development to the eyed embryo stage, but no hatching success, at egg selenium concentrations between 46-76 µg/g dw. These results were unusual in that previous studies have not reported significant effects of selenium on fertilization or hatching success in fish (reviewed by Lemly 2004c). In addition, Rudolph *et al*. (2007) reported no relationship between egg selenium concentration and the frequency or severity of characteristic selenium-associated larval deformities (i.e., skeletal, craniofacial and fin). In contrast, Rudolph *et al*. (2007) reported that the threshold for WCT embryo survival was 46 µg/g dw. The disparity between the viability of embryos noted in these two studies cannot be readily explained.

Similar to the Kennedy *et al*. (2000) study, Holm *et al*. (2005) reported no increase in larval deformities in brook trout (*Salvelinus fontinalis*) collected from two sites in Alberta with mean egg selenium concentrations of 6.6 and 7.8 µg/g wet weight (approximately 16.9 and 20.0 µg/g dry weight based on 61% moisture). However, Holm *et al*. (2005) also reported that larval deformities were elevated in rainbow trout (*Oncorhynchus mykiss*) at a threshold (i.e. EC15) egg selenium concentration of between 8.8 - 10.5 µg/g wet weights (22.6 – 26.9 µg/g dry weight). As noted, a similar egg selenium threshold (EC20 = 33.6 µg/g dry weight) was reported in northern pike collected from areas receiving mine discharge in northern Saskatchewan (Muscatello *et al*., 2006). Taken together, these studies involving coldwater fishes illustrate the need for further investigation of potential selenium-related early life stage toxicity endpoints. Thus, for future studies to resolve effects threshold for selenium, they must use a suitable two-way ANOVA to resolve the role of the key variables: maternal source and site water. Also, the studies reported for northern Saskatchewan that demonstrated no or unexpected responses (those prior to 2006) were comparable to the observations previously reported for the Elk Valley over the last decade.

As a consequence of the past studies in northern Saskatchewan and the Elk River Valley, a suite of observations have been identified along with unclear dose-response relationships for selenium exposure to fishes. First, the observations identify these fish do not conform to the patterns observed in southern locations for selenium accumulation in tissues, so site-specific analyses are warranted to understand these chemical partitioning and effect thresholds. Second, the role of different selenium partitioning patterns in flowing compared with standing water habitats must be explicitly considered in the analysis, as these physical processes strongly influence the fraction of selenium available to exposed fishes, even at low concentrations of selenium (e.g., Simmons and Wallschläger, 2005). Indeed, the pronounced difference in the fate and potential impact of selenium across habitats generally has been reported, and this particularly applies to sites within the Elk Valley (Kennedy *et al*., 2000; Hamilton and Palace, 2001; McDonald and Kennedy, 2002). Third, the unclear dose-response relationship between selenium concentrations of unfertilized eggs and the subsequent survival rates of embryos requires focused investigations. Fourth, the role of fluctuating tissue levels in species such as fish deserves further study (Chapman, 2007).

In summary, the observations from fish in northern Saskatchewan and northern habitats such as the Elk River Valley along with the other studies has revealed that site-specific and species-dependent relationships exist for bioaccumulation of selenium in fishes, and that this pattern is particularly clear for northern habitats. These observations need to be integrated within modeling exercises focused on management of water-borne selenium concentrations. Further, this awareness, provided by past studies, generates information that can be used to frame future studies, and test hypotheses to better resolve the effects threshold for selenium exposure on northern fish species. The proposed future studies for Elk Valley, scheduled for 2008, will focus on the processes involved in the transformation, mobility, and bioavailability of selenium and other environmental parameters that determine fate and effects to WCT in this complex ecosystem (P. Chapman, pers. comm.).

It is this awareness and the natural gradient of selenium in the Elk Valley that provides an opportunity for learning within this managed environment and experiment (Walters and Holling, 1990; McCarty, 2002). This awareness needs to be extended to other environments where the natural selenium concentrations have been enhanced due to anthropogenic or natural activities, or a combination thereof.

Summary

Consideration of these case studies for different sites and species acts to identify some key findings. These include:

- the environmental fate and degree of uptake of selenium is highly dependent on the specific conditions and circumstances experienced at a given site;
- the presence of selenium at concentrations exceeding reported thresholds in ambient media does not necessarily translate to elevated levels in biota;

- the presence of selenium in biological tissues at levels that exceed suggested thresholds is not necessarily indicative of the occurrence of adverse effects on biota; and
- the presence of selenium at concentrations exceeding reported thresholds in ambient media does not necessarily translate to elevated levels in biota.

5.2 Molybdenum

5.2.1 Existing Guidelines

Molybdenum is ubiquitous in the environment and an essential element required for life by microorganisms, plants, and animals. Because molybdenum can accumulate to toxic levels in exposed species, numerous regulatory criteria have been established to minimize the occurrence of adverse effects in Canada. These regulatory guidelines are in step with those identified by other countries and groups (e.g., USA, WHO, 2006, also see below).

The Canadian environmental quality guidelines for molybdenum in water (CCME, 2002) are as follows:

- 73 µg/L (or 0.073 mg/L) in freshwater to protect aquatic life,
- 10 – 50 µg/L (or 0.010 to 0.050 mg/L) in irrigation water, and
- 500 µg/L (or 0.5 mg/L) for livestock watering.

Numerous agencies have developed threshold levels that are intended as general limits of exposure beyond which impacts may occur. The regulatory guidelines and exposure thresholds for physical media, animals and plants for Canada were previously noted (Table 4.4). When these guidelines are contrasted with comparable information from the USA (U.S. DOI, 1998), it reveals that there are considerable differences in responses across species of animals to molybdenum in the environment. Thus, it is important to understand these responses when assessing potential trigger points that may cause health effects. It is also important to exercise caution when comparing different animal groups such as ruminants, rodents and birds as their gut morphology, physiology and microflora affect how trace metal uptake and metabolism occurs (e.g., Eisler 1989). Also the behaviour of the animals will determine their exposure and will vary across habitats and seasons. Differences will be particularly clear between resident versus migratory species.

The relative lack of toxicity from molybdenum exposure is tacitly indicated by the absence of this element in Health Canada's (1996) Guidelines for Canadian Drinking Water Quality. This absence in national guidelines led to the identification of Provincial guidelines, for the protection of aquatic life. For example, Fletcher *et al*. (1997) recommended an interim molybdenum guideline in freshwater of 73 µg/L. This value was derived from the lowest

observed chronic toxicity value of 0.73 mg/L for rainbow trout (Birge, 1978) then scaling it with a safely factor of 0.1 factor from this LOAEL.

Disparity exists between the Canadian and Provincial guidelines for molybdenum. For example, in British Columbia, the 0.05 mg/L criterion is low relative to other regulatory benchmarks. This criterion reflects a series of very conservative assumptions that are intended to result in levels of molybdenum exposure that are protective of even the most sensitive species. These assumptions for characterizing exposure include very high rates of ingestion involving both food and water ingestion (typical of lactating dairy cows in commercial dairy operations). An additional assumption is that the animals are also exposed to molybdenum in the food that may also contribute to a state of molybdenosis (B.C. MOE, 1986; CCME, 1987). Further, the BC criterion reflects the absolute lowest levels of total molybdenum intake that might cause a minor effect in sensitive animals. No consideration is given to the molybdenum form or its status relative to copper or sulfates in the animal diet or drinking water that may modify molybdenum toxicity. If the assumption of molybdenum contribution through food is removed, the British Columbia criterion would increase to 0.08 mg/L. If typical water intake rates for cattle are considered (i.e., 75 L/d vs. 205 L/d), the criterion would further increase to about 0.2 mg/L for this sensitive species. At this level, although still considered conservative, the BC criterion approaches criteria that have been accepted in other jurisdictions (e.g., the CCME guideline of 0.5 mg/L).

The benchmark for molybdenum in mammals is derived from multigenerational testing on a strain of laboratory mice bred to be free of potentially toxic metals, who continuously were given water containing 10 mg/L molybdenum (Schroeder and Mitchener, 1971). The adverse effects were observed mainly in the third generation and consisted of failure to breed and mortality of young. It was noted in the concluding comments that “partial breeding out of the strain (of mice) given molybdenum was unexpected, for no adverse metabolic effects have resulted from this dose fed to rats for life”. These results and comments would suggest that the observations may not be expected in other species, especially wild species that are generally much more resilient than laboratory strains.

The NRC (1984) set a limit of 6 mg/kg of molybdenum in commercial feed as the maximum tolerable limit for cattle (also see Schalscha *et al*., 1987). Further, because molybdenum toxicity is dependent on available copper levels, the simultaneous presence of sulfate can counteract potential toxic effects of molybdenum. This observation is predicated on various studies that suggested copper:molybdenum ratios less than 4 are likely to produce copper deficiencies (Schalscha *et al*., 1987; Eisler, 1989). The maximum tolerable level of molybdenum for long-term grazing is 10 to 100 mg/kg in forage. By contrast, the general limit for molybdenum in forage matter for livestock protection is 10 mg/kg (NRC, 1980).

Since some plants readily accumulate molybdenum, the toxicity thresholds are quite variable. Generally, any concentration of molybdenum in soil greater than 100 mg/kg is considered toxic. This trend of accumulation and tolerance to molybdenum by plants has led to regulatory limits for soils, sewage, compost, and related products.

Soils, Processed Sewage, Compost and Related Products

The Government of Canada has identified guidelines for molybdenum in soils that are analogous to those for selenium. These guidelines identify a maximum cumulative addition of molybdenum to soils as 4.0 kg/ha and the maximum concentration in soil as 20 mg/kg (dw). This guidance applies to the following media that may be applied to soils: processed sewage waste, compost, raw or processed manure, and other similar products (e.g., sewage residues, fertilizer, supplements; also see www.inspection.gc.ca/ for additional details). In addition, O'Connor *et al*. (2001ab) presented a risk assessment that identified molybdenum standards for the application of biosolids to different land types.

5.2.2 Case Studies

Some mining operations in North America have elevated levels of molybdenum in effluent waters or in solid wastes, such as tailings and rock piles (Ward, 1978). The combination of elevated levels in waste rock, tailings and water together with, in some cases, high natural background levels, has resulted in elevated levels of molybdenum in terrestrial plants. This has resulted in some impacts, reported by ranchers, for domestic cattle grazing on these lands. The case studies reported here focus on examples associated with mining sites in Canada and other related information.

Toxicity of Molybdenum to Domestic and Wild Animals

As noted, elevated levels of molybdenum have been shown to be toxic to some animals, particularly ruminants. The mode of action involves molybdenum limiting the absorption of copper, an essential metal, in the digestive tract. This interference can lead to molybdenosis, which has been linked to serious adverse health effects and, in some cases, may be fatal to the animal (Eisler, 1989; U.S. DOI, 1998; NAS, 2003). The documented occurrences of molybdenosis were found in domestic livestock or wildlife, such as moose, feeding on lands with naturally elevated levels of molybdenum. In these situations, it is common to have naturally alkaline soils that result in conditions that make molybdenum readily available. Soils affected by waste mine rock are commonly treated with alkaline materials (e.g., lime) to counteract any acidic drainage. However, this process was shown to negatively alter the copper:molybdenum ratios in terrestrial plants to potentially toxic levels (Erdman *et al*., 1978; Opresko, 1993b).

Brenda Mine Example

The Brenda copper-molybdenum mine, located near Kelowna, British Columbia, opened in 1970 and exhausted the ore reserves in 1990. Site reclamation was conducted, based on studies that involved a careful analysis of molybdenum partitioning in the environment (Taylor and McKee, 2003). This analysis considered vegetation, tailings, and mule deer faeces. Sampling identified that the mean copper:molybdenum ratio in the vegetation ranged from a low of 0.06 in sainfoin (*Onobrychis viciaefolia*) to 0.16 in willow (*Salix* spp.) compared to 2.6 in the tailings (Taylor and McKee, 2000). In contrast, the mule deer faeces

collected on the site showed copper:molybdenum ratios of 0.1:1 to 4.2:1. This range of ratios identifies large variation in the mass of copper and molybdenum that is defecated by these ruminants. Monitoring studies also identified that all the deer at the mine site appeared to be in good health, and individuals breeding on site produced apparently healthy young. In terms of exposure, these mule deer arrive in the spring (June), during the period of rapid vegetation growth, and then spend approximately four to five months there. After this time, the deer descend to the valleys to winter.

Moose also reside at the Brenda Mine site and use both the aquatic macrophytes in several ponds and consume browse. This feeding on macrophytes by moose is common from June through mid-September (Taylor and McKee, 2000). At this site, leaves from deciduous trees and shrubs are the common summer diet of moose while aquatic forage makes up the remaining 25% of the diet. Monitoring data indicated the moose did not show any symptoms of molybdenosis, and were observed with calves indicating that reproduction was occurring (Taylor and McKee, 2000). In spite of a considerable number of hours of observation, the ruminants did not show any symptoms of molybdenosis, and no dead or dying animals were found. If animals were sickened by molybdenum toxicity one would have expected to find remains of dead animals or carcasses from cougar kills, but none were found in spite of observations of cougar from the site (confirmed by tracks and photographs; Taylor and McKee, 2000).

Highland Valley Mine Example

The Highland Valley copper mine is an active site located south of Kamloops, British Columbia. This is the largest base metal mine operating in Canada and processes rock that generally contains about 0.40% copper and 0.006% molybdenum (see www.teckcominco.com/operations/hvc/ for additional site details). Studies have shown the average recoveries for copper and molybdenum are 91% and 55% respectively, in processed ore. Hence, the presence of residual molybdenum in mine tailings is a concern.

Reclamation efforts at Highland Valley have been extensive. For example, in 1997, the mine was presented with the 1996 British Columbia Reclamation Citation Award for its reclamation activities. That year, the mine planted 700,000 native trees and shrubs on 1000 ha of reclaimed land. Work to re-establish aquatic species like fish stocks in different water bodies on the property is on-going. To date, over 2000 ha of land has been reclaimed and is routinely monitored for molybdenum concentrations in different environmental media (soil, water, animals).

The presence of molybdenum at this site has led to active treatment of mine effluent to reduce molybdenum releases to the environment. This treatment process involves the promotion of algae in tailing pits. Specifically, the algae incorporate the molybdenum from the water into their tissues, and then when these plants die, they settle to the bottom of the pit. This settling significantly reduces the water column concentration of molybdenum. This treatment approach to lower molybdenum levels is relatively simple and requires: 1) the

addition of a fertilizer, like phosphorus, to promote rapid growth of algae, 2) a deep pit, so the algae can settle out of the water column, and 3) removal of sediment-bound molybdenum from the pit. This approach is considered a low-cost option to reduce the molybdenum concentration but it is only practical for use in deep pits where the algae can settle to anoxic depths. Specifically, in shallow waters, like rivers, after the algae die, the molybdenum becomes oxidized and returns to the water column. This approach reflects understanding of reclamation methods used successfully at Island Copper Mine (see below).

Studies at Highland Valley have shown the routes for transformation of molybdenum by terrestrial plants on the mine sites. Investigations of alfalfa (*Medicado sativa*) grown on reclaimed land identified the actual uptake pathways for molybdenum. These studies also identified the typical chemical forms of molybdenum in these plants (Surridge *et al*., 2001). In addition, controlled experiments were used at Highland Valley to assess the risk of molybdenosis to cattle that forage at the site. The experiments extended over three years and identified no significant differences in weight gain, elevated liver molybdenum, or signs of copper deficiency in the cattle (Gardner *et al*., 2003). However, it is possible to argue that this result was confounded by the copper in the diet provided to the cattle. This finding is also consistent with the observation of no reports of molybdenosis in wild ruminants (and other mammals) at this site (Majak pers. comm., 2003; Majak *et al*., 2004)

Endako Mine Example

The Endako molybdenum mine is located west of Prince George, British Columbia. At this site, the mining occurs in three separate pits, so the potential for molybdenum exposure across large areas is high. However, no field evidence of molybdenosis has been identified in small mammals (Mathieu, 1995, 1996) or larger wildlife (Riordon, 2003). Studies of aquatic invertebrates and fish associated with this site revealed little risk from elevated molybdenum in water (Davies *et al*., 2003, 2005). Site reclamation activities are on-going and involve treatment of effluent (also see: www.endakomines.com/main2.htm).

Taseko Gibraltar Mine Example

The Gibraltar open-pit copper and molybdenum mine extends across more than 100 km^2, and is located north of Williams Lake, British Columbia. This mine operated from 1972 to 1998, and then reopened during October 2004 due to favorable copper and molybdenum prices. Currently, the mine extracts 15,000 tonnes of ore per day. The content of molybdenum in the ore at this site ranges from 0.008-0.010%. As a consequence, site activities include extensive groundwater and surface water monitoring along with treatment of mine effluent. The Gibraltar Mine is projected to produce 100 million pounds of copper by 2008 along with large quantities of molybdenum. Activities at this site have involved local First Nations peoples to resolve the consequences on mine activities on the surrounding ecosystems.

Marine Isolation of Mine Tailings with High Molybdenum Concentrations

It is feasible to dispose some mine tailings in the oceanic environment, and is referred to as submarine tailings disposal (STD). The now-closed Island Copper Mine, located at the northern end of Vancouver Island, used this option for disposal of their tailings. These tailings, which contained high molybdenum concentrations, were placed in deep water locations at the bottom of Rupert Inlet, and in an on-site pit filled with ocean water. This practice was conducted through the mine's entire life and continued during site reclamation (Island Copper Mine, 1997). The STD approach was used because it was assumed the tailings with high molybdenum concentrations and residual copper would be readily assimilated in the marine food web, as these two elements are present only in trace quantities in salt water (Howarth, 1988; Island Copper Mine, 1997). Because sulfate can inhibit the uptake of molybdenum in water by bacteria in sea water (Marino *et al*., 2003), it may directly inhibit the remediation of mine tailings high in molybdenum that are placed in marine environments. Recent studies indicate that benthic invertebrates colonized the mine tailings in Rupert Inlet after active deposition ended, and density and diversity of invertebrates is now at pre-mining levels; fish and shellfish populations have responded in a similar way (Welchman and Aspinall, 2000). Documents that detail this process and consider the consequences of STD for the Island Copper Mine site are available (see www.gateway.uvic.ca/archives/featured_collections/esa/fonds_island_copper_mines/default.html).

Molycorp Mine Example

Molycorp Inc. operates a large molybdenum mine in the southwest, near Questa, New Mexico, USA [refer to Nordstrom (2005) for full site description]. This site includes molybdenum mining and processing; tailings are transported from the mine via pipeline to a storage and treatment facility. Activities started at the site in the 1920s. During the 1980s, the mine started to collect groundwater and surface runoff from the site for treatment at an ion exchange plant. However, the periodic releases to the environment over time have led to very high concentrations of molybdenum and other metals in the groundwater, soil, and surface water in the vicinity of the mine. The site was placed on the U.S. National Priority List in 2000 and is now referred to as a Superfund site. This watershed, therefore, provides an interesting case study to resolve possible consequences of high molybdenum concentrations and other metals (e.g., aluminum, boron, iron, lead, magnesium, manganese, zinc) from the mine.

Routine studies in the vicinity of the Molycorp mine site resolved the time line of environmental degradation. The first baseline studies were done in 1966, just after the start of open-pit mining, and identified that the adjacent Red River received little impact from the mine. Surveys during 1971 noted some degradation of the river, due to periodic releases of tailings from the pipelines that run along the shore of the river. At that time, the absence of fish in the river in the vicinity of the mine was noted, where populations were previously abundant. By the early 1980s, large impacts were documented in the river, upstream and downstream of the mine. These impacts extended from water quality to benthic

invertebrates to fish (e.g., Lynch *et al*., 1988). In 1992, the State of New Mexico submitted a report that identified these major impacts in the river and the presence of elevated levels of metals, including cadmium, copper, lead, silver, and zinc. This report requested federal action to clean up the site. Other studies indicated a similar need for government action for this site given the broad extent of the degradation. For example, it was reported in the mid-1990s that at least eight miles of the Red River could be considered biologically dead due to the activities at the Molycorp mine (New Mexico Surface Water Quality Bureau, 1996). These studies were recently reviewed (Nordstrom, 2005). Today, the Red River is regarded as severely impacted from this mine's activities. Despite these impacts, the river near the mine is still used for recreation and the water is used for irrigation and livestock watering. Additional details are available (www.epa.gov/superfund/sites/npl/nar1599.htm).

The extensive environmental degradation near the mine motivated a study of possible human health consequences, as reported in ATSDR (2005). This study indicated that if consumption of contaminated groundwater resumed by residents, adverse health effects were likely to occur. These residents generally stopped drinking groundwater during the mid-1970s due to awareness of impacts to the Red River (New Mexico Surface Water Quality Bureau, 1996). An additional facet of the ATSDR (2005) study was the consideration of dust that moves downwind of the waste rock and tailings sites. Risk from the dust currently originating from the mine is considered minimal, as dust prevention measures were started in the early 1990s. Risk assessment from dust in the past from the mine was also completed. Based on the available but limited data, ATSDR (2005:19) stated: '…short-term adverse health effects, including eye and respiratory irritation and respiratory problems in sensitive groups, were possible during periods of high dust levels.' By contrast, the long-term consequences of inhaled dust from the site were not expected to have any adverse health effects. The Molycorp molybdenum mine continues to operate but is attempting to rehabilitate the environmental contamination and reduce additional impacts.

Summary

An understanding of the implications of molybdenum exposure at different mine sites is required since new mines will be developed, particularly because of the current record high commodity prices for molybdenum. One such site under development is the Preissac Molybdenum Mine, located near Cadillac, Abitibi County, Québec. The need to remediate molybdenum contamination at mine sites has led to the development of private companies that offer this service. For example, microbe and phytoplankton approaches are currently used (for an example see: http://www.microbialtech.com/water.html).

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

For both selenium and molybdenum, there is a clear potential for adverse effects on aquatic biota as a result of excessive loading to the aquatic receiving environment. There is also some potential for adverse effects on terrestrial biota that have a food web association with the affected water body. For both of these compounds, bioavailability processes occurring at lower levels (e.g., uptake into invertebrates and plants) have a great influence on exposure of higher trophic level animals.

Selenium is recognized as a highly bioaccumulative substance, whereas molybdenum is less so (U.S. EPA, 2004b). For this reason, there is a high potential for foodchain exposure to selenium present in the ambient environment, but a much lower potential for molybdenum.

For both selenium and molybdenum, uptake into plant tissues is generally not sufficient to cause plant toxicities, but risk to animals consuming these tissues and resulting specific toxic impacts (e.g., teart, molybdenosis; Eisler, 1989; Lemly, 2004c; McGeer *et al*, 2003). The documented occurrences of these specific impacts are frequently represented by livestock or wildlife foraging in areas with naturally elevated levels of selenium or molybdenum in soil. The role of irrigation water, as a vector that can move large concentrations of selenium and molybdenum across habitats over short periods of time, always needs to be considered in SSRAs.

Studies have shown different species are susceptible to selenium and molybdenum. For selenium, the toxicological profiles and case studies also reveal a potential for food web impacts to predatory fish as well as birds and animals that feed on any class of biota (plants, invertebrates, fish) from the aquatic environment. For molybdenum, ruminants are the most susceptible animals to toxicity effects. The potential also exists for similar but local impacts arising from exposure of animals feeding on aquatic vegetation with elevated concentrations in water.

For both of these compounds, accumulation at lower levels (e.g., uptake into invertebrates and plants) has a great influence on exposure and potential impacts on animals at higher trophic levels. This process clearly differs from biomagnification (i.e., a process that is associated with increasing concentrations of organic substances through three or more trophic levels through dietary uptake; Rand *et al*., 1995). Specific environmental management strategies for preventing such effects need to reflect key aspects of environmental fate and toxicity of these two elements. For selenium, the food web exposure concerns biota of all classes and trophic levels. For molybdenum, the key concern is the ingestion of aquatic plants by certain herbivorous mammals.

6.1.1 Selenium

The following major aspects of the environmental fate and toxicity of selenium are key factors to consider in the determination of environmental management criteria (release limits, environmental quality objectives, monitoring, remediation).

- The biogeochemistry of selenium is complex and variable, and highly dependent on the interactive influence of a number of site-specific characteristics. Key factors in aquatic environments include productivity, water depth, flow rate, and content of sediments.
- Concentrations of selenium in water are proportional to sediment concentrations; increases or decreases of selenium in the water will likely be reflected within a period of months to years in the sediment load.
- Selenium is highly bioaccumulative, although not subject to biomagnification. The degree that selenium is taken up by various types of biota is also highly variable, and strongly influenced by biogeochemical dynamics. Uptake is also dependent on the food web and diet composition of organisms.
- The exposure and potential impacts of selenium on higher trophic level animals are greatly influenced by the bioavailability processes at the base of the food web (e.g., uptake into invertebrates and plants) and this can have a great influence on exposure and risk to higher trophic level animals.
- The potential for adverse effects due to selenium toxicity are primarily dependent on the degree of accumulation in target tissues of the affected organisms.
- There are well documented cases where selenium has accumulated significantly in animal tissue, causing measurable and ecologically-meaningful effects, often on the reproductive success of the exposed organism. These cases (e.g. Kesterson Reservoir) represent extreme circumstances.
- Current regulatory limits and exposure threshold recommendations for selenium in the aquatic environment are very low. They are typically derived either from study of these extreme cases, or from laboratory studies.
- Examination of other exposures to anthropogenic releases of selenium under conditions different from the extreme cases suggests that environmental concentrations of selenium can exceed limits without evidence of effects.

The regulatory limits currently in effect for selenium in water or sediment are based on assumptions of high rates of uptake. Given the degree of variability and uncertainty that exists regarding uptake, and the relatively narrow range between levels of selenium that are sufficient and those that are toxic, the application of these guidelines to some cases is extremely conservative. For this reason, the most effective approach to the management of

environmental implications of selenium is to develop site-specific criteria. These analyses need to include multi-season analyses and migratory species

6.1.2 Molybdenum

The environmental implications of molybdenum in the aquatic environment are well-defined relative to those from exposure to selenium. The toxicity of molybdenum to aquatic species is well documented. Unlike selenium, molybdenum does not readily undergo biomagnification and the potential for significant food web exposure of animals of high trophic status is low. The key pathway of possible concern is the uptake of molybdenum by ingestion of aquatic vegetation and incidental ingestion of lake sediment by sensitive herbivores (i.e., ruminants). This recognition and complementary experimental studies led Davies *et al*. (2005) to encourage a review of the toxicity guidelines for aquatic species. Thus it is likely that revised government guidelines will be produced in the near future.

Available literature and case studies reviewed herein suggests that a conservative BCF for aquatic macrophytes is in the order of 3,000 L/kg (dw). This is representative of the upper end of the range. The NRC (1976) suggests a dietary limit of 10 mg/kg in feed for the protection of sensitive species of livestock.

6.2 Recommendations

6.2.1 Site-specific Selenium Criteria

Numerous authors (e.g., McDonald and Strosher, 1998; Simmons and Wallschläger, 2005) recognize that generic criterion for selenium are not well suited to all sites, and recommend the development of site-specific guidelines or limits in the aquatic environment. The current regulatory guidance (e.g., CCME) also acknowledges that site-specific modifications of generic criteria are appropriate for selenium.

To determine a site-specific release limit, acceptable ambient concentrations are first identified, and then the release limit is estimated as a function of that concentration and the anticipated levels of effluent assimilation in the receiving environment. In both cases, the process is primarily oriented to determining what level of selenium may exist in the specific waterbodies in question without an expectation of measurable adverse effects.

The following is an outline of a tiered approach for determining acceptable ambient levels or releases limits for selenium in the aquatic environment in a site-specific manner. The approach is broadly consistent with approaches documented in the literature (Lemly, 1987; Adams *et al*., 2000; Brix *et al*., 2004; Toll *et al*., 2005). Indeed, this tiered, risk-based approach has rapidly evolved in the last decade, based on recent field observations of organismal responses and a greater awareness of different modifying factors that shape toxicity. This approach follows the principles and specific techniques of risk assessment (e.g. Chapman and Wang, 2000). It contains several steps that are conceptually similar to

the step-wise process for determining Total Maximum Daily Load limits (TMDLs) within the US EPA's regulatory framework, as detailed previously (e.g., Lemly, 2001a,b, 2004b).

Step 1 - Site Characterization

The extent of biomagnification of selenium that may occur in any given location is largely dependent on habitat type. For a given level of selenium in the water column, the end result in terms of tissue concentrations in wildlife will be much lower in some habitats than others. In particular, the pronounced difference in the fate and potential impact of selenium between lentic (slow flowing) and lotic (fast flowing) systems has been well documented (Adams *et al*., 2000; Hamilton and Palace, 2001; Harding *et al*., 2005). A review conducted by Simmons and Wallschläger (2005) examined the environmental dynamics and potential implications of selenium in lentic and lotic environments. In this review, it was concluded that lentic environments exhibit a propensity for the uptake and subsequent food web transfer of selenium that is minimally ten times greater than that in lotic environments.

The potential importance of habitat requires key variables to be effectively characterized prior to the determination of a site-specific selenium criteria. In following TMDL process (see Lemly, 2001a), the key site attributes can be assessed and assigned a ranking relative to the retention and biomagnification of selenium in the system. This characterization scheme focuses on productivity, flow, and sediment type and the ranking system is as follows:

1. productivity: oligotrophic (ranks low), mesotrophic (ranks medium), and eutrophic (ranks high);
2. flow: fast (low), moderate (medium), and slow or near-zero flow (high); and
3. sediment type: mineral (low), mixed (medium), organic (high).

An overall rating for selenium retention and bioaccumulation potential of a water body is determined by combining the three factor ratings as follows:

- 3 low ratings = low
- 2 low and 1 medium = low
- 2 low and 1 high = medium
- 2 medium and 1 low = medium
- 2 medium and 1 high = medium
- 3 medium ratings = medium
- 2 high and 1 low = medium
- 2 high and 1 medium = high
- 3 high ratings = high

In addition to this general classification, other information should be gathered at the initial stages of assessment to serve in later more-detailed stages of the criterion development process, should they be required. Information of relevance would include:

- species inventories of fish, mammals, and birds; for birds, nesting status at the site should be recorded;
- sediment characteristics (pH, redox, organic matter content, general chemistry);
- water chemistry, especially with respect to substances that are known to influence the uptake and/or toxicity of selenium (phosphate, sulfate, cadmium, arsenic mercury, etc.), and
- prevailing climate patterns (i.e., northern versus southern locales, high or low precipitation).

It is important to include all potentially affected water bodies in the characterization. For example, if the initial receiving water is a fast flowing river, but discharges to a nutrient rich lake within a short distance, then both the river and the lake need to be considered.

Step 2 – Initial Screening

As a first comparison, ambient concentrations (measured or expected) can be compared with the most restrictive generic limits for selenium in water (i.e., 1 µg/L or 0.001 mg/L) and/or sediment (i.e., 2 µg/g dw). It is important to compare these ambient concentrations to the guidelines (CCME, 2002) in order to resolve the importance of natural background concentrations for the site. For example, for areas adjacent to some mine sites, the natural background of selenium may be at or above the 1 µg/L guideline, and so little discrimination of other sources of selenium will be feasible. Such scenarios where high background concentrations of selenium are present have proved to be problematic for resolution of time trends and presence of dose:response relationships with exposed organisms (e.g., in northern Saskatchewan, in the Elk Valley, B.C., and in the western USA).

In light of the importance of the selenium load associated with sediments, and that food web exposure is strongly driven by sediments, the use of sediment-related criteria for selenium has received substantial support among researchers and regulatory advisors (e.g., Canton and Van Derveer, 1997). However, for predictive assessment of the impacts of future effluent discharges, any sediment-based criterion would require extrapolation to a water-based value to govern the discharge. Further insight could be provided if this value was then compared with assessments based on other criteria (e.g., fish tissue residues, Toll *et al*., 2005). Such comparisons will resolve if the predictive assessments are comparable and representative of the habitat(s) in question.

If ambient existing water concentrations and sediment concentrations of selenium are below the threshold, then conditions are acceptable. For predictive assessments, only the water concentration can be initially considered. If the concentration of selenium in the water exceeds 1 µg/L but is less than 5 µg/L (i.e., within the U.S. FWS “level of concern” –

see Table 5.1) it is reasonable to stop the assessment and prescribe an appropriate monitoring program for water bodies ranked as “low”. For “medium” and “high”, or for “low” if the concentration exceeds 5 µg/L, further and more detailed examination is warranted.

Step 3 – First Tier Risk Assessment

If the initial screening warrants further examination, a simplified risk assessment should be initiated. This level of assessment should consider the transfer of selenium from the water column to other environmental media. All major forms of aquatic biota should be considered for the site within the SSRA framework.

The transfer factors (Kd, BAF) that are used to quantify selenium partitioning and exposure have been discussed in this report. The first stage SSRA should use transfer factors conservatively derived from the generic data. The selection of transfer factors should reflect the rating of the water body (high, medium, low). This can be recognized largely in the selection of bioaccumulation factors (BAFs). Based on reported differences of 10-fold between lentic and lotic habitats, assuming a 2-fold to 5-fold range in BAFs between the “low” and “high” rankings is not unreasonable. The transfer parameter values provided in Table 6.1 follow such an assumption. These values have been derived through examination of the literature and available case studies. These values have been derived to conservatively reflect systems of high, medium, and low selenium accumulation potential. Further, this is not a comprehensive list and relevant studies should be considered, where feasible, for a site.

If the first tier assessment identifies any concentrations in sediment or aquatic biota that exceed the noted thresholds (Table 5.1), progression to a more detailed and site-specific SSRA is warranted. Terrestrial biota (birds and mammals) is only assessed if concentrations in potential food items exceeds the noted threshold for diets. If this exercise suggests that selenium in biota will remain below respective thresholds, an appropriate monitoring program should be developed and implemented for the site.

Step 4 – Detailed SSRA

As recommended by Schlekat *et al*. (2004), the assessment of potential adverse effects of selenium, particularly if higher trophic levels are of relevance, should involve a full pathways analysis to ensure that all key ecosystem components are considered. It is a common contention that for various reasons, avian reproduction is the most sensitive endpoint when considering selenium in the aquatic environment. For this reason, all bird types should be included as receptors in the detailed risk assessment. The identification of focal species for detailed study reflects the view that selenium can act on many different species across multiple trophic levels an ecosystem whereas risk from molybdenum is particularly relevant for terrestrial species. If preliminary studies were completed at a site, it may be possible to refine the species identified for detailed study. Similarly, relevant laboratory studies may exist that could be used to generate information to narrow this analysis.

Overall, the receptor species to include in the risk assessment should represent the following major niches:

- piscivorous bird (e.g., kingfisher, osprey, merganser);
- benthivorous bird (e.g., shorebirds like sandpipers, diving waterfowl such as scaup);
- herbivorous bird (e.g., mallard);
- piscivorous mammals (e.g., otter);
- carnivorous mammals (e.g., mink);
- omnivorous mammal (e.g., coot);
- small herbivorous mammals, feeding on aquatic vegetation (e.g., muskrat); and
- large herbivorous mammal, feeding on aquatic vegetation (e.g., moose).

The procedure for determining the concentrations of selenium that may occur in the diet and/or tissues of receptors is to be determined at the discretion of the assessor. The need for pathways-discrete assessment of selenium exposure has lead to the development of several modeling processes. Specifically, the USGS (2004) developed a multi-media model for assessments of selenium, and Luoma and Presser (2004) propose a biodynamic model for uptake and exposure to trace metals, including selenium. There are also several other models that have been applied to pathways analyses with a focus on selenium, including the IMPACT model (e.g., see EcoMetrix, 2005a). Regardless of the model selected, parameter inputs should reflect site-specific data to the greatest extent possible.

At higher trophic levels, exposure can be assessed in the form of dose, or as tissue concentrations. Depending on the nature of the pathways model and the data availability, tissue concentrations can be difficult to predict. Generally, an assumption of 100% assimilation efficiency could be used to estimate body burdens from assumed diet intake if data are not available. In absence of this conservative assumption or any reliable capacity to determine tissue concentrations in birds and mammals, the estimated selenium *dose* (i.e., amount of selenium ingested per unit body weight per day) can be calculated. This expected dose can be compared to doses that are threshold doses for toxic effects (i.e., benchmark doses). Tables 6.2 and 6.3 provide body-weight adjusted benchmark doses for focal wildlife species that can be used in a dose-based assessment. For tissue-based assessments, Table 5.1 identified thresholds for fish, bird and mammal tissues.

6.2.2 Molybdenum Release Limits

In the case of molybdenum, threshold levels for effects on aquatic biota (fish, invertebrates) are not usually of concern. In contrast, the benchmarks for small mammals and larger ruminants that consume aquatic plants (muskrat and moose) may flag assessment of those species in the downstream environment.

Because molybdenum does not readily bioaccumulate, there is not generally a serious concern for food web effects. However, for any site where there is a proposed discharge of molybdenum to the aquatic environment, key site characteristics should be examined, including:

- the potential presence of large herbivorous mammals (deer or moose) in the area;
- alkalinity of the receiving waters;
- site-specific alterations to soil or water chemistry;
- the copper status (measured or projected) of the receiving environment; and
- site history pertaining to livestock/wildlife health.

At these sites, the following themes need to be considered. First, what is the concentration of copper? If copper status is low, and certain sensitive animals are present, then there is a need to consider the possibility of food web exposure of these animals to levels of molybdenum that result in potentially harmful copper:molybdenum ratios in diet (i.e., copper:molybdenum <2:1 – see Table 4.4). The possibility of such exposure is greater in alkaline environments, where molybdenum is mobilized for uptake into plant tissues. If the possibility for such molybdenum-related impacts is identified, based on the ratio between these elements or generic guidelines for soil or plants consumed by livestock (i.e., 10 mg/kg), then a site-specific predictive assessment of animal exposure to molybdenum through the food web may be necessary. The preceding review of literature indicates it is likely most assessments will only need to evaluate the pathway involving the ingestion of aquatic and terrestrial plants by herbivores. Second, what other site-specific modifying factors may alter the expected relationships for short-term exposure? For example, as noted, the addition of lime to lakes of Sweden rapidly changed the molybdenum dynamics across entire watersheds and was identified as the cause for problems with moose there. Third, what, if any, past observations exist, that concern the health (i.e., nutritional) status of domestic animals like sheep at the site? If a long history of healthy animals exists, then precedent exists to regard the site as having low risk for causing effects on wildlife.

As with selenium, the assessment of molybdenum could use a stage-based approach. This strategy would be predicated on an initial conservative screening for a site against water quality guidelines, and then through to a pathways-based, site-specific assessment as warranted by the comparisons with government limits. The main focus of initial stages of the assessment should be the potential for occurrence of molybdenum in plant tissues at levels that may be problematic for sensitive herbivores. If there is an initial indication of significantly elevated exposure of sensitive animals to molybdenum via the diet, other receptors and pathways should be added to the assessment. To assist with this process, Tables 6.2 and 6.3 include body-weight adjusted benchmark doses for molybdenum for the assessment of representative receptor species.

6.2.3 Environmental Monitoring

In all cases, environmental monitoring at a site should be undertaken at the very least to confirm the reliability of the assumptions made in the risk assessment. Such direct evidence is a key step for accurately representing dynamic ecological systems and food webs. These studies will also provide insight on potential ecological impacts associated with release limits, based on actual observations. This insight can be used to gauge proposed changes in release limits and possible effect(s) on receptor species. An environmental monitoring program should be tiered, with a level of detail and coverage consistent with the expected effect(s). Even if the assessment suggests no impacts at a given ambient concentration or releases rate, monitoring should be conducted over time to ensure the reliability of the initial assessment and to confirm assumptions.

An environmental monitoring program design should reflect the issues identified in the pathways analysis. Some general principals would include:

- At a minimum, water, sediment, plants, and invertebrates should be assessed in the program. This will adequately indicate if there is any initial concern for uptake of selenium or molybdenum into the food web.
- In the monitoring of sediment, sampling design should account for spatial heterogeneity that is likely to exist.
- Sediment sampling should assess the thin detrital layer at the sediment surface (if present), as this can play a key role in initial exposure and bioaccumulation.
- Sediment monitoring should include analysis of organic matter, and sediment texture, among other parameters, to allow for an understanding of the potential availability of sediment-borne molybdenum or selenium.
- The monitoring program (frequency, spatial extent) may need to be evaluated if stochastic (e.g., forest fire) events occur in the watershed that may modify the loading of selenium or molybdenum to local watersheds.
- If the assessment indicated no likely effects on higher trophic level species (e.g., aquatic: different fish species, terrestrial: moose), the initial monitoring need only target water, sediment, invertebrates and plants.
- If significant risk for biomagnification and food web effects is indicated, sampling and analysis of fish could be added to the monitoring. Fish collection for tissue analysis should be accompanied by measures of physiological indicators of possible effects on fish health, especially reproductive tissues (e.g., maturity status, fecundity, gonad somatic index for males and females).
- Sampling or monitoring of terrestrial biota should only be initiated if all evidence regarding diet suggests that selenium exposure will exceed threshold levels.

- Monitoring of birds should be as non-destructive as possible, targeting early season eggs of species that are indeterminate (i.e., will lay new eggs to replace eggs that are lost).
- In all cases, monitoring should recur over time, to assess exposure and bioaccumulation in the sediments and food web over time.

6.3 Further Research Needs

Available evidence suggests that the risks from exposure of selenium to species are higher than exposure to molybdenum. As a consequence, the present research needs focus primarily on selenium.

- Forms of selenium such as dimethyl selenide, dimethyl diselenide, methane selanone, methane selenol, and dimethyl selenyl sulfide have not been well studied in terms of persistence in the environment. As noted, these chemical species all tend to be short-lived in the aquatic environment as a result of volatilization to the atmosphere. Because this type of loss can account for perhaps 20% or more of the selenium at a site (Gao and Tanji, 1995; Azaizeh *et al*., 1997), it would be useful to identify the actual rather than perceived destination for these selenium species in aquatic ecosystems. Such a study would test the assumption these forms of selenium do move to the atmosphere rather than remaining in an aquatic environment. It is possible that some of this selenium does not volatize and as such, could confound estimates of TMDL.

- Further study of the movement of selenium in different terrestrial environments is likely warranted. Such a study could also be structured to represent different precipitation regimes, as the movement of water through soil acts to influence the form of selenium and destination (e.g., what proportion will move to surface waters or remain bound to the sediment). However, He *et al*. (2005) have attempted to resolve facets of this relationship, but further study would be useful.

- Resolution of the role of sediment content and uptake of selenium and molybdenum is warranted to more accurately and precisely frame accumulation patterns and SSRAs.

- Accumulation of selenium and molybdenum (and associated dose-response relationships) are lacking for amphibians. For example, no definitive studies appear to exist and some inferences suggest that fish show similar responses as amphibians while others imply the opposite relationship. Resolving such a relationship would be a worthy avenue for future investigations. A comprehensive study would require the consideration of different amphibians (e.g., frogs, salamanders, toads) associated with contrasting types of sediments (e.g., variable clay content, variable organic content etc.) and co-variation of water-borne selenium concentrations. An ideal study design would also

consider amphibians simultaneously from different habitats (e.g., boreal forest, prairies, mountains, etc.).

- Similarly, reptiles have been understudied in terms of accumulation or dose-responses to selenium and molybdenum. Thus, a study as noted for amphibians would be appropriate for different reptiles (e.g., lizards, snakes, turtles).

- Resolution of the accumulation patterns of selenium and molybdenum among different types of lakes is warranted. Specifically, the role of lake productivity (i.e., oligotrophic, mesotrophic, eutrophic) has only been inferred from studies across habitats. A focused study is needed to better determine if the low to high rankings noted above are justified.

- Focused studies that resolve the role of adaptation compared with a combined response of acclimation and adaptation. Such studies would also require the resolution of the energetic costs of adaptation (likely low cost) relative to acclimation (likely high cost and associated trade offs)

- Studies that resolve the modifying factors that influence molybdenum toxicity are warranted

- Evidence suggests that there are different responses across species of animals to selenium and molybdenum, as represented by different toxicity responses (e.g., different NOAELs) across species and between species in Canada and those studied elsewhere (e.g., USA). Thus, it would be useful to complete wide-scale studies to better understand the toxicity response patterns of species that differ in life history, habitat use, and other factors.

- Finally, because site specific risk assessments are recommended, it would be instructive to provide a guide to the development of site-specific transfer coefficients using available site data. This exercise is generally precluded by the lack of data at many Greenfield sites. However, there are existing operations where monitoring data have been compiled for many years. Data that includes concentrations in water, sediment, aquatic plants and fish tissue provide a basis on which to develop site-specific transfer parameters such as water-sediment distribution coefficients (i.e., K_d), bioaccumulation coefficients (BAF) in fish and other transfer coefficients. A guide to the application of such data to derive such coefficients would be instructive.

7.0 CITED AND OTHER REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. Toxicological Profile for Selenium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Available online at http://www.atsdr.cdc.gov/toxprofiles/tp92.html

Agency for Toxic Substances and Disease Registry (ATSDR). 2005. Public health assessment for Molycorp, Inc. Questa, Taos County, New Mexico; facility identification: NMD002899094. February 28, 2005. U.S. Department of Health and Human Services. Division of Health Assessment and Consultation. Atlanta, Georgia. 92 p.

Adams, W.J., K.V. Brix, M. Edwards, L.M. Tear, D.K. DeForest, and A. Fairbrother. 2003. Analysis of field and laboratory data to derive selenium toxicity thresholds for birds. Environmental Toxicology and Chemistry 22:2020–2029.

Adams, W.J., J.E. Toll, K.V. Brix, L.M. Tear, and D.K. DeForest. 2000. Site-specific approach for setting water quality criteria for selenium: differences between lotic and lentic systems. Proceedings of the 24th Annual British Columbia Mine Reclamation Symposium. Williams Lake B.C., 2000.

Adams, W.J., K.V. Brix, K.A. Cothern, L.M. Tear, R.D. Cardwell, A. Fairbrother, and J.E. Toll. 1998. Assessment of selenium food chain transfer and critical exposure factors for avian wildlife species: Need for site-specific data. Environmental Toxicology and Risk Assessment: Seventh Volume. ASTM STP 1333, E.E. Little, A.J. DeLonay, and B.M. Greenberg (Eds.). American Society of Testing and Materials, Philadelphia, PA.

Allen, E.W., E.E. Prepas, S. Gabos, W. Strachan, and W. Chen. 2003. Surface water chemistry of burned and undisturbed watersheds on the Boreal Plain: an ecoregion approach. Journal of Environmental Engineering Science 2:S73-86.

Australia New Zealand Food Authority (ANZFA) 2002. Food surveillance Australia New Zealand. Canberra, Government of Australia.

Arrington, L.R. and G.K. Davies. 1953. Molybdenum toxicity in the rabbit. Journal of Nutrition 51: 295-304.

Ashworth, D.J., and G. Shaw. 2006. Soil migration, plant uptake and volitalisation of radio-selenium from a contaminated water table. In press Science of the Total Environment 370:506-514.

Azaizeh, H.A., S. Gowthaman, and N. Terry. 1997. Microbial selenium volatilization in rhizosphere and bulk soils from a constructed wetland. J. Environ. Quality 26(3):666-672.

Bailey, F.C., A.W. Knight, R.S. Ogle, and S.J. Laine. 1995. Effect of sulphate levels on selenium uptake by *Ruppia maritime*. Chemosphere 30:579-591.

Bañuelos, G.S. 2002. Irrigation of broccoli and canola with boron- and selenium-laden effluent. Journal of Environmental Quality 31:1802–1808.

Bañuelos, G.S. and D.W. Meek. 1990. Accumulation of selenium in plants grown on selenium - treated soil. Journal of Environmental Quality 19:772-777.

Bañuelos, G.S., H.A. Ajwa, B. Mackey, L. Wu, C. Cook, S. Akohoue, and S. Zambruzuski. 1997. Evaluation of different plant species used for phytoremediation of high soil selenium. Journal of Environmental Quality 26(3): 639-646.

Bañuelos, G.S., A. Zayed, N. Terry, L. Wu, S. Akohoue and S. Zambrzuski. 1996. Accumulation of selenium by different plant species grown under increasing sodium and calcium chloride salinity. Plant and Soil 183:49-59.

Bañuelos, G.S., G. Cardon, B. Mackey, J. Ben-Asher, L. Wu, P. Beuselinck, and S. Akohoue. 1993. Boron and selenium removal in boron-laden soils by four sprinkler-irrigated plant species. Journal of Environmental Quality 22:786-792.

Barceloux. D.G. 1999a. Selenium. Clinical Toxicology 37:145-172.

Barceloux D.G. 1999b. Molybdenum. Clinical Toxicology 37:231-237

Barrie, L.A. 1988. Aspects of atmospheric pollutant origin and deposition revealed by multielemental observations at a rural location in Eastern Canada. *Journal of Geophysical Research 93*:3773-3788.

Barwick D.H. and R.D. Harrell. 1997. Recovery of fish populations in Belews Lake following selenium contamination. Proc Ann Conf SEAFWA 51:209-216.

Becker, G.C. 2001. *Fishes of Wisconsin*. University of Wisconsin Press, Madison, WI.

Belovsky G.E., P.A. Jordan, and D.V. Botkin. 1975. Proceedings of an International Symposium on Moose Ecology, Quebec City, March 1973. Laval University Press, Quebec City.

Bennett, P.M. and D.M. Janz. 2007. Bioenergetics and growth of young-of-the-year northern pike (*Esox lucius*) and burbot (*Lota lota*) exposed to metal mining effluent. Ecotoxicology and Environmental Safety 68, 1-12.

Birge, W.J. 1978. Aquatic toxicology of trace elements of coal and fly ash. IN Energy and environmental stress in aquatic ecosystems. Edited by J.H. Thorp and J.W. Gibbons. Department of Energy Symposium Series. CONF 771114.

Birge, W.J., J.A. Black, A.G. Westerman, and J.E. Hudson. 1980. Aquatic toxicity tests on inorganic elements occurring in oil shale. Pages 519-534 in C. Gale (ed.) Oil shale symposium: sampling, analysis and quality assurance. National Technical Information Service, Springfield, VA. EPA-600/9–80–022.

Boila R.J., T.J. Devlin and K.M. Wittenberg. 1987. Geographical variation of the total sulfur content of forages grown in northwestern Manitoba. Canadian Journal of Animal Science 67: 869-872.

Braune, B.M. and B.J. Malone. 2006. Mercury and selenium in livers of waterfowl harvested in Northern Canada. Archives of Environmental Contamination and Toxicology 50:284-289.

British Columbia Ministry of Environment (BC MOE). 1986. Ambient Water Quality Objectives. Resource Quality Section. Water Management Branch.

Brix, K.V., D.K. DeForest, A. Fairbrother, and W.J. Adams. 2000. Critical review of tissue-based toxicity thresholds for fish and birds. Proceedings Mine Reclamation Symposium: Selenium Session; Sponsored by Ministry of Energy and Mines, Williams Lake, British Columbia, Canada, June 21-22, 2000.

Brix, K.V., D.K. DeForest, R.D. Cardwell, and W.J. Adams. 2004. Derivation of a chronic site-specific water quality standard for selenium in the Great Salt Lake, Utah, USA. Environmental Toxicology and Chemistry 23:606-612.

Brown, T. and A. Shrift. 1981. Exclusion of selenium from proteins in selenium-tolerant *Astragalus* species. Plant Physiology 67: 1951–1953.

Buck W.B. 1978. Copper/molybdenum toxicity in animals. Pages 491-515, F.W. Oehme (ed.). Toxicity of heavy metals in the environment. Part 1. Marcel Dekker, New York.

Buhl, K.J. and S.J. Hamilton. 2000. The chronic toxicity of dietary and waterborne selenium to adult Colorado pikeminow (*Ptychocheilus lucius*) in a water quality simulating that of the San Juan River. Prepared for the San Juan River Basin Recovery Implementation Program and the National Irrigation Water Quality Program.

Canadian Council of Ministers of the Environment (CCME). 2007. *Canadian Environmental Quality Guidelines*. Environment Canada, Ottawa. Available at: http://www.ccme.ca/publications/cegg_rcqe.html

Canadian Council of Ministers of the Environment (CCME). 2002. Summary of Existing Canadian Environmental Quality Guidelines. Updated 2002.

Canadian Council of Ministers of the Environment (CCME). 1987. Canadian Water Quality Guidelines (plus updates). Canadian Council of Resource and Environment Ministers (CCREM), now Canadian Council of Ministers of the Environment. Winnipeg.

Canadian Water Quality Guidelines for the Protection of Aquatic Life: Molybdenum (1999). Ottawa, Canada.

Canton, S.P. and W.D. Van Derveer. 1997. Selenium toxicity to aquatic life: An argument for sediment-based water quality criteria. *Environmental Toxicology and Chemistry* 16(6):1255-1259.

Carigan, R., P. D'Arcy, and S. Lamontagne. 2000. Comparative impacts of fire and forest harvesting on water quality in Boreal Shield lakes. Canadian Journal of Fisheries and Aquatic Sciences 57(Suppl. 2):105-117.

Carmichael, N.B., and P.M. Chapman. 2006. Baseline selenium in sculpins related to the northeast British Columbia coal zone. Proceedings, B.C.'s 30th Annual Mine Reclamation Symposium. The British Columbia Technial and Research Committee on Reclamation.

Carpenter, S.R. 1990. Large-scale perturbations: opportunities for innovation. Ecology 71:2038–2043.

Carvalho, K.M. and D.F. Martin. 2001. Removal of aqueous selenium by four aquatic plants. Journal of Aquatic Plant Management 39:33-36.

Chapman, P.M. 2007. Selenium thresholds for fish from cold freshwaters. Human and Ecological Risk Assessment 13: 1-5.

Chapman P.M. 2005. Selenium status – Elk River Valley, BC. In: W. Price, B. Hart, B. Dixon, P. Jarman, B. Riordan, M. Freberg, C. Howell (eds.), Proceedings of the 29th Annual British Columbia Mining Reclamation Symposium – "The Many Facets of Mine Reclamation", British Columbia Technical and Research Committee on Reclamation, Victoria, BC, Canada.

Chapman, P.M. 2004. Selenium from coal mining in the Elk River Valley. Mine Reclamation Symposium Proceedings. British Columbia Technical and Research Committee on Mine Reclamation. Also available at: http://www.trcr.bc.ca/ .

Chapman, P. and F. Wang. 2000. Issues in ecological risk assessment of inorganic metals and metalloids. Human Ecol. Risk Assess. 6:1-24.

Chappell, W.R. 1975. Transport and biological effects of molybdenum in the environment. Pages 167-188 P.A. Krenkel, (ed.) *Heavy Metals in the Aquatic Environment*. Pergamon Press, New York.

Chappell, W.R. and Peterson, K.K. (eds.) 1976. *Molybdenum in the Environment. Volume 1, The biology of molybdenum*. Marcel Dekker, New York.

Chau, Y.K., P.T.S. Wong, B.A. Silverberg, P.L. Luxon, and G.A. Bengert. 1976. Methylation of selenium in the aquatic environment. *Science* 192:1130–1131.

Cherry, D.S., R.J. Currie, and D.J. Soucek. 2000. Review of the global adverse environmental impacts to groundwater and aquatic ecosystems form coal combustion wastes. Report prepared for Hoosier Environmental Council and Citizens Coal Council. Indianapolis, Indiana.

Clemens, S. 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. In press, Biochimie.

Collier, R.W. 1985. Molybdenum in the Northeast Pacific ocean. Limnology and Oceanography. 30, 1351–1354.

Crusius, J., S. Calvert, T. Pedersen, and D. Sage. 1996. Rhenium and molybdenum enrichments in sediments as indicators of oxic, suboxic, and sulfidic conditions of deposition. Earth and Planetary Science Letters 145:65-78.

Cumbie, P.M. and S.L. Van Horn. 1978. Selenium Accumulation Associated with Fish Mortality and Reproductive Failure. Proc. Annual Conf. Southeast Assoc. Fish Wildlife Agencies 32:612.

Custer, T.W. 2003. Trace elements in moose *(Alces alces*) livers from northwestern Minnesota. Presentation at the 11th Annual Meeting of the Society of Environmental Toxicology and Chemistry, January 29-30, 2003, Treehaven Research Station – Rhinelander/Tomahawk, Wisconsin.

Custer, T.W., E. Cox, and B. Gray. 2004. Trace elements in moose (*Alces alces*) found dead in northwestern Minnesota, USA. Science of the Total Environment 330:81-87.

Custer, C.M. and T.W. Custer. 2000. Organochlorine and trace element contamination in wintering and migrating diving ducks in the southern Great Lakes, USA, since the zebra mussel invasion. Environmental Toxicology and Chemistry 19:2821–2829.

Davies, T.D., J. Pickard, and K.J. Hall. 2005. Acute molybdenum toxicity to rainbow trout and other fish. Journal of Environmental and Engineering Science 4:481-485.

Davies, T.D., J.S. Pickard, and K.J. Hall. 2003. Sulphate toxicity to freshwater organisms and molybdenum toxicity to rainbow trout embryos/alevins. Mine Reclamation Symposium Proceedings. British Columbia Technical and Research Committee on Mine Reclamation. Also available at: http://www.trcr.bc.ca/ .

DeMayo, A., E. Brien and K.W. Taylor. 1979. The concentration of elements in crude oils, coals and fly ash. Unpublished literature review. Water Qual. Branch, IWD, Environment Canada, Ottawa.

De Rosemond, S.C., K. Liber and A. Rosaasen. 2005. Relationship between embryo selenium concentration and early life stage development in white sucker (*Catostomus commersoni*) from a northern Canadian lake. Bull. Environ. Contam. Toxicol. 74: 1134-1142.

Desy, J.C., M. Amyota, B. Pinel-Alloula, P.G.C. Campbell. 2002. Relating cadmium concentrations in three macrophyte-associated freshwater invertebrates to those in macrophytes, water and sediments. Environmental Pollution 120:759–769.

Diamantino,T., L. Guilhermino, E. Almeida, and A.M.V.M. Soares. 2000. Toxicity of sodium molbydate and sodium dichromate to *Dapnia magna* Straus evaluated in acute, chronic, and acetycholinesterase inhibition texts. Ecotoxicology and Environmental Safety 45:253-259.

Dickerson, K. and P. Ramirez. 1997. An investigation of irrigation related contaminants in water, bottom sediment, and biota from Goshen County, Wyoming. US FWS Contaminant Report # R6/711C/97.

Driscoll, C.T., J.K. Otton, and Å. Iverfeldt. 1994. Trace metals speciation and cycling. Pages 299 to 322 in B. Moldan and J. Černŷ (eds.), *Biogeochemistry of Small Catchments: A Tool for Environmental Research*. John Wiley and Sons, Ltd. New York.

EcoMetrix Incorporated (EcoMetrix). 2005a. Key Lake Project: Environmental risk assessment 2005. Report prepared by EcoMetrix Incorporated, Brampton, Ontario. Report prepared for Cameco Corporation, Saskatoon, Saskatchewan.

EcoMetrix Incorporated (EcoMetrix). 2005b. McArthur River Operation: Environmental risk assessment 2005. Report prepared by EcoMetrix Incorporated, Brampton, Ontario. Report prepared for Cameco Corporation, Saskatoon, Saskatchewan.

Erdman, J.A., R.J. Ebens, and A.A. Case. 1978. Molybdenosis: A potential problem in ruminants grazing on coal mine spoils. J. Range Manage. 31:34-36.

Efroymson, R.A., M.E. Will, G.W. Suter II, and A.C. Wooten. 1997. Toxicological Benchmarks for Screening Contaminants of Potential Concern for Effects on Terrestrial Plants: 1997 Revision. Report prepared for the US Department of Energy. ES/ER/TM-85/R3.

Eisler, R. 1989. Molybdenum Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. U.S. Fish and Wildlife Service Biological Report 85(1.19). Contaminant Hazard Reviews Report No. 19, U. S. Department of the Interior, Washington, D.C., 61 pp.

Eisler, R. 1985. Selenium Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. U.S. Fish and Wildlife Service Biological Report 85(1.5). Contaminant Hazard Reviews Report No. 5, U. S. Department of the Interior, Washington, D.C., 57 pp.

Erdman, J.A., R.J. Ebens, and A.A. Case. 1978. Molybdenosis: A potential problem in ruminants grazing on coal mine spoils. J. Range Manage. 31:34-36.

ESG International Inc. 1999. Aquatic Effects Technology Evaluation (AETE) Program Synthesis Report of Selected Technologies for Cost-Effective Environmental Monitoring of Mine Effluent Impacts in Canada. Report prepared for Canada Centre for Mining and Energy Technology and Mining Association of Canada. AETE Project 4.1.4.

Evans, D.H. 1993. Osmotic and ionic regulation. Pages 315 to 342 in D.H. Evans, (ed.), *Fish Physiology*. CRC Press, Boca Raton, FL.

EVS Environment Consultants (EVS). 2005. Elk Valley selenium lotic monitoring program (2001-2003). Report prepared for the Elk Valley Mines Environmental Management Committee.

Expert Group on Vitamins and Minerals (EVM). 2000. Review of Molybdenum. Report prepared for the United Kingdom Food Standards Agency. Report EVM/00/09P. Available on-line at http://archive.food.gov.uk/committees/evm/papers/evm9.pdf.

Fairhall, L.T., R.C. Dunn, N.E. Sharpless, and E.A. Pritchard.1945. The Toxicity of Molybdenum. U.S. Public Health Bull. No. 293.

Fan, W.-M., S.J. Teh, D.E. Hinton, and R.M. Higashi., 2002a. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium laden drainage waters in California. Aquatic Toxicology 57:65–84.

Fan, W.-M., E. Pruszkowski, and S. Shuttleworth. 2002b. Speciation of selenoproteins in Se-contaminated wildlife by gel electrophoresis and laser ablation-ICP-MS. Journal of Analytical Atomic Spectrometry 17:1621–1623.

Finley, K. and R. Garrett, 2007. Recovery at Belews and Hyco Lakes: Implications forfish tissue selenium thresholds. Integrated Environmental Assessment and Management 3(2):297-299.

Fisk, A.T. C.A. de Wit, M. Wayland, Z.Z. Kuzyk, N. Burgess, R. Letcher, B. Braune, R. Norstrom, S. Polischuk Blum, C. Sandau, E. Lie, H.J.S. Larsen, J. Utne Skaare, and D.C.G. Muir. 2005. An assessment of the toxicological significance of anthropogenic contaminants in Canadian arctic wildlife. *Science of the Total Environment* 351–352:57–93.

Fitzgerald D., R. Nicholson, P. McKee, and K. Himbeault. 2006. Identification of site-specific and species-dependent uptake of selenium in fishes of northern Saskatchewan: implications for modeling and management. Proceedings of the 33rd Aquatic Toxicity Workshop, Jasper, AB, October 1-4, 2006.

Fletcher, T., G.L. Stephenson, B. Muncaster, C.D. Wren, and D.J. Spry. 1997. Scientific criteria document for the development of an interim provincial water quality objective for molybdenum. Ontario Ministry of Environment and Energy. Standards Development Branch, Toronto.

Food and Agriculture Organization (FAO) and World Health Organization (WHO). 2001. Selenium. Pages 235-255. Chapter 15 in Human and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation. Bangkok, Thailand.

Fournier, E., C. Adam, J.C. Massabuau, and J. Garnier-Laplace. 2005. Effect of selenium exposure on the freshwater bivalve *Corbicula fluminea*. Radioprotection, Suppl. 1, vol. 40 (2005) S3-S9.

Fox, P.M. and H.E. Doner. 2002. Trace element retention and release on minerals and soil in a constructed wetland. *Journal of Environmental Quality* 31:331–338.

Foy, C., R. Chaney, and M. White, 1978. The physiology of metal toxicity in plants. Annual Review of Plant Physiology, 29, 511–566.

Frank, A. 2004. A Review of the “Mysterious” Wasting Disease in Swedish Moose (*Alces alces* L.) Related to Molybdenosis and Disturbances in Copper Metabolism. Biological Trace Element Research. 102;143-159.

Frank, A. 1998. Mysterious moose disease in Sweden. Similarities to copper deficiency and/or molybdenosis in cattle and sheep. Biochemical background of clinical signs and organ lesions. *Science of the Total Environment* 209:17-26.

Frank, A., R. Danielsson, and B. Jones. 2000a. The ‘mysterious’ disease in Swedish moose. Concentrations of trace elements in liver and kidneys and clinical chemistry. Comparison with experimental molybdenosis and copper deficiency in the goat.

Frank, A., R. Danielsson, and B. Jones. 2000b. Experimental copper and chromium deficiency and additional molybdenum supplementation in goats. II. Concentrations of trace and minor elements in the liver, kidneys, and ribs: haematology and clinical chemistry. *Science of the Total Environment* 249:143-170.

Frank, A., D.R. Sell, R. Danielsson, J.F. Fogarty, and V.M. Monnier. 2000c. A syndrome of molybdenosis, copper deficiency and type 2 diabetes in the moose population of south-west Sweden. *Science of the Total Environment* 249:123-131.

Frank, A., R. Wibom, and R. Danielsson. 2002. Myocardial cytochrome c oxidase activity in Swedish moose (*Alces alces* L.) affected by molybdenosis. *Science of the Total Environment* 290:121-129.

Frankenberger, W.T. Jr., and Richard A. Engberg, eds., 1998, Environmental Chemistry of Selenium: New York, Marcel Dekker, Inc., 713 p.

Franson, J.C, T. Hollmén, R.H. Poppenga, M. Hario, M. Kilpi, and M.R. Smith. 2000. Selected trace elements and organochlorines: Some findings in blood and eggs of nesting common eiders (*Somateria mollissima*) from Finland. Environ. Toxicol. Chem. 19:1340-1347.

Fungwe, T.V., F. Buddingh, D.S. Demick, C.D. Lox, M.T. Yang, and SW.P. Yang. 1990. The role of dietary molybdenum on estrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats. Nutrition Research 10: 515-524.

Gailer, J. 2002. Reactive selenium metabolites as targets of toxic metals/metalloids in mammals: a molecular toxicological perspective. *Environment, Biology and Toxcicology* 16:701-707.

Gamberg, M., G. Boila, G. Stern, and P. Roach. 2005. Cadmium, mercury and selenium concentrations in mink (*Mustela vison*) from Yukon, Canada. Science of the Total Environment 351-352:523-529.

Gao, S. and K.K. Tanji. 1995. Model for biomethylation and volatilization of selenium from agricultural evaporation ponds. Journal of Environmental Quality 24:191-197.

Gao, S., K.K. Tanji, Z.Q. Lin, N. Terry, and D.W. Peters. 2003. Selenium Removal and Mass Balance in a Constructed Flow-Through Wetland System. Journal of Environmental Quality 32:1557–1570.

Garcia-Hernandez, J., K.A. King, A.L. Velasco, E. Shumilin, M.A. Mora, and E.P. Glenn. 2001. Selenium, selected inorganic elements, and organochlorine pesticides in bottom material and biota from the Colorado River delta. *Journal of Arid Environments* 49: 65-89.

Gardner, W. and K. Broersma. 1999. Pages 66-75, i*n*: Molybdenum Issues in Reclamation: Proceedings of the 1999 Workshop. Kamloops, British Columbia, September 24, 1999.

Gardner, W.C., K. Broersma, J.D. Popp, Z. Mir, P.S. Mir, and W.T. Buckley. 2003. Copper and health status of cattle grazing high-molybdenum forage from a reclaimed mine tailing site. Canadian Journal of Animal Science 83:479-485.

Garrett, G.I. and C.R. Inman. 1984. Selenium-Induced Changes in the Fish Populations of a Heated Reservoir, *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies* 38: 291-301.

Gaskin, J.G., R.B. Brobst, W.P. Miller, and E.W. Tollner. 2003. Long-term biosolids application effects on metal concentrations in soil and bermudagrass forage. Journal of Environmental Quality 32:146-152

Gélinas, Y., M. Lucotte, and J-P. Schmit. 2000. History of the atmospheric deposition of major and trace elements in the industrialized St. Lawrence Valley, Quebec, Canada. *Atmospheric Environment* 34:1797-1810.

Gélinas, Y. and J-P. Schmit. 1998. Estimation of the bulk atmospheric deposition of major and trace elements to a rural watershed. *Atmospheric Environment* 32:1473-1483.

Goldberg, S., and H.S. Forster. 1998. Factors affecting molybdenum adsorption by soils and minerals. Soil Science 163:109-114.

Golder Associates (Golder). 2007. Elk River Valle, B.C. Selenium Status Report 2005/2006. Prepared by Golder Associates Ltd., for the Elk Valley Selenium Task Force (EVSTF).

Golder Associates (Golder). 2006. Selenium trend analysis Elk River at Sparwood and Highway 93. Report prepared for Elk Valley Coal Corporation, Sparwood, BC, Canada.

Glooschenko W.A. and N. Arafat. 1988. Atmospheric deposition of arsenic and selenium using *Sphagnum* moss as a biomonitor. Science of the Total Environment 73:269-275.

Goonerante, S.R. and D.A. Christensen. 1989. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Canadian Journal of Animal Science 69:141-150.

Goonerante, S.R., W.T. Buckely, and D.A. Christensen. 1989. Review of copper deficiency and metabolism in ruminants. Canadian Journal of Animal Science 69:891-845.

Gray, L.F. and L.J. Daniel. 1954. Some effects of excess molybdenum on the nutrition of the rat. Journal of Nutrition 53:43-51.

Gupta, U.C. 1997. Soil and plant factors affecting molybdenum uptake by plants. Pages 71-92 *in* U.C. Gupta, (ed.) Molybdenum in agriculture. Cambridge University Press, Cambridge, U.K.,

Hamilton, S.J., and K.J. Buhl. 1990. Acute toxicity of boron, molybdenum, and selenium to fry Chinook salmon and coho salmon. Archives of Environmental Contamination 19:366-373.

Hamilton, S.J. and A. D. Lemly. 1999. Water-sediment controversy in setting environmental standards for selenium. Ecotoxicology and Environmental Safety 44:227-235.

Hamilton, S.J. and V.P. Palace. 2001. Assessment of selenium in lotic ecosystems. Ecotoxicology and Environmental Safety 50:161-166.

Hansen, D., P.J. Duda, A. Zayed, and N. Terry. 1998. Selenium removal by constructed wetlands: role of biological volatilization. Environmental Science and Technology 32:591-597.

Harding, L.E., M. Graham and D. Paton. 2005. Accumulation of selenium and lack of severe effects on productivity of American dippers (*Cinclus mexicanus*) and spotted sandpipers (*Actitis macularia*). Archives of Environmental Contamination and Toxicology 48:414-423.

Harrison, S.E. and J.F. Klaverkamp. 1990. Metal contamination in liver and muscle of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) from lakes near the smelter at Flin Flon, Manitoba Canada. Environmental Toxicology and Chemistry 9:941-956.

Hartikainen, H. 2005. Biogeochemistry of selenium and its impact on food chain quality and human health. Journal of Trace Elements in Medicine and Biology 18:309-318.

He, Z.L., X.E. Yang, and P.J. Stofella. 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology 19:125-140.

Health Canada. 1996. Guidelines for Canadian Drinking Water Quality. Ottawa, ON.

Health Canada. 1992. Guidelines for Canadian Drinking Water Quality: Supporting Document – Selenium. April, 1979. Updated December 1992. Available online at http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/doc_sup-appui/selenium/index_e.html.

Heinz, G.H. and A.D. Hoffman. 1998. Methylmercury chloride and selenomethionine interactions on health and reproduction in mallards. Environ Toxicol Chem 17:139-145.

Heinz, G.H. and A.D. Hoffman. 1987. Reproduction in mallards fed selenium. Environmental Toxicology and Chemistry 6:423-433.

Heinz, G.H., D.J. Hoffman, and L.G. Gold. 1989. Impaired reproduction of mallards fed an organic form of selenium. *Journal of Wildlife Management* 53:418-428.

Hill, C.H. 1980. Influence of Time of Exposure to High Levels of Minerals on the Susceptibility of Chicks to *Salmonella gallinarum*. Journal of Nutrition 110:433-436.

Hill, C.H. 1974. Influence of High Levels of Minerals on the Susceptibility of Chicks to *Salmonella gallinarum*. Journal of Nutrition 104:1221-1226

Hill, C.H. 1979a. Studies on the Ameliorating Effect of Ascorbic Acid on Mineral Toxicities in the Chick. Journal of Nutrition 109:84-90.

Hill, C.H. 1979b. The Effects of Dietary Protein Levels on Mineral Toxicity in Chicks. Journal of Nutrition 109:501-507.

Hillwalker, W.E., P.C. Jepson, and K.A. Anderson. 2006. Selenium accumulation patterns in lotic and lentic aquatic systems. Science of the Total Environment 366:367-379.

Hoffman, D.J. 2002. Role of selenium and toxicity and oxidative stress in aquatic birds. Aquatic Toxicology 57:11-26.

Hoffman, D.J., H.M. Ohlendorf, C.A. Marn, and G.W. Pendleton. 1998. Association of mercury and selenium with altered glutathione metabolism and oxidative stress in diving ducks from the San Francisco Bay Region, USA. Environmental Toxicology and Chemistry 17:167-172.

Hollister, V.F. (editor). 1991. Porphyry copper, molybdenum , and gold deposits, volcanogenic deposits (massive sulfides), and deposits in layered rock. Society for Mining, Metallurgy, and Exploration, Littleton, Colo. 256 p.

Holm, J., V.P. Palace, P. Siwik, G. Sterling, R.E. Evans, C.L. Baron, J. Werner, and K. Wautier. 2005. Development effects of bioaccumulated selenium in eggs and larvae of two salmonid species. *Environmental Toxicology and Chemistry* 24, 2373-81.

Hopkins, W.A., B.P. Staub, J.A. Baionno, B.P. Jackson and L.G. Talent. 2005. Transfer of selenium from prey to predators in a simulated terrestrial foodchain. Environmental Pollution 134:447-456.

Hopkins, W.A., S.E. DuRant, B.P. Staub, C.L. Rowe, and B.P. Jackson. 2006. Reproduction, embryonic development, and maternal transfer of contaminants in the amphibian *Gastrophryne carolinensis*. Environmental Health Perspectives 114:661-666.

Hopkins, W.A., J.W. Snodgrass, J.H. Roe, B. Jackson, J. Gariboldi, and J.D. Congdon. 2000. Detrimental effects associated with contaminants in lake chubsuckers (*Erimyzon sucetta*) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology 39:193-199.

Howarth, R.W. and J.J. Cole, 1985. Molybdenum availability, nitrogen limitation and phytoplankton growth in natural waters. *Science* 229:653-655.

Howarth, R.W. 1988. Nutrient limitation of net primary production in marine ecosystems. Annual Reviews of Ecology and Systematics 19:89-110.

Hren, J. and H.R. Feltz. 1998. Effects of irrigation on the environment of selected areas of the western United States and implications to world population growth and food production. Journal of Environmental Management 52:353-360.

Huang, Z.Z. and L. Wu. 1991. Species richness and selenium accumulation of plants in soils with elevated concentration of selenium and salinity. Ecotoxicology and Environmental Safety 22:251-266.

Indian and Northern Affairs Canada (INAC). 2003. Canadian Arctic Contaminants Assessment Report II – Contaminant Levels, trend and Effects in the Biological Environment. ISBN 0-662-33467-1.

International Atomic Energy Agency (IAEA). 1994. Handbook of Parameter Values for the Prediction of Radionuclide Transfer in Temperate Environments. International Atomic Energy Agency, Vienna.

International Atomic Energy Association (IAEA). 2004. Sediment Distribution Coefficients and Concentration Factors for Biota in the Marine Environment. IAEA Technical Report Series No. 422, International Atomic Energy Agency.

International Joint Commission (IJC). 1982. First Biennial Report under the Great Lakes Water Quality Agreement of 1978. International Joint Commission, Washington, D.C. and Ottawa, ON.

Island Copper Mine, BHP Minerals Canada Ltd. 1997. Reclamation Report: Operation and Surface Work, Permit No. M-9. 37+ pp.

Jeter, M.A. and G.K. Davis. 1954. The effect of dietary molybdenum upon growth, haemoglobin, reproduction and lactation of rats. Journal of Nutrition. 54: 215-220.

Josephy, P.D. 2006. Molecular Toxicology. Second Edition. Oxford University Press. 608 p.

Jones, D.S., G.W. Suter II, and R.N. Hull. 1997. Toxicological benchmarks for screening contaminants of potential concern for effects on sediment-associated biota: 1997 revision. Oak Ridge National Laboratory, Oak Ridge TN. 34 pp. ES/ER/TM-95/R4.

Kálmán, E. 1994. Routes to the development of low toxicity corrosion inhibitors for use in neutral solutions. Pages 12-18 *in* A Working Party Report on Corrosion Inhibitors. European Federation of Corrosion, Institute of Materials.

Karlsson, S., M. Meili, and U. Bergström. 2002. Bioaccumulation factors in aquatic ecosystems - A critical review. SKB Rapport R-02-36. ISSN 1402-3091. Stockholm, Sweden. July 2002

Karnacky, Jr., K.J. 1997. Osmotic and ionic regulation. Pages 157-176 in D.H. Evans (ed.) *The Physiology of Fishes*, CRC Press, Boca Raton, FL.

Kapustka, L.A., W.H. Clements, L. Ziccardi, P.R. Paquin, M. Sprenger, and D. Wall. 2003. Issue Paper on the Ecological Effects of Metals. Draft Report Prepared for the U.S. Environmental Protection Agency, Risk Assessment Forum. August, 2003.

Kawakubo, S., S. Hashi, and M. Iwatsuki. 2001. Physiochemical speciation of molybdenum in rain water. *Water Research* 35:2489-2495.

Kehoe, R.A., J. Chalak, and E.J. Largent. 1944. The concentration of certain trace metals in drinking waters. Journal of the American Water Works Association 36:637-644.

Kennedy C.J., L.E. McDonald, R. Loveridge, and M.M. Strosher. 2000. The effects of bioaccumulated selenium on mortalities and deformities in the eggs, larvae and fry of a wild population of cutthroat trout (*Oncorhynchus clarki lewisi*). Archives of Environmental Contamination and Toxicology 39:46-53.

Khattak, R.A., A.L. Page, D.R. Parlr, and D. Bakhtar. 1991. Accumulation and interactions of arsenic, selenium, molybdenum, and phosphorus in alfalfa. Journal of Environmental Quality 20:165-168.

King, K.A., C.L.H. Marr, A.L. Velasco, and H.M. Schotborgh. 2003. Contaminants in waterbirds, grackles, and swallows nesting on the lower Colorado River, Arizona. 2000-2001. U.S. Fish and Wildlife Service, Arizona Ecological Services Field Office, Phoenix. 42 pp.

Kufel, I. 1991. Lead and molybdenum in reed and cattail -- open versus closed type of metal cycling. *Aquatic Botany*. 40:275-288.

Kubachka, K.M., J. Meija, D. L. Leduc, and J. A. Caruso. 2007. Selenium volatiles as proxy to the metabolic pathways of selenium in genetically modified *Brassica juncea*. Environmental Science and Technology 41:1863-1869.

Lam, J.C.W., S. Tanabe, M.H.W. Lam, and P.K. Lam. 2005. Risk to breeding success of waterbirds by contaminants in Hong Kong: evidence form trace elements in eggs. Environmental Pollution 135(3):481-490.

Langmuir, D., P. Chrostowski, B. Vigneault, and R. Chaney. 2004. Issue Paper on the Environmental Chemistry of Metals. Report prepared for the U.S. Environmental Protection Agency Risk Assessment Forum.

Latshaw, J.D., T.Y. Morishita, C.F. Sarver, and J. Thilsted. 2004. Selenium toxicity in breeding ring-necked pheasants (*Phasianus colchicus*). Journal of Avian Disease 48:935–939.

Lawson, S. and J.M. Macy. 1995. Bioremediation of selenite in oil refinery wastewater. Applied Microbiology and Biotechnology 43:762-765.

Lemly, A.D. and G.J. Smith. 1987. Aquatic cycling of selenium: implications for fish and wildlife. United States Fish and Wildlife Service (USFWS) Fish and Wildlife Leaflet 12. United States Department of the Interior. Washington, D.C..

Lemly, A.D. 2004a. Implications of selenium in proposed wastewater discharges to Great Salt Lake. Report prepared for Friends of Great Salt Lake, Salt Lake City, Utah.

Lemly, A.D. 2004b. Recommendations for Pre-Mine Assessment of Selenium Hazards Associated With Coal Mining in West Virginia.

Lemly, A.D. 2004c. Aquatic selenium pollution is a global environmental safety issue. Ecotoxicology and Environmental Safety 59:44–56.

Lemly, A.D. 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquatic Toxicology 57, 39-49.

Lemly, A.D. 2001a. A technical framework for implementing aquatic ecosystem loading limits (TMDLs) to reduce selenium pollution from phosphate mining wastes on Caribou National Forest, Idaho. Report prepared for the United States Department of Justice, Environment and Natural Resources Division. January 2001.

Lemly, A.D. 2001b. A procedure for setting environmentally safe TMDLs for selenium in San Diego Creek and Newport Bay, California. Report prepared for the California State Water Quality Control Board, Santa Ana Region, Riverside, California.

Lemly, A.D. 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicology and Environmental Safety 37 :259-266.

Lemly, A.D. 1997. Role of season in aquatic hazard assessment. Journal Environmental Monitoring and Assessment 45:88-98.

Lemly, A.D. 1993a. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. Environmental Monitoring and Assessment 28:83-100.

Lemly A.D. 1993b. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27:133-158.

Lemly, A.D. 1987. Aquatic cycling of selenium: implications for fish and wildlife. U.S. Department of the Interior, Fish and Wildlife Service, Rpt. 12,. Washington, DC.

Lemly A.D. 1985. Toxicology of selenium in a freshwater reservoir: Implications for environmental hazard evaluation and safety. Ecotoxicology and Environmental Safety 10:314–338.

LeResche, R.E. and J.L. Davis. 1973. Importance of nonbrowse foods to moose on the Kenai Peninsula, Alaska. Journal of Wildlife Management. 37: 279-287.

Levander, O.A. 1977. Metabolic Interactions between arsenic and selenium. Environmental Health Perspectives 19:159-164.

Lin, Z-Q. and N. Terry. 2003. Selenium removal by constructed wetlands: quantitative importance of biological volatilization in the treatment of selenium-laden agricultural drainage water. Environmental Science and Technology 37:606-615.

Logan, T.J., A.C. Chang, A.L. Page, and T.J. Ganje. 1987. Accumulation of selenium in crops grown on sludge-treated soil. Journal of Environmental Quality 16(4):349-352.

Lohner, T.W., R.J. Reash, V.E. Willet, and L.A. Rose. 2001. Assessment of tolerant sunfish populations (*Lepomis* sp.) inhabiting selenium-laden coal ash effluents. 1. Hematological and population-level assessment. Ecotoxicology and Environmental Safety 50:203-216.

Luoma, S.N. and T.S. Presser. 2004. Linking Selenium Sources to Ecosystems: San Francisco Bay-Delta Model: U.S. Geological Survey Fact Sheet 2004-3091.

Luoma, S.N. and T.S. Presser. 2002. Beyond the San Luis Drain: understanding the present and understanding the linkages, *in* Borgonovo, R., ed., Proceedings of the Beyond the Drain Conference, Sustaining Agriculture and Improving Water Quality in California's San Joaquin Valley, March 27, 2001, Sacramento, California: San Francisco Bay Estuary Project, Oakland, California, 13 p.

Luoma, S. N. and T.S. Presser. 2000. Forecasting selenium discharges to the San Francisco Bay-Delta Estuary: ecological effects of a proposed San Luis drain extension; United States Geological Survey Open-File Report 00-416; Menlo Park, CA. 358 pp.

Luoma, S.N and P.S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environmental Science and Technology* 39:1921-1931.

Lynch, T.R., C.J. Popp, and G.Z. Jacobi. 1988. Aquatic insects as environmental monitors of trace metal contamination: Red River, New Mexico. *Water, Air, & Soil Pollution* 42:19-31.

MacNicol, R.D. and P.H.T. Beckett. 1985. Critical tissue concentrations of potentially toxic elements. Plant and Soil 85:107-129.

Maier, K. J., C. Foe, R.S. Ogle, M.J. Williams, A.W. Knight, P. Kiffney, and L.A. Melton. 1987. The Dynamics of Selenium in Aquatic Ecosystems. Pages 361-408, D.D. Hemphill (ed.) *Trace Substances in Environmental Health and Medicine XXI, 1987, A Symposium.* University of Missouri, Columbia.

Majak, W. Scientist, Kamloops Range Research Station. Personal communication during 2003.

Majak, W., D. Steinke, J. McGillivray, and T. Lysyk. 2004. Clinical signs in cattle grazing high molybdenum forage. Journal of Range Management 57:269-274.

Malisa, E.P. 2001. The behaviour of selenium in geological processes. Environmental Geochemistry and Health 23:137-158.

Marino, R., R.W. Howarth, F. Chan, J.J. Cole, and G. E. Likens. 2003. Sulfate inhibition of molybdenum-dependent nitrogen fixation by planktonic cyanobacteria under seawater conditions: a non-reversible effect. Hydrobiologia 500: 277–293

Martinez, C.T. 1994. Selenium levels in selected species of aquatic birds on Imperial National Wildlife Refuge. Master's thesis. School of Renewable Resources, University of Arizona, Tucson.

Mathieu, S.F. 1996. Toxic Effects of Molybdenum on Forest Small Mammals at the Endako Mine in North Central British Columbia. University of Saskatchewan. Master's Thesis. 84 pp.

Mathieu, S. 1995. Molybdenum compilation. Summary of Environmental Research Conducted at Placer Dome Canada, Endako Mines Division from 1989-1994. Endako, B.C. Submitted to Placer Dome, Endako Mine. 82 pp.

Maurice, D.V. and L.S. Jensen. 1979. Reduction of hepatic lipid deposition in laying hens by dietary selenium-yeast interaction. Poultry Science 58:1548-1556.

McConnell, K.P. and O.W. Portman. 1952. Toxicity of dimethyl selenide in the rat and mouse. Proceeding of the Society of Experimental Biological Medicine 79:277-282.

McDonald, B.G. and P.M. Chapman. 2007. Assessing selenium effects: A weight of evidence approach. Integrated Environmental Assessment and Management 3: 129-136.

McDonald, L.E. and C.J. Kennedy. 2002. Reply to commentary on the effects of selenium bioaccumulation in a wild population of cutthroat trout. SETACGlobe 3(4): 36-27.

McDonald, L.E. and M.M. Strosher. 1998. Selenium Mobilization from Surface Coal Mining in the Elk River Basin, British Columbia: A survey of Water, Sediment and Biota. Report from the B.C. Ministry of Environment, Lands and Parks – Pollution Prevention Branch.

McDowell, L.R. and J.H. Conrad. 1979. Trace mineral nutrition in Latin America. Florida Agricultural Station Series No. 671. Department of Animal Science, University of Florida, Gainesville, Fla.

McGeer,J., G. Henningsen, R. Lanno, N. Fisher, K. Sappington, and J. Drexler. 2003. Issue Paper on the bioavailability and bioaccumulation of metals. Report prepared for the U.S. Environmental Protection Agency. Risk Assessment Forum.

McGrath, S.P., A.M. Chaudri, and K.E. Giller. 1995. Long-term effects of metals in sewage sludge on soils, microorganisms and plants. Journal of Industrial Microbiology and Biotechnology 14: 94-104.

Mendel, R. and G. Schwartz. 1999. Molybdoenzymes and molybdenum cofactor in plants. Critical Reviews in Plant Science 18:33-69.

Mikkelsen, R.L., G.H. Haghnia, A.L. Page and F.T. Bingham. 1988. The influence of selenium, salinity, and boron on alfalfa tissue composition and yield. J. Environ. Qual. 17:85-88.

Mills, C.F. and I. Bremner. 1980. Nutritional aspects of molybdenum in animals. Pages 517-542 in M.P. Coughlan (ed.) Molybdenum and Molybdenum-Containing Enzymes. Pergamon Press, Oxford.

Mills, C.F. and G.K. Davis. 1987. Molybdenum. Pages 429–463 in Trace Elements in Human and Animal Nutrition, Vol. 1, W. Mertz, (ed.) New York: Academic.

Ministry of Environment and Energy (MOEE). 1994. Ontario Drinking Water Objectives (Revised 1994). 68 pp. Toronto, ON.

Minster of Supply and Services Canada (MSSC). 1996. Fact Sheet No. 3. Shell Canada Products Limited. 10501 Sherbrooke St. East. Montreal East, Quebec. HB 1B3. Minister of Environment. Catalogue No.: EN153-6/3-1996E.

Mine Environment Neutral Drainage (MEND) Program. 2004. Review of water quality issues in neutral pH drainage: examples and emerging priorities for the mining industry in Canada. MEND Report 10.1. Prepared by Stantec Consulting Ltd. (Brampton). November 2004.

Mochizuki, M. and eight co-authors. 2002. The distribution of molybdenum in the tissues of wild ducks. Environmental Monitoring and Assessment 77:155-161.

Moller, G. 1995. Biogeochemical interactions affecting hepatic trace element levels in aquatic birds. Environmental Toxicology and Chemistry 7:1025-1033.

Morford, J.L. and S.R. Emerson. 1999. The geochemistry of redox sensitive trace metals in sediments. Geochimica et Comochimica Acta 63:1735-1750.

Morlon, H., C. Fortin, M. Floriani, C. Adam, J. Garnier-Laplace, and A. Boudou, 2005. Toxicity of selenite in the unicellular green alga *Chlamydomonas reinhardtii*: comparison between effects at the population and sub-cellular level. Aquatic Toxicology, 73, 65-78.

Muscatello, J.R., P.M. Bennett, K.T. Himbeault, A.M. Belknap and D.M. Janz. 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. Environmental Science and Technology 40, 6506-6512.

Nagpal, N.K. and K. Howell. 2001. Water Quality Guidelines for Selenium – Technical Appendix. Prepared for the Ministry of Water, Lands and Air Protection (MWLAP), Victoria, British Columbia. Available online at http://wlapwww.gov.bc.ca/wat/wq/BCguidelines/selenium/

Nagpal, N.K., L.W. Pommen, and L.G. Swain. 1995. Approved and working criteria for water quality. ISBN 0-7726-2522-0. Water Quality Branch. Ministry of Environment, Lands and Parks. Victoria, British Columbia. 45 pp.

Naiman, R.J. and J.J. Latterell. 2005. Principles for linking fish habitat to fisheries management and conservation. *Journal of Fish Biology* 67(Supplement B):166-185.

National Academy of Science (NAS). 2003. *Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications*. National Academies Press, Washington, D.C. ISBN 0-309-08625-6.

National Academy of Science (NAS). 1977. Drinking water and health. Washington, D.C. 1977:279-285.

National Research Council (NRC). 1976. Nutrient requirements of beef cattle. No. 4. Fifth revised edition. National Academy of Sciences, Washington, DC. USA. 56 pp.

National Research Council. 1980. Mineral tolerances of domestic animals. National Academy Press, Washington, DC. ISBN 0-309-03022-6.

National Research Council. 1989. Irrigation Induced Water Quality Problems: What can be learned from the San Joaquin Valley Experience. National Academy Press, 157 p.

National Research Council. 1983. Selenium in nutrition. Committee on Animal Nutrition, National. Academy of Science, Washington, D.C.

National Council on Radiation Protection and Measurements (NCRP). 1996. A guide for uncertainty analysis in dose and risk assessments related to environmental contamination. NCRP Commentary No. 14, NCRP, Bethesda, Maryland.

National Research Council. 1989. Irrigation induced water quality problems: What can be learned from the San Joaquin Valley experience. National Academy Press, 157 p.

National Research Council (NRC). 1984. Nutrient Requirements of Beef Cattle, Sixth Revised Edition. Washington, D.C.: National Academy Press.

National Research Council. 1983. Selenium in nutrition. Committee on Animal Nutrition, National. Academy of Science, Washington, D.C.

National Research Council. 1980. Mineral tolerances of domestic animals. National Academy Press, Washington, DC. ISBN 0-309-03022-6.

Neilands, J.B., F. M. Strong, and C. A. Elvhejem. 1948. Molybdenum in the nutrition of the rat. J. Biol. Chem., 173: 431-439.

New Mexico Environment Department/Surface Water Quality Bureau. 1996. *Red River groundwater investigation final report*. Final Report Submitted to USEPA, Region VI. March.

Newman, M.C., G.L. Diamond, C. Menzie, J. Moya, and J. Nriagu. 2004. Issue paper on metal exposure assessment. Report prepared for the U.S. Environmental Protection Agency. Risk Assessment Forum.

Nobbs, D.M., J.P. Barford, B.I. Carroll, and G.M. Peters. 1997. An integrated investigation of anthropogenic selenium contamination in Lake Macquarie, New South Wales. Pure and Applied Chemistry 69:2387-2401.

Nordberg, G., B. Sandstrom, G. Becking, and R.A. Goyer. 2000. Essentiality and toxicity of trace elements: Principles and methods for assessment of risk from human exposure to essential trace elements. Journal of Trace Elements in Experimental Medicine 13:141–153.

Nordstrom, D.K. 2005. A river on the edge--Water quality in the Red River and the USGS background study, *in* Price, L.G., Bland, D., McLemore, V.T., and Barker, J.M., eds., Mining in New Mexico, Decision-Makers Conference 2005, Taos region, Socorro, New Mexico: New Mexico Bureau of Geology and Mineral Resources, p. 64-67.

Oak Ridge National Laboratory (ORNL) – Risk Assessment Information System (RAIS). Internet Database at http://risk.lsd.ornl.gov/tox/tox_values.shtml. March 2003.

O'Connor, G.A., and seven co-authors. 2001a. A modified risk assessment to establish molybdenum standards for land application of biosolids. Journal of Environmental Quality 30:1490-1507.

O'Connor, G.A., T.C. Granato, and R.H. Dowdy. 2001b. Plant and environmental interactions. Bioavailability of biosolids molybdenum to corn. Journal of Environmental Quality 30:140-146.

O'Hara, T.M., J.C. George, J. Blake, K. Burek, G. Carroll, J. Dau, L. Bennett, C.P. McCoy, P. Gerard, and V. Woshner. 2003. Health Assessment of Western Arctic and Teshekpuk Lake Caribou of Northern Alaska in Response to a Mortality Event. Arctic 56 (2): 125-135.

Ohlendorf, H.M., R.L. Hothem, C.M. Bunck, and K.C. Marois. 1990. Bioaccumulation of selenium in birds at Kesterson reservoir, California. Archives of Environmental Contamination and Toxicology 19:495-507.

Opresko, D.M. 1993a. *Toxicity summary for selenium and selenium compounds*. Report prepared for the Oak Ridge Reservation Environmental Restoration Program. March, 1993.

Opresko, D.M. 1993b. *Toxicity summary for molybdenum and molybdenum compounds*. Report prepared for the Oak Ridge Reservation Environmental Restoration Program. January, 1993.

Osman, N.H.I and A.R. Sykes. 1989. Comparative effects of dietary molybdenum. concentration on distribution of copper in plasma of sheep and red deer (*Cervus elephas*). New Zealand Soc. Anim. Prod. 49: 15-19.

Oster, J.D., J.E. Tracy, J.L. Meyer, and M.J. Snyder. 1988. Selenium in or near the Southern Coast Range: well waters and vegetable crops. Pages 51 to 55, in Tanji, K.K., Valoppi, L., and Woodring, R.C., (eds.), *Selenium Contents in Animal and Human Food Crops Grown in California*. University of California, Division of Agriculture and Natural Resources Publication 3330.

Ostrom, C.A., R.Van Reen, and C.W. Miller. 1961. Changes in the connective tissue of rats fed toxic diets containing molybdenum salts. Journal of Dental Research 40:520-528.

Outridge, P.M., A.M. Scheuhammer, G.A. Fox, B.M. Braune, L.M. White, L.J. Gregorich, and C. Keddy. 1999. An assessment of the potential hazards of environmental selenium for Canadian water birds. Environmental Reviews 7:81-96.

Pais, I. and J.B. Jones,Jr. 1997. The Handbook of Trace Elements. St. Lucie Press, Boca Raton, Florida.

Palace, V.P., J. E. Spallholz, J. Holm, K. Wautier, K., R.E. Evans, and C.L. Baron. 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. Ecotoxicology and Environmental Safety 58:17–21.

Paulsson, K. and K. Lundberg. 1989. Treatment of mercury contaminated fish by selenium addition. Journal of Water Air and Soil Pollution. 56:833-841.

Peters, G.M., W.A. Maher, J.P. Barford, and V.G. Gomes. 1997. Selenium associations in estuarine sediments: Redox effects. Journal Water, Air, and Soil Pollution 99:275-282.

Peters, R.H. 1991. A critique for ecology. Cambridge University Press, Cambridge. 382 p.

Peterson, J. A. and A. V. Nebeker. 1992. Estimation of waterborne selenium concentrations that are toxicity thresholds for wildlife. Archives of Environmental Contamination and Toxicology 23: 154-162.

Peterson, M.J., J.G. Smith, M.G. Southworth, M.G. Ryon, and G.K. Eddlemon. 2002. Trace element contamination in benthic macroinvertebrates from a small stream near a uranium mill tailings site. Environmental Monitoring and Assessment 74:193-208.

Phillippo, M., W.R. Humphries, and P.H. Garthwaite. 1987. The effect of dietary molybdenum and iron on copper status and growth in cattle. J. Agric. Sci. 109:315–320.

Pickard, J., P. McKee, and J. Stroiazzo. 1999. Site specific multi-species toxicity testing of sulphate and molybdenum spiked mining effluent and receiving water. Pages 86-95 in W.A. Price, B. Hart, and C. Howell (eds.), Proceedings of the 1999 Workshop on Molybdenum Issues in Reclamation. Kamloops, B.C. 24 September.

Pilon-Smits, E.A.H., M.P. De-Souza, G. Hong, A. Amini, R.C. Bravo, S.T. Payabyab, and N. Terry. 1999. Selenium accumulation by twenty aquatic plants species. Journal of Environmental Quality 28:1011–1018.

Pipken, B.W., D.D. Trent, and R. Hazlett, 2004. Geology and the Environment. Fourth Edition. Thomson Brooks Cole, Nelson, British Columbia, Canada.

Prange, A. and K. Kremling. 1985. Distribution of dissolved molybdenum, uranium and vanadium in Baltic Sea waters. Marine Chemistry 16:259-274.

Presser, T.S. and H.W. Ohlendorf. 1987. Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA: Environmental Management, 11:805-821.

Presser, T.S., M.A. Sylvester, and W.H. Low. 1994, Bioaccumulation of selenium from natural geologic sources in western states and its potential consequences: Environmental Management 18:423-436.

Rainbow, P.S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 120: 497-507.

Rand, G.M., P.G. Wells, and L.S. McCarty. 1995. Introduction to Aquatic Toxicology. Pages 3 – 70 in G.M. Rand (ed.), Fundamentals of Aquatic Toxicology Effects, Environmental Fate, and Risk Assessment. Taylor and Francis, London.

Rieberger, K. 1992. Metal concentrations in bottom sediments from uncontaminated B.C. lakes. Water Quality Section, Water Quality Branch, Ministry of Environment, Lands and Parks. Province of British Columbia.

Reash, R.J., T.W. Lohner and K.V. Wood. 2006. Selenium and other trace metals in fish inhabiting a fly ash stream: Implications for regulatory tissue thresholds. Environmental Pollution 142: 397-408.

Reid, S.D. 2002. Physiological impact of acute molybdenum exposure in juvenile kokanee salmon (*Oncorhynchus nerka*). Comparative Biochemistry and Physiology Part C 133:355-367.

Retana, J., D.R. Parker, C. Amrhein, and A.L. Page. 1993. Growth and trace element concentrations of five plant species grown in a highly saline soil. Journal of Environmental Quality 22:805-811.

Rieberger, K. 1992. Metal concentrations in bottom sediments from uncontaminated B.C. lakes. Water Quality Section, Water Quality Branch, Ministry of Environment, Lands and Parks. Province of British Columbia.

Riordan, B. 2003. A comparison of pre-development and present molybdenum concentrations in vegetation on the Endako mine site and implications to ungulate health. Presented at the 27th Annual British Columbia Mine Reclamation Symposium, Kamloops, September 15-18, 2003.

Roe, J. H., W.A. Hopkins, W.A. Hopkins, J.A. Baionno, B.P. Staub, C.L. Rowe, and B.P. Jackson. 2004. Maternal transfer of selenium in *Alligator mississippiensis* nesting downstream from a coal-burning power plant. Environmental Toxicology and Chemistry 23: 1969-1972.

Rosenfeld, I. and O.A. Beath. 1954. Effect of selenium on reproduction in rats. Proceedings Society of Experimental and Biological Medicine 87:295-297.

Rossman R. and J. Barres J. 1988. Trace element concentrations in near-surface waters of the great lakes and methods of collection storage and analysis. Journal of Great Lakes Research 14:188-204.

Rudolph, B., C.J. Kennedy, and I. Andreller. 2007. The effects of selenium on westslope cutthroat trout reproduction and development in fish captured at Clode Pond and O'Rourke Lake. Report prepared for the Elk Valley Selenium Task Force.

Sample, B.E., D.M. Opresko and G.W. Suter. 1996. Toxicological benchmarks for wildlife: 1996 Revision. Risk Assessment Program, Health Sciences Research Division. Oak Ridge, Tennessee. ES/ER/TM-86/R3.

Sappington K, J. McGeer, C. Delos, R. Goulet, R. Lipnick, A. Rea, P. Shaw-Allen, and R. Sugatt. 2006. Critical review and synthesis of metals bioaccumulation models for use in risk assessment. Poster presentation at the 27th Annual Meeting of the Society of Environmental Toxicology and Chemistry, November 13-17, 2005, Baltimore, MD, USA.

Saskatchewan Geological Survey (SGS). 2003. Geology and Mineral and Petroleum Resources of Saskatchewan. Miscellaneous report 2003-7, 173 + viii pages.

Schalscha, E.B., M. Morales, and P. F. Pratt. 1987. Lead and molybdenum in soils and forage near an atmospheric source. Journal of Environmental Quality 16:313-315.

Schlekat, C.E., D.G Purkerson and S.N. Luoma. 2004. Modeling selenium bioaccumulation through arthropod food webs in San Francisco Bay, California, USA. Environmental Toxicology and Chemistry 23:3003-3010.

Schroeder, M.D. and M. Mitchener. 1971. Toxic effects of trace elements on the reproduction of mice and rats. Archives of Environmental Health 23:102-106.

Sharmasarkar, S. and G.F. Vance. 2002. Soil and Plant Selenium at a Reclaimed Uranium Mine. Journal of Environmental Quality 31:1516–1521.

Sheppard, S.C., C. Gaudet, M.I. Sheppard, P.M. Cureton, and M.P. Wong. 1992. The development of assessment and remediation guidelines for contaminated soils, a review of the science. Canadian Journal of Soil Science 72: 359-394.

Sheppard, M.I. and D.H. Thibault. 1990. Default soil solid/liquid partition coefficients, Kds, for four major soil types: a compendium. Health Physics 59:471-82.

Simmons, D.B. and D. Wallschläger. 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. Environmental Toxicology and Chemistry 24:1331-1343.

Skorupa, J.P. and H.M. Ohlendorf. 1991. Contaminants in drainage water and avian risk thresholds. Pages 345-368 in D. Zilberman (eds), The economics and management of water and drainage in agriculture. Kluwer Academic Publishers, Boston.

Smart, M.E., R. Cohen, D.A. Christensen and C.M. Williams. 1986. The effects of sulphate removal from the drinking water on the plasma and liver copper and zinc concentrations of beef cows and their calves. Canadian Journal of Animal Science 66: 669-680.

Smith, G.J., G.H. Heinz, D.J. Hoffman, J.W. Spann, and A.J. Krynitsky. 1988. Reproduction in Black-Crowned Night-Herons Fed Selenium. Lake and Reservoir Management 4(2):175-180.

Staven, L.H., B.A. Napier, K. Rhoads, and D.L. Strenge. 2003. A compendium of transfer factors for agricultural and animal products. PNNL-13421.Pacific Northwest National Laboratory, Richland, Washington.

Stokinger, H.E. 1981. The metals. *In*: Patty's industrial hygiene and toxicology, 3rd edition. Editors, G.D. Clayton and F.E. Clayton. John Wiley and Sons, New York.

Surridge, B., T.S. Sorenson, and W. Majak. 2001. Purification and characterization of molybdenum species from *Medicago sativa* (alfalfa) grown on mine tailings. Journal of Agriculture and Food Chemistry 49:3853-3858.

Suter, G.W. and C.L. Tsao. 1996. Toxicological benchmarks for screening potential contaminants of concern for effects on aquatic biota: 1996 Revision. Risk Assessment Program, Health Sciences Research Division. Oak Ridge, Tennessee.

Taylor, M.E. and P. McKee. 2003. Wild ruminant study at Brenda Mines. Presented at the 27th Annual British Columbia Mine Reclamation Symposium. Kamloops, B.C. Sept. 15-18, 2003.

Taylor, M.E. and P. McKee. 2000. Brenda Mines: Wild ruminant study. Prepared for Noranda Inc. 28 pp.

Thomas, P.A. 1996. Baseline concentration of heavy metals and radionuclides in nine moose livers from the Hudson Bay area of east central Saskatchewan including implications for risk assessment. Saskatchewan Health. 32 pp.

Thomas, P., J. Irvine, L. Lyster, and R. Beaulie. 2005. Radionuclides and trace metals in Canadian moose near uranium mines: comparison of radiation doses and food chain transfer with cattle and caribou. Health Physics 88:423-438.

Tri-Star Environmental Consulting (Tri-Star). 2005. Site-Specific Water Quality Guidelines for the Kootenay River at Kootenay Crossing for the Purpose of National Reporting. Prepared for Environment Canada. 32 p.

Toll, J.E. 2005. Statistical regression model for setting site-specific selenium water quality objectives. Report prepared for the Canadian Nuclear Safety Commission. Toll Environmental, Seatle, WA. Contract No. 877055-05-0179.

Toll, J.E., L.M. Tear, D.K. DeForest, K.V. Brix, and W.J. Adams. 2005. Setting site-specific water quality standards using tissue residue criteria and bioaccumulation data. Part 1: Methodology. Environmental Toxicology and Chemistry 24:224-230.

Twidwell, L.G., J. McCloskey, P. Miranda, and M. Gale. 2000. Technologies and potential technologies for removing selenium from process and wastewater: update. Proceedings Minor Elements 2000, Editor, C. Young, SME, Salt Lake City, UT. pp 53-66.

Tyler, G. 2005. Changes in the concentrations of major, minor and rare-earth elements during leaf senescence and decomposition in a *Fagus sylvatica* forest. Forest Ecology and Management 206: 167-177.

Underwood, E.J. 1971. Trace elements in human and animal nutrition. Pages 116 to 140 in Molybdenum. Academic Press, New York.

U.S. Environmental Protection Agency (U.S. EPA). 2006. Life cycle assessment: principles and practice. Prepared by Scientific Applications International Corporation for U.S. EPA, National Risk Management Research Laboratory. Contract 68-C02-067.

U.S. Environmental Protection Agency (U.S. EPA). 2005a. Integrated Risk Information System (IRIS).

U.S. Environmental Protection Agency (U.S. EPA). 2005b. Review of EPA's Draft Framework for Inorganic Metals Risk Assessment (Internal Review Draft). Science Advisory Board, Washington, DC, USA (available at http://www.epa.gov/sab/fiscal06.htm).

U.S. Environmental Protection Agency (U.S.EPA). 2004a. Draft aquatic life water quality criteria for selenium. EPA-822-D-04-001. Nov. 2004.

U.S. Environmental Protection Agency (U.S.EPA). 2004b. Report on bioavailability of chemical wastes with respect to the potential for soil bioremediation. Report prepared for by E. L. Madsen, Department of Microbiology, Cornell University.

U.S. Environmental Protection Agency (U.S.EPA). 2002. National recommended water quality criteria: 2002 Human health criteria calculation matrix. EPA-822-R-02-012. November, 2002.

U.S. Environmental Protection Agency (U.S.EPA). 2000a. Introduction to phytoremediation. National Risk Management Research Laboratory. Office of Research and Development, U.S. Environmental Protection Agency. EPA/600/R-99/107. February 2000.

U.S. Environmental Protection Agency (U.S.EPA). 2000b. Bioaccumulation testing and interpretation for the purpose of sediment quality assessment - Status and needs. EPA-823-R-00-001.

U.S. Environmental Protection Agency (U.S.EPA). 1999. Understanding variation in partition coefficient values: Vol 1 - The Kd Model, Methods of Measurement, and Application of Chemical Reaction Codes. EPA 402-R-99-004A.

U.S. Environmental Protection Agency (U.S.EPA). 1993. Wildlife exposure factors handbook. Vol. 1, U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/R-93/187a.

U.S. Department of Interior (U.S. DOI). 1998. Guidelines for interpretation of the biological effects of selected constituents in biota, water, and sediment. National Irrigation Water Quality Program (NIWQP) – Information Report No. 3.

U.S. Geological Survey (USGS). 2004. Forecasting selenium discharges to the San Francisco Bay-Delta Estuary: Ecological Effects of A Proposed San Luis Drain Extension, Prepared by Samuel N. Luoma and Theresa S. Presser, U.S. Geological Survey. Open-File Report 00-416, available at http://water.usgs.gov/pubs/ofr00-416/

U.S. Geological Survey (USGS). 2006. Molybdenum. Mineral Commodity Summaries, January 2006. U.S. Department of the Interior, Washington, D.C. Copy available at: http://minerals.usgs.gov. Prepared by Michael J. Maguer.

U.S. Geological Survey (USGS). 2006. Selenium. Mineral Commodity Summaries, January 2006. U.S. Department of the Interior, Washington, D.C. Copy available at: http://minerals.usgs.gov. Prepared by Michael W. George.

Vidal, D.E., S.M. Bay., and D. Schlenk. 2005. Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). Archives of Environmental Toxicology and Chemistry 49: 71-75.

Vyskocil, A. and C. Viau. 1999. Assessment of molybdenum toxicity in humans. Journal of Applied Toxicology 19:185–192.

Walker, H.H., J.H. Runnels, and R. Merryfield. 1976. Determination of trace quantities of selenium in petroleum and petroleum products by atomic absorption spectrophotometry. Analytical Chemistry 48:2056-2060.

Walters, C.J. and C.S. Holling, 1990. Large-scale management experiments and learning by doing. Ecology 71:2060-2068.

Wan, H.F., R.L. Mikkelsen, and A.L. Page. 1988. Selenium uptake by some agricultural crops from central California soils. Journal of Environmental Quality 17:269-272.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: a review. Journal of Animal Science 46:1078-1085.

Wayland, M., H.G. Gilchristb, and E. Neugebauer. 2005. Concentrations of cadmium, mercury and selenium in common eider ducks in the eastern Canadian arctic: Influence of reproductive stage. Science of the Total Environment 351-352:323-332.

Welchman, B. and C. Aspinall 2000. Mine Closure and Sustainable Development: Island Copper Mine: a Case History. Prepared for the World Bank; Mine Closure and Sustainable Development Workshop (Washington, D.C.).

Wide, M. 1984. Effect of short-term exposure to five industrial metals on the embryonic and fetal development of the mouse. Environmental Research 33:47-53.

World Health Organization (WHO). 2006. Guidelines for drinking-water quality incorporating first addendum. Vol. 1, Recommendations. – 3rd ed. 1.Potable water – standards. 2.Water – standards. 3.Water quality – standards. 4.Guidelines. (available at http://www.who.int/)..

World Health Organization (WHO). 1996. Molybdenum in drinking water. Background document for development of WHO guidelines for drinking-water quality guidelines In: Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. World Health Organization, Geneva, 1996. Available at: http://www.who.int/).

World Health Organization (WHO). 1996. Selenium in drinking water. Background document for development of WHO guidelines for drinking-water quality guidelines In: Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. World Health Organization, Geneva, 1996. .

World Health Organization (WHO). 1986. International Programme on Chemical Safety – Environmental Health Criterion 58: Selenium. Available at: http://www.who.int/).

Wu, L. 2005. Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium. Ecotoxicology and Environmental Safety 57:257-269.

Xie, R., H.M. Seip, G. Wibetoe, S. Nori, and C.W. Mcleod. 2006. Heavy coal combustion as the dominant source of particulate pollution in Taiyuan, China, corroborated by high concentrations of arsenic and selenium in PM_{10}. Science of the Total Environment.370:409-415.

Yudovich, Ya.E., and M.P. Ketris. 2006. Selenium in coal: A review. International Journal of Coal Geology 67:112-126.

Zhang, Y. and J.N. Moore. 1996. Selenium fractionation and speciation in a wetland system. Environmental Science and Technology 30:2613-2619.

Zhang, Y., Z.A. Zahir, and W.T. Frankenberger, Jr. 2004. Fate of colloidal-particulate elemental selenium in aquatic systems. Journal of Environmental Quality 33:559-564.

Zhang, Y. and W.T. Frankenberger, Jr. 2005. Removal of selenium from river water by a microbial community enhanced with *Enterobacter taylorae* in organic carbon coated sand columns. Science of the Total Environment 346:280-285.

TABLES

Table 3.1: Typical Background Concentrations of Selenium in Different Media and Biological Tissues

Media		Background Range	
		Lower	Upper
Physical Media	Water	0.1	0.4
	Sediment	0.2	2
Plants	Terrestrial plants	0.01	0.6
	Freshwater algae	0.1	1.5
	Freshwater macrophytes	0.1	2
Fish	Whole body	NA	NA
	Muscle	NA	NA
	Liver	1	4
Birds	Liver	4	10
	Muscle	1	3
	Eggs (MES)	<5	
Mammals	Muscle	<1	
	Liver	1	10

Values pertaining to water are in units of µg/L, all other values are in units of ug/g (dw)
All values taken from U.S. DOI (1998), except for bird liver, taken from USGS, 2004a
MES-mean egg selenium

Table 3.2: Freshwater Bioconcentration Factors (BCF) and Bioaccumulation Factors (BAF) for Various Forms of Selenium for Organisms that Differ in Size and Habitat Use

	Selenate		Selenite		Selenomethionine		Unspecified	
Biota Type	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Cyanobacteria BCF	30	115	267	1,004	1,520	12,139		
Algae BCF	-	428	102.7	1,440	-		100	2,600
Periphyton BCF	-	141	-	755	-	16,836	628	8,667
Rooted macrophyte BCF	72	72	363	1,200	-	3,266	174	
Zooplankton BCF	-	351	-	1,087	-	28,870	-	-
Macro-invertebrates BCF	71	322	570	1,800	-	-	172	~4,000
Macro-invertebrates BAF	-	-	1,399	1,957	-	-	969	31,800
Fish BCF	6.97	56	2	470	0.5	1	200	5,333
Fish BAF	-	-	1,930	6,800	-	-	273	6,538
Water Birds BAF	-	-	-	-	-	10.45	-	-

All values in units of L/kg (dry weight), taken from Nagpal and Howell, 2001
Fish are either whole body or muscle

Table 3.3: Summary of Selenium BAFs and BCFs for Freshwater Biota Across Study Sites and Default Range

Biota Type	Staven et al., 2003	Karlsson et al, 2002	Nagpal and Howell, 2001	EPA, 2004a	Conservative Default
Cyanobacteria	-	-	30 - 12,139	-	10,000
Algae	-	-	102.7 - 2,600	428 - 1,440	2500
Zooplankton	-	218 - 149,000	351 - 28,870	91 - 570	100,000
Periphyton	-	-	141 - 16,836	628 - 8,667	15,000
Rooted Aquatic Macrophytes	1000	-	72 - 3,266	-	3,000
Macro-invertebrates BCF	8000	-	71 - 4,000	91 - 1,440	6,000
Macro-invertebrates BAF	-	-	-	969 - 31,800	30,000
Fish BCF	680	-	0.5 to 5,333	2 - 470	5,000
Fish BAF	-	985 - 13,000	273 - 6,800	273 - 6,538	10,000
Birds - BAF	-	-	10.45	-	10

All values in units of L/kg (dry weight)
Fish are either whole body or muscle

Table 4.1: Summary of Toxicity Values for Selenium as Selenate to Aquatic Species That Differ in Size and Habitat Use

Species		LC50 (ug/L)		Number of
Common name	Scientific name	Mean	S.D.	Studies
Insects:				
Midge	Chironomus thummi	8,045	6,255	2
Zooplankton:				
Calanoid copepod	Acartia clausi	2,100	-	2
Calanoid copepod	Acartia tonsa	850	-	2
Opossum shrimp	Americamysis bahia	600	-	1
Rotifer	Brachionus calyciflorus	16,100	100	2
Rotifer	Brachionus plicatilis	17,000	-	1
Water flea	Daphnia magna	880	786.6	6
Scud	Hyalella azteca	450	363.6	3
Amphibians:				
Eastern Narrow-Mouthed Toad	Gastrophryne carolinensis	90	-	1
Fish:				
Sheepshead minnow	Cyprinodon variegatus	25,425	18,975	4
Coho salmon,silver salmon	Oncorhynchus kisutch	24,214	3,475	7
Rainbow trout,donaldson trout	Oncorhynchus mykiss	24,400	18,252	5
Chinook salmon	Oncorhynchus tshawytscha	67,838	16,681	8
Fathead minnow	Pimephales promelas	800	200	2

Data obtained from U.S. EPA ECOTOX Database (2006)

Table 4.2: Summary of Toxicity Values for Different Forms of Selenium to Invertebrates and Fish in Freshwater and Marine Habitats: I

Duration	Selenium species	Freshwater		Marine	
		Invertebrates	Fish	Invertebrates	Fish
Acute[1]	Selenite	6 - 68,000	62 - 126,000	127 to >10,000	599 - 36,700
	Selenate	57 - 25,000	5,500 - 82,000	No data	1,600 - 85,840
	Unspecified or mixed	220 - 56,700	620 - 87,300	800 - 6,200	600 - 67,100
Chronic[2]	Selenite	3 - 3,000	10 - 32,700	212 (1 value)	675 (1 value)
	Selenate	90 - 15,000	566 - 8,780	400 (NOAEC)	39 - 1,360
	Unspecified or mixed	2 - 8,000	5 - 5,600	135 - 100,000	90 (1 value)

All values reported in units of µg/L, after Nagpal and Howell (2001)
1 - acute values include LC50 or EC50
2 - includes effects of uncertain ecological relevance (e.g. behavioural and metabolic effects)

Reported by Nagpal and Howell (2001)

Table 4.3: Summary of Toxicity Values for Different Forms of Selenium to Invertebrates and Fish in Freshwater and Marine Habitats: II

Value	Selenium species	Freshwater		Marine	
		Invertebrates	Fish	Invertebrates	Fish
SMAV	Selenite	440 - 203,000	1,783 - 35,000	255 - 10,000	599 - 17,350
	Selenate	5.93 - ~1,500,000	10,305 - 226,320	NA	9,790 (1 species)
FAV	Selenite	514.9		253.4	
	Selenate	834.4 (417.2 - adjusted)*		NA	
GMCV	Total Selenium	42.36 (1 species)	9.5 to >23.28	NA	
FCV	Total Selenium	7.91		NA	

Toxicity data adapted from the EPA (2004a)
* adjusted to 100 mg/L sulphate
Acute values reported as Species Mean Acute (toxicity) Values (SMAV) and Final Acute (toxicity) Values (FAV) in units of µg/L
Chronic values reported as Genus Mean Chornic Value (GMCV) and Final Chronic (toxicity) Value (FCV) in units of µg/g tissue (dw)

Table 4.4: Summary of Regulatory Benchmarks and Suggested Toxicity Threshold Levels for Molybdenum for Organisms that Differ in Size and Habitat Use

Media		Regulatory Benchmarks		US DOI (1998)			Most Restrictive
		CCME	BC	No Effect Level	Toxic Threshold	Note	
Physical Media	Water - fish	0.073	2	0.02	0.12	For fish. 0.02, upper limit of natural background (Eisler 1989); 0.12, LC10 for larval trout (Birge et al. 1980)	0.02
	Water - aqautic plants	-	-	-	>50	-	50
	Water - livestock	0.5	0.08	-	-	-	0.08
	Water - wildlife	-	0.05	-	-	-	0.05
Birds	Diet	-	-	500	>6000	Adverse effects on reproduction and on survival, respectively	500
	Eggs (MES)	-	-	23	33	Normal egg concentration is <1. Emryotoxic threshold is 23 to 33 mg/kg in egg.	23
Mammals	Diet - (concentration in food)	-	-	-	10	Recommend limit in livestock forage to protect sensitive ruminants (NRC, 1976)	10
	Diet - (Copper:Molybdenum ratio in feed)	-	-	6:1 to 10:1	<2:1 or >10:1	Ratios found to lead to either Cu deficiency or Cu toxicosis	-

Values pertaining to water are in units of mg/L, all other values are in units of mg/kg (dw)
MES-mean egg selenium

Table 4.5: Review of the Observed Toxicity of Molybdenum, as Sodium Molybdate, to Different Fish Species from Laboratory and Field Studies

Species	Endpoint	Effect Concentration (mg/L Mo)	Reference
Oncorhyncus mykiss	32-d LC50	>400	Davies *et al*. (2005)
“”	28-d LC50	0.73	Birge (1978)
“”	28-d LC50	0.79	Birge *et al*. (1980)
Oncorhyncus clarki	30-d LC50	>90	Pickard *et al*. (1999)
Oncorhyncus kisutch	96-h LC50	>1000	Hamilton and Buhl (1990)
Oncorhyncus nerka	96-h LC50	>2000	Reid (2002)
Carassius auratus	7-d LC50	60	Birge (1978)

Toxicity data adapted from Davies *et al*., (2005).

Table 5.1: Summary of Regulatory Benchmarks and Suggested Toxicity Threshold Levels for Selenium for Organisms that Differ in Size and Habitat Use

Media		Regulatory Benchmarks			Suggested Thresholds							
					US DOI, 1998		US FWS, 2001[1]					
		CCME (2002)	BC (2001)	EPA Draft (2004a)	No Effect Level	Threshold	No Effect Level	Threshold	Lemly and Smith, 1987	Lemly, 2001 and 2004	Brix et al., 2005	Most Limiting
Physical Media	Water	1	1	-	<1	>2	<2	>5	2	2 (filtered)	-	1
	Sediment	-	4	-	<1	>4	<2	>4	4	2	-	2
Fish	Diet	-	-	-	<2	>3	<2	>7	5	3	10	3
	Whole body	-	-	7.91	<3	>4	<4	>12	12	4	6	4
	Muscle	-	-	-	-	-	-	-	8	8	-	8
	Liver	-	-	-	-	-	-	-	-	12	-	12
	Ovaries/eggs	-	-	-	-	-	-	-	-	10	17	10
Birds	Diet	-	-	-	<2	>3	<2	>7	3	3	-	3
	Liver	-	-	-	-	-	-	-	-	10	-	10
	Eggs (MES)	-	-	-	<3	>6	<3	>8	15	3	16	3
Mammals	Diet	-	-	-	<2	>3	-	-	-	-	-	3
	Muscle	-	-	-	-	-	-	-	-	-	-	-
	Liver*	-	-	-	-	-	-	-	-	-	-	7

*WHO (1987) benchmark for selenium in animal liver is >7 ug/g ww

Values pertaining to water are in units of ug/L, all other values are in units of ug/g (dw)

1 - concentrations higher than the "No effect level" yet lower than the "threshold" are considered to be within a "level of concern".

Table 5.2: Environmental Distribution of Selenium in the Elk River Downstream of Coal Mines for Different Media, Biota, and Fish

Environmental Compartment		Screening Threshold (See Table 5.1)	Site						
			745	750	748	746	749	751	747
			Main Channel - Upstream Reference	Main Channel - Upstream of 1st tributary	1st Exposed Tributary - Upstream	1st Exposed Tributary - Downstream	2nd Exposed Tributary	3rd Exposed Tributary	Main Channel - Downstream of 3rd tributary
Physical Media	Water Se	1	0.1	0.4	NS	**8.6**	**10.5**	**7.1**	2.2
	Sediment Se	2	1.28	0.7	0.57	**2.41**	1.74	**2.32**	NS
	TOC (%)[1]	-	3.1	0.8	1.4	8.1	35	2.7	NS
	Kd (L/kg)[2]	-	12,800	1,750	NC	280	166	327	NC
Primary Biota	Periphyton Se	3	0.31	0.78	NS	1.56	1.28	1.26	1.28
	BAF (L/kg)[3]	-	3,100	7,800	NC	15,600	12,800	12,600	12,800
	Invertebrates	3	2.74	**4.62**	**6.84**	**10.7**	**8.69**	**6.82**	**4.29**
	BAF	-	884	1,490	NC	3,452	2,803	2,200	1,384
Fish[4]	Muscle	3	**3.9**	**9.4**	NS	NS	NS	NS	**4.4**
	BAF (L/kg)	-	12,560	30,161	NC	NC	NC	NC	14,093
	Liver	8	**18.9**	**40.0**	NS	NS	NS	NS	**19.8**
	BAF (L/kg)	-	6,882	14,586	NC	NC	NC	NC	7,236
	Gonad	10	**21.0**	**28.2**	NS	NS	NS	NS	**31**
	BAF (L/kg)	-	5,393	7,243	NC	NC	NC	NC	8,013

All values are site means, reported by McDonald and Strosher (1998)
Selenium concentrations in water are in units of ug/L, all other values are in units of ug/g (dw)
NS/NC - not sampled/not calculated
1 - TOC is total organic carbon content of sediments
2 - Kd is the distribution coefficient, calculated as the concentration of selenium in sediment (converted to µg/kg) to that in water (µg/L)
3 - BAF is the bioaccumulation factor, calculated as the concentration of selenium in biota (converted to µg/kg) to that in water (µg/L)
4 - Fish species were cutthroat trout (*Oncorhynchus clarki lewisi*) and mountain whitefish (*Coregonus lavaretus*)

Table 5.3: Summary of Obsrved Selenium Distribution in Receiving Environments Across Media, Biota, and Fish

Reference		Lemly, 1985	Presser and Ohlendorf, 1987	Dickerson and Ramirez, 1997				
Location		Belews Lake, North Carolina	Kesterson Reservoir, California	Wildlife Management Areas, Wyoming				
Waterbody type		Reservoir	Reservoir	Ponds and drainage basins				
Anthropogenic influence(s)		Coal effluents	Irrigation Drainage	Irrigation Drainage				
Other COPCs		Metals	Metals, Nutrients, Pesticides	Metals, pesticides				
Source concentraton of selenium (ug/L)		150 - 200	330	Not Reported				
Physical media	Water	10	15 - 350	6.0	3.0	<2	<2	2
	Sediment	14	12	0.8	0.8	0.4	2.0	0.5
	Kd (L/kg)[1]	1,400	~100	133	267	>200	>1000	250
Primary biota	Plankton	30	18.1 - 36.5	-	-	-	-	-
	BAF (L/kg) (dw)[2]	-	~2000	-	-	-	-	-
	Macrophytes	-	12.9 - 35.4	4.4	2.5	4.3	3.5	3.8
	BAF (L/kg) (dw)[2]	-	~2,000	733	833	>2150	>1750	1900
	Invertebrates	20 - 50	6.4 - 96.3	3.8	2	9	2.4	2.9
	BAF (L/kg) (dw)[2]	~2,000 - 5,000	~200	633	667	>4500	>1200	1450
Fish	Whole	40 - 125	115 - 283	-	-	-	-	-
	Muscle	20 - 40	-	-	-	2.1	-	-
	Liver	-	-	-	-	-	-	-
	Gonad	20 - 170	-	-	-	-	-	-
Birds	Liver	-	2 - 180	-	-	-	-	-
	Egg	-	3 - 360	3.2	4	4	-	-
Notes			Selenium load mostly as selenate.	Five separate sites reported. Macrophytes sampled were pondweed. Bird spcies included mallards and avocets.				
Effects		Extirpation of multiple fish species.Teratogenic abnormalities in surviving species.	Suspected extirpation fo fish species, reproductive failure of several bird species	Measures of effect included nest success and embryo deformities. No evidence of adverse effects reported.				

Values are reported site means or ranges

Water concentrations are total selenium (µg/L). All other selenium concentrations (sediment and biota) are µg/g dry weight

1 - Kd is the distribution coefficient, calculated as the mean or mid concentration of selenium in sediment (converted to ug/kg) to that in water (ug/L)

2 - BAF is the bioaccumulation factor, calculated as the mean or mid concentration of selenium in biota (converted to µg/kg) to that in water (µg/L)

Table 6.1: Estimated Selenium Transfer Parameters for Different Organisms Used in SSRA at Sites with Low to High Selenium

Environmental Compartment	High	Medium	Low
Sediment	10,000	5,000	2,500
Algae	2,500	1,500	1,000
Zooplankton	100,000	100,000	100,000
Periphyton	15,000	10,000	5,000
Aquatic Macrophytes	3,000	2,000	1,000
Benthic Invertebrates	30,000	20,000	10,000
Forage Fish	15,000	10,000	5,000
Predatory Fish	30,000	20,000	10,000

Sediment value is the partition coefficient (Kd) in unts of L/kg (dw)
All biological values are BAFs, in units of L/kg (dry weight)
Fish BAFS are whole body
Derived from various sources (e.g., U.S. DOI, 1998; U.S. EPA, 1999; U.S. EPA 2004a, U.S. EPA 2005b)

Table 6.2: Summary of Suggested Toxicity Threshold Levels for Representative Species of Mammals

Reference Dose				Reference Organism		Benchmark Dose for Representative Receptor Species				
Reference	Substance	Dose Type	Dose (ug/kg/d)	Species	Weight (kg)	Moose (400 kg)	Black bear (85 kg)	River Otter (8 kg)	Muskrat (1.4 kg)	Mink (1 kg)
Opresko, 1993	selenium (unspecified)	Chronic LOAEL	15	human	55	9.1	13	24	38	41
Opresko, 1993	selenium (unspecified)	Chronic NOAEL	23	human	55	14	21	37	58	63
Sample et al., 1996	selenate	Chronic LOAEL	760	mouse	0.03	71	104	188	291	316
Sample et al., 1996	selenate	Chronic NOAEL	76	mouse	0.03	7.1	10	19	29	32
Sample et al., 1996	selenite	Chronic LOAEL	4600	mouse	0.03	428	630	1138	1760	1914
Sample et al., 1996	selenite	Chronic NOAEL	460	mouse	0.03	43	63	114	176	191
Sample et al., 1996	selenomethionine	Chronic NOAEL	25	macaque	4.25	8.0	12	21	33	36
Sample et al., 1996	selenate	Chronic LOAEL	330	rat	0.35	57	84	151	233	254
Sample et al., 1996	selenate	Chronic NOAEL	200	rat	0.35	34	51	91	141	154
Sample et al., 1996	molybdate	Chronic LOAEL	2600	mouse	0.03	242	356	643	995	1082
Sample et al., 1996	molybdate	Chronic NOAEL	260	mouse	0.03	24	36	64	99	108

All doses in units of ug/kg/day

In the calcalution of benchmark doses for other animals, $Dose_2=Dose_1*(weight_1/weight_2)^{1/4}$, as per Sample et al., 1996

Table 6.3: Summary ofToxicity Thresholds for Representative Avian Fauna

Substance	Reference Organism	LOAEL (ug/kg/day)	NOAEL (ug/kg/day)
selenite	Mallard	1000	500
selenomethionine	Mallard	800	400
selenomethionine	Screech owl	1500	440
selenomethionine	Night-heron	NA	1800
Molybdate	Chicken	35300	3530

No body-weight adjustment required for birds (Sample *et al*., 1996)

FIGURES

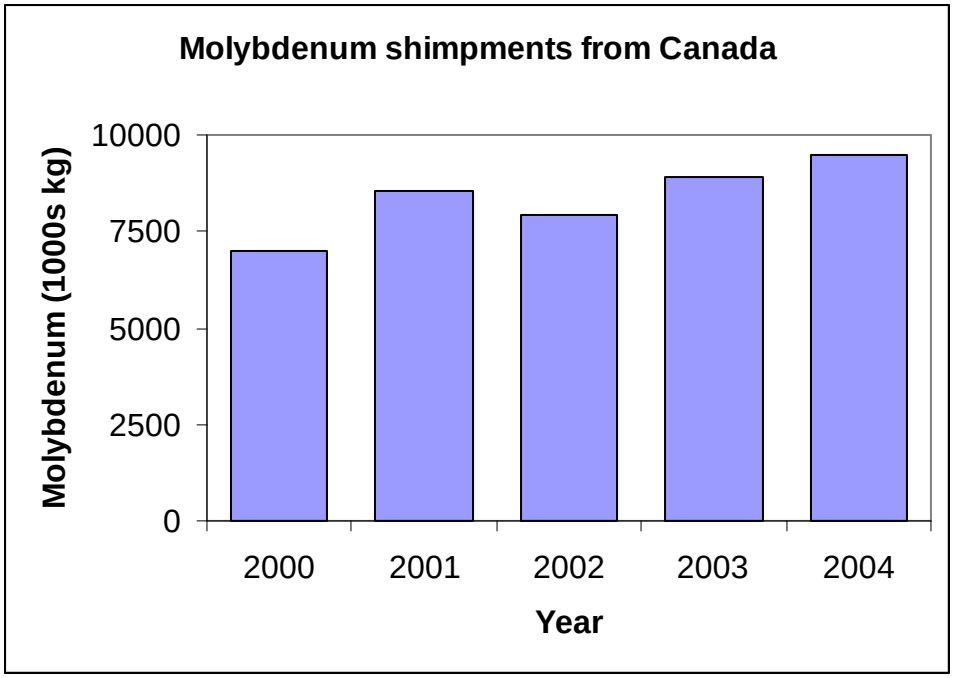
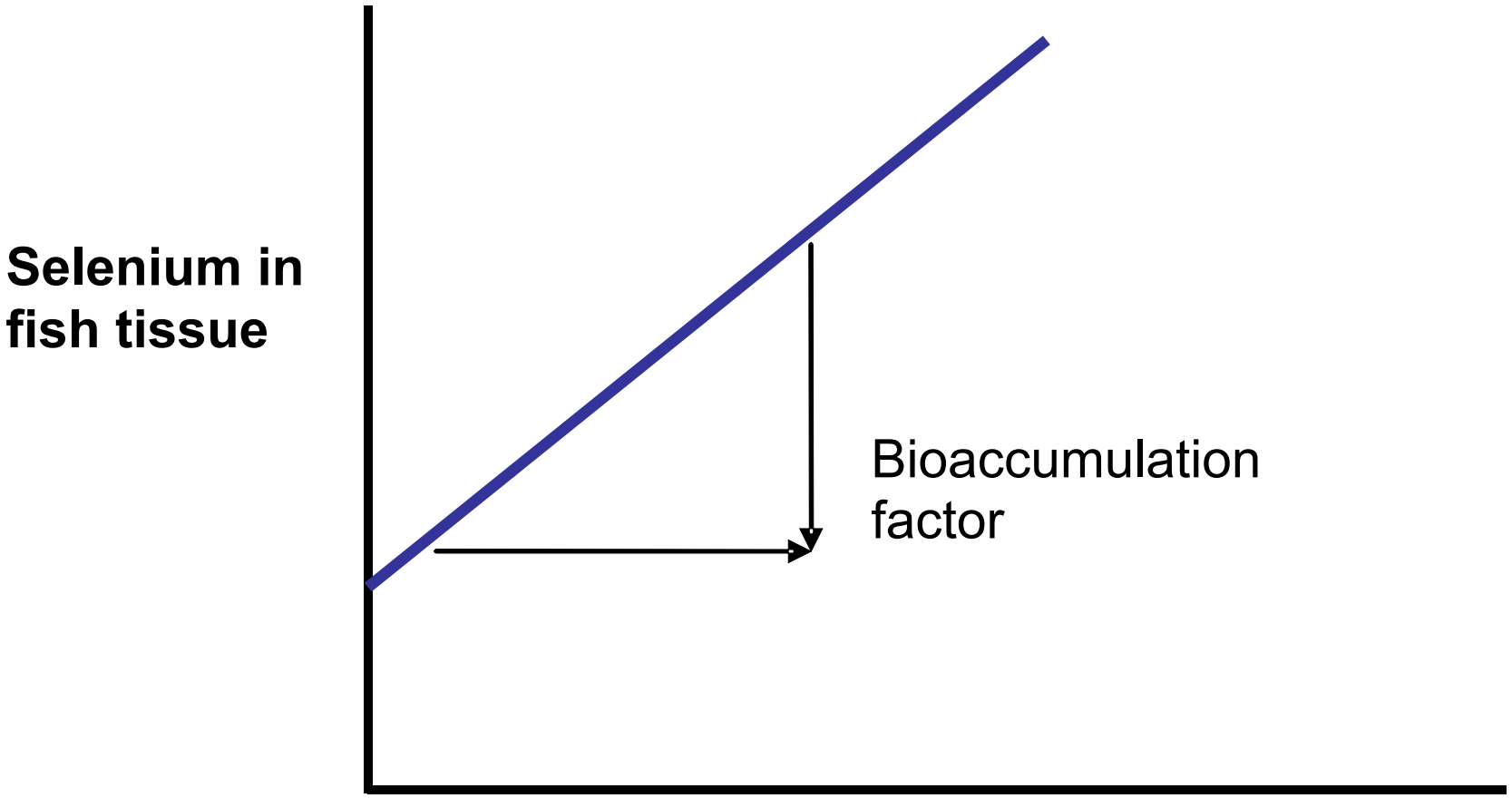


Figure 2.1: Molybdenum shipments, by weight, from Canada, 2000-2004.



Note;
This relationship is predicated on the view the fraction of selenium in the fish tissue is representative of the bioavailable fraction causing the dose-response relationship.

Hypothetical Linear Dose-response Representing the Relationship Between the Concentration of Selenium in Water and the Resulting Selenium in Fish Tissue.

EcoMetrix INCORPORATED | November 2007 | **Figure 3.1**

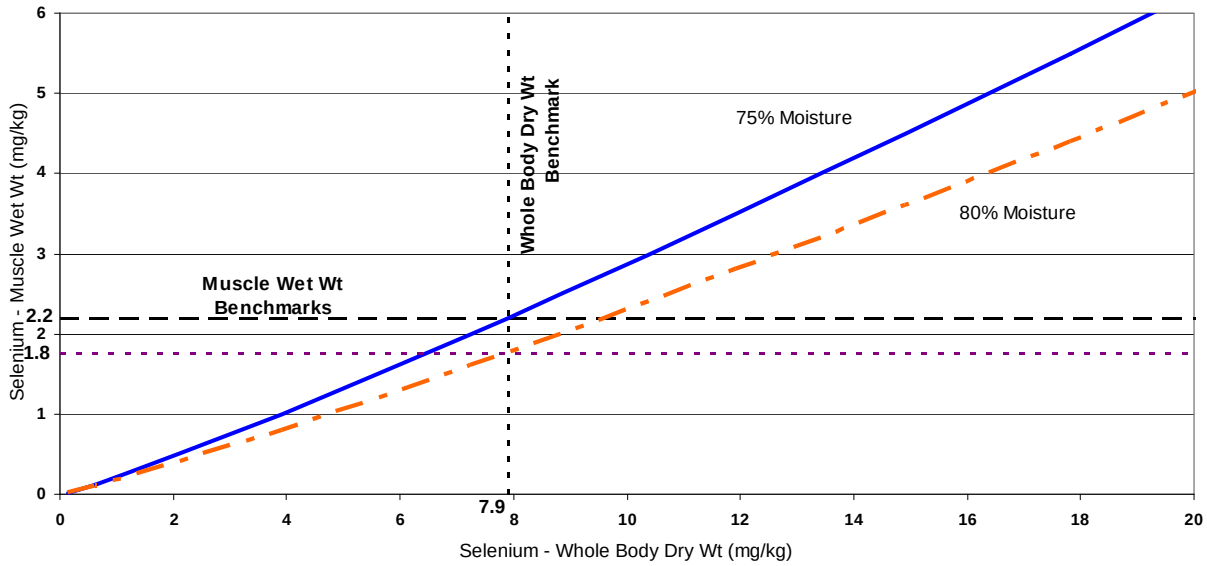


Figure 3.2: Expected Relationship between Selenium and Muscle for Water Content in Fish Ranging from 75% (blue line) to 80% (red line). Plot developed from guidance provided from different sources (U.S. EPA 2004a; Toll *et al*., 2005).

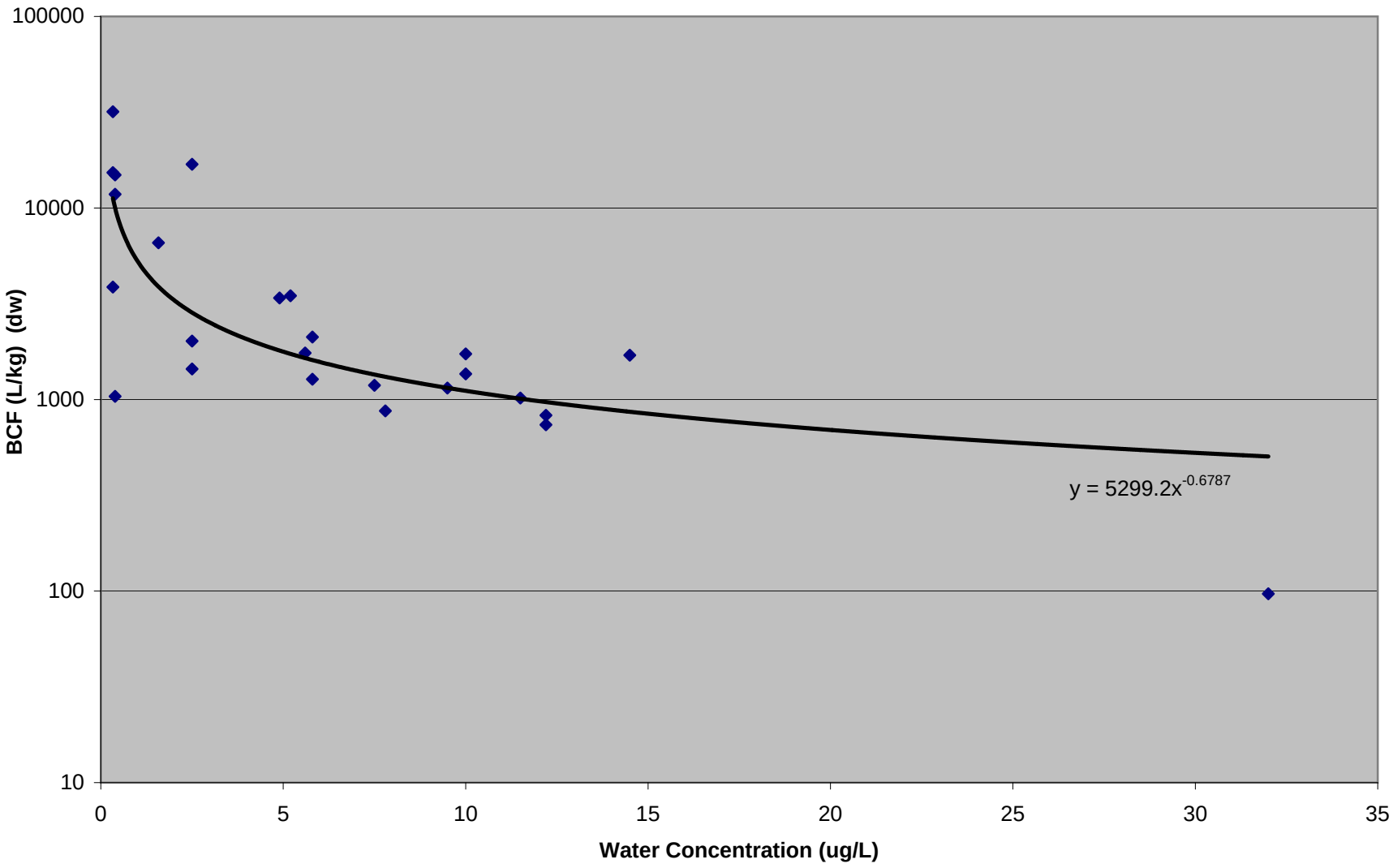
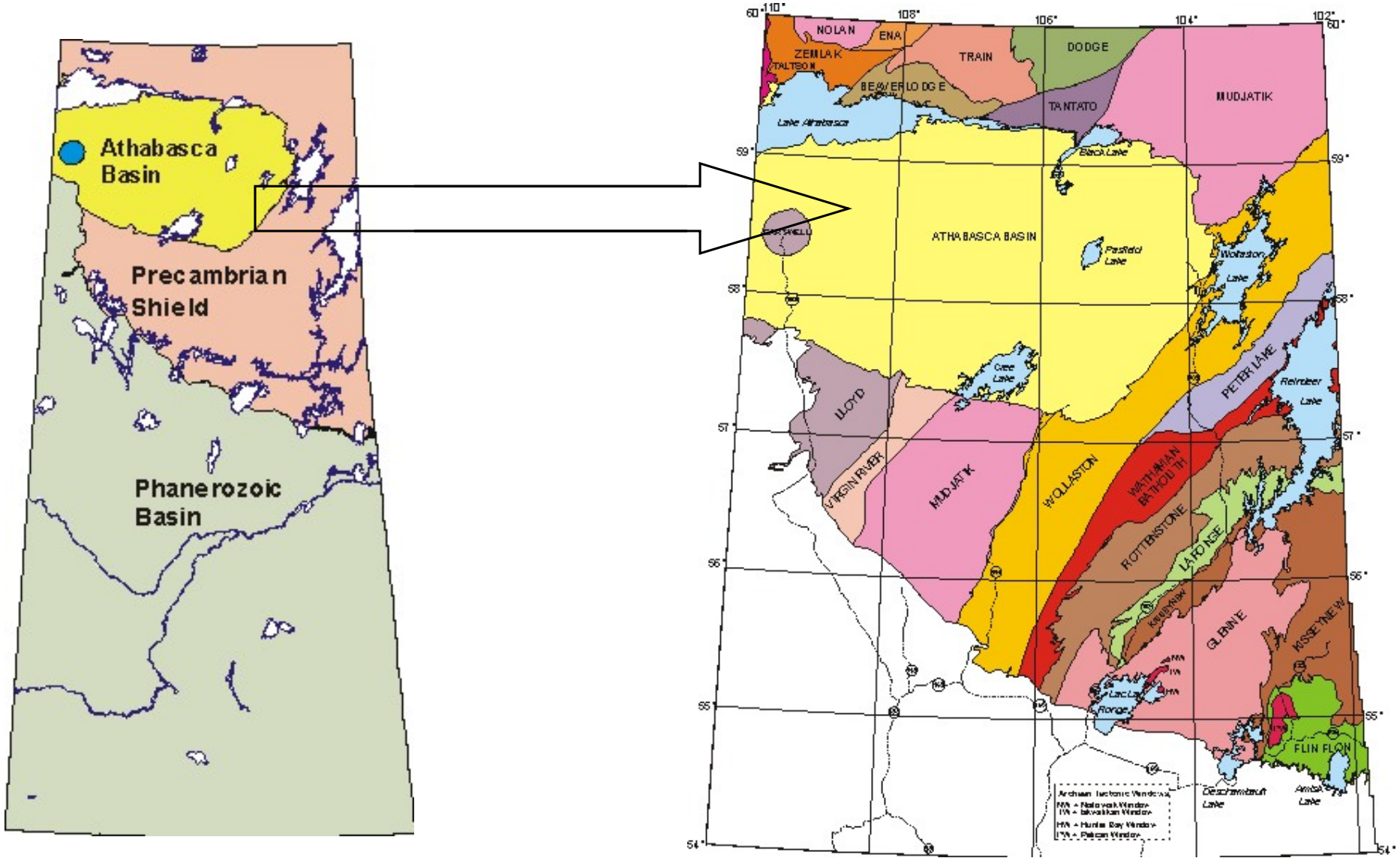


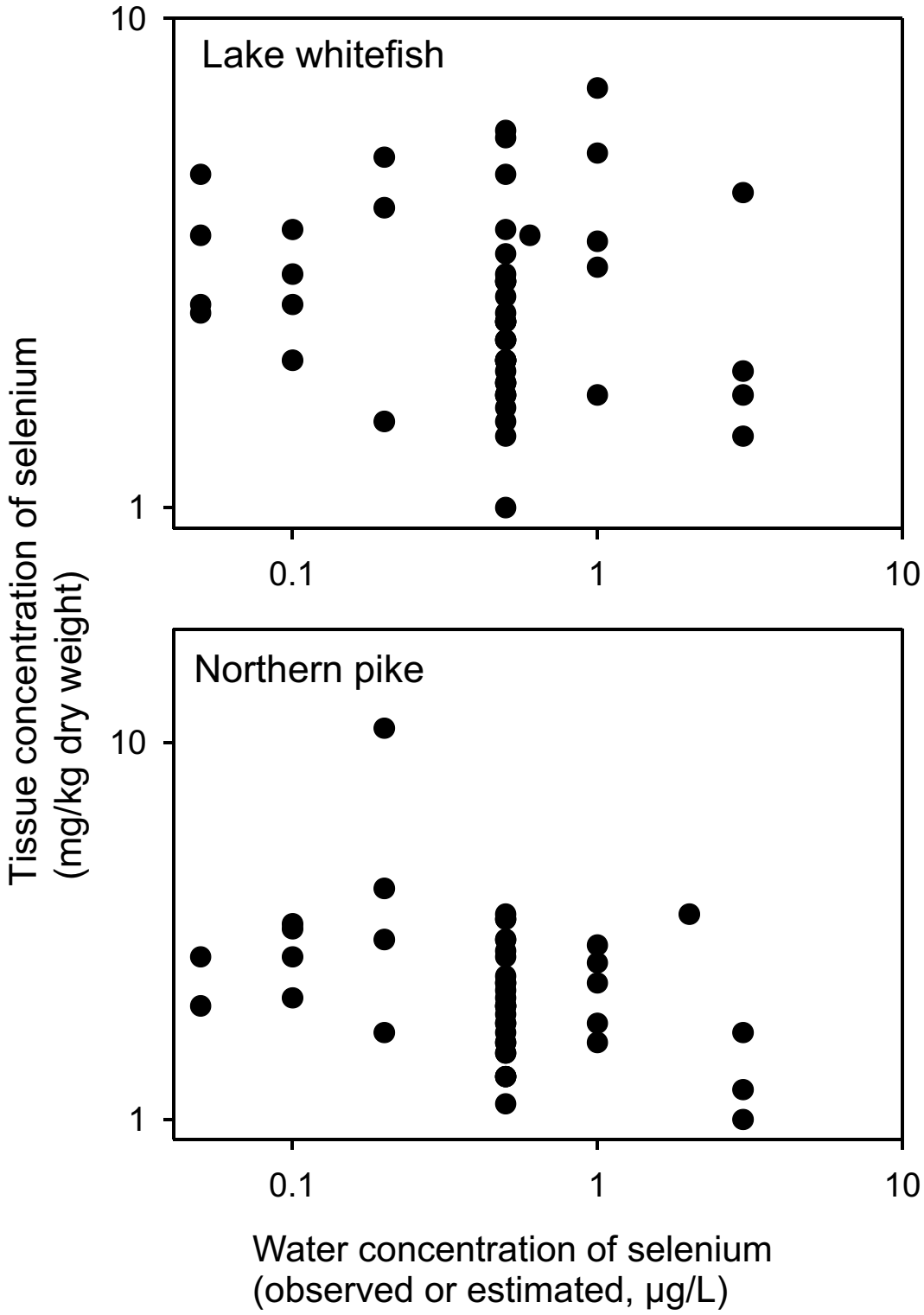
Figure 3.3: Selenium bioconcentration factor (BCF) for Invertebrates as a Function of Water Concentration (combined data from EPA, 2004a and Fan *et al* ., 2002a)



Note:
The dominance of sandstone within the Athabaska basin (in yellow on the maps) is unique relative to adjacent areas. These images were from a Saskatchewan government website (i.e., www.ir.gov.sk.ca/).

Maps that show the general geology of Saskatchewan (left) and the northern portion including the Athabaska Basin (right).

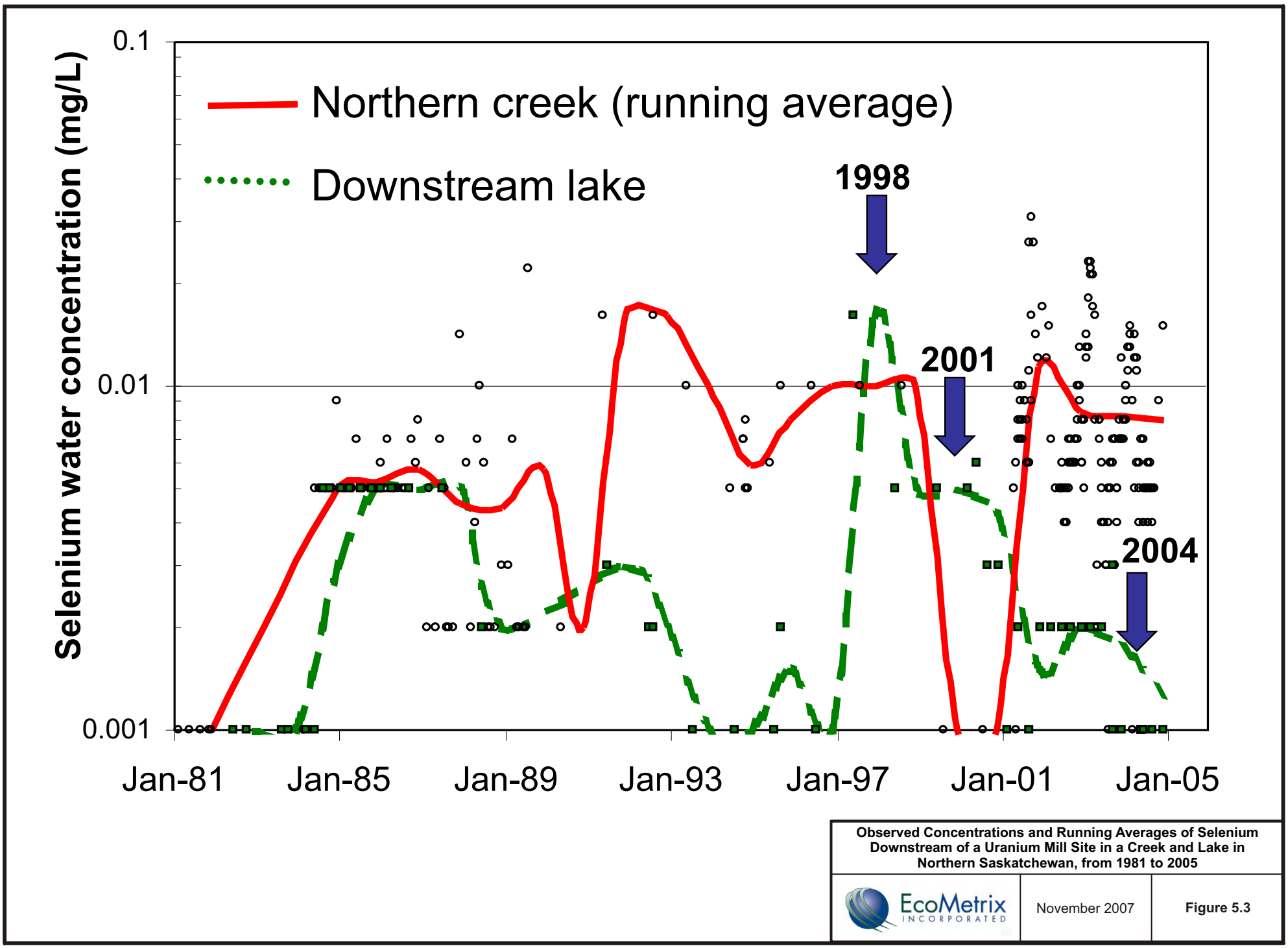
EcoMetrix INCORPORATED	November 2007	**Figure 5.1**



This plot concerns lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*) sampled from reference lakes across the Athabasca basin of Saskatchewan. Consideration of this distribution with linear regression identifies no linear or other statistically-significant pattern in the distribution of the observations for both species.

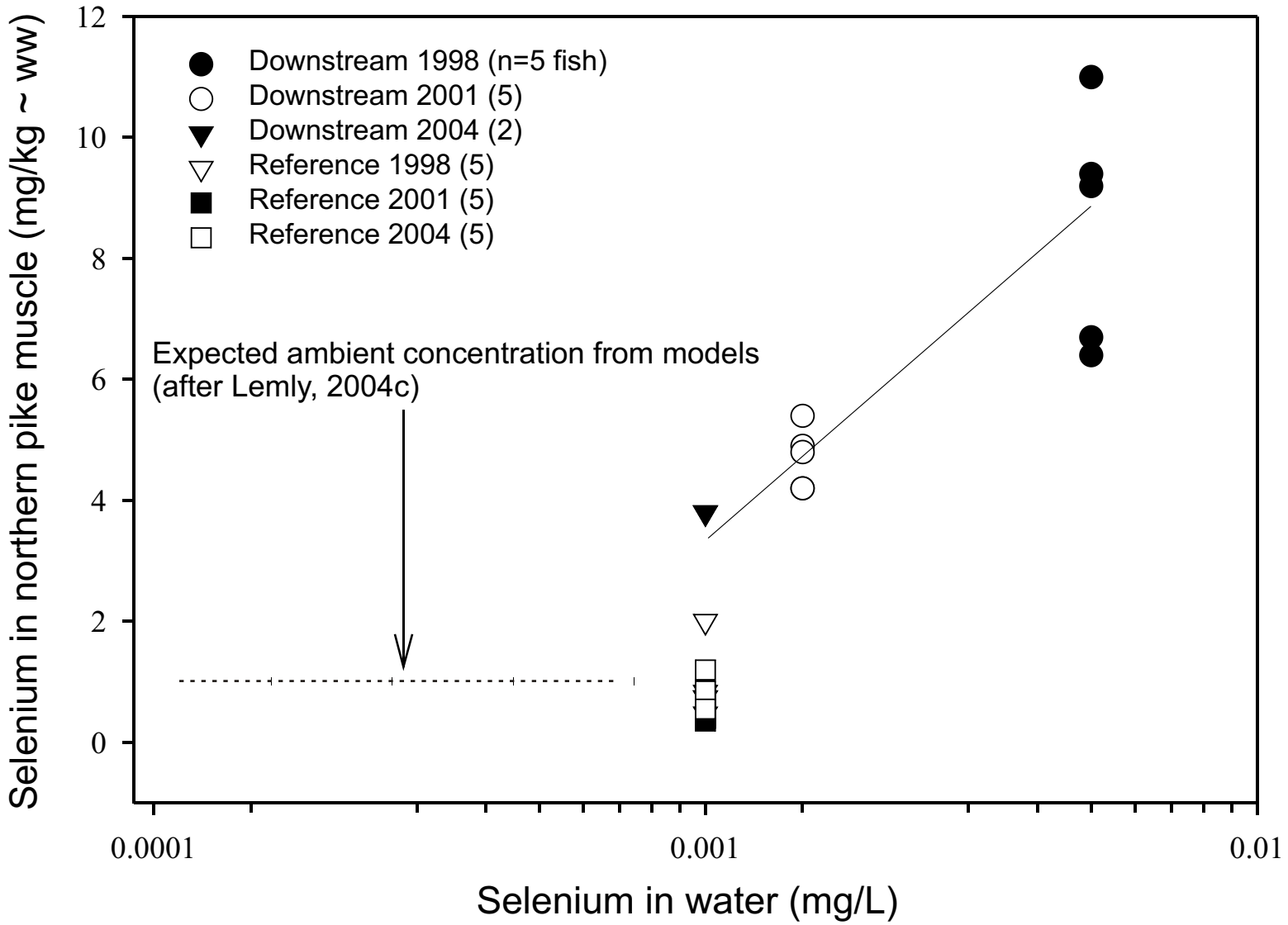
Observed Dose-response Representing the Relationship Between the Concentration of Selenium in Water and Selenium in Fish Tissue

EcoMetrix INCORPORATED | November 2007 | **Figure 5.2**



Observed Concentrations and Running Averages of Selenium Downstream of a Uranium Mill Site in a Creek and Lake in Northern Saskatchewan, from 1981 to 2005

EcoMetrix INCORPORATED | November 2007 | **Figure 5.3**



Observed Concentrations of Selenium in Pike from Downstream of a uranium mine and from a Reference Lake in Northern Saskatchewan from 1998 and 2004.

EcoMetrix INCORPORATED | November 2007 | **Figure 5.4**

APPENDIX A

INTERNATIONAL MOLYBDENUM ASSOCIATION

8th February 2007

Mr Tremblay
Program Manager, Special Projects/
The Mend Initiative
Natural Resources Canada
555 Booth Street
Ottawa, Ontario
K1A 0G1 Canada

Dear Mr Tremblay,

MEND Initiative's 'Review of Environmental Management Criteria for Selenium and Molybdenum'

The International Molybdenum Association is aware that the MEND initiative has commissioned a report called "A Review of Environmental Management Criteria for Selenium and Molybdenum", dated January 2007.

With respect to the aquatic compartment, we have noted the following;
The CCME guideline of 73 ug/L for Mo in freshwaters is based on a study by Birge et al., 1978 (LOAEC for rainbow trout of 730 ug/L and then divided by 10). There is further reference to the Davies et al 2005 study (which tried to reproduce the Birge study and resulted in much higher effect levels). The Davies' recommendation that the CCME guideline needs to be re-evaluated is also cited.

To this end, I would like to describe to you the work that the International Molybdenum Association (IMOA) is currently conducting on the ecotoxicity of molybdenum in freshwaters.

As part of industry's obligations under REACH (the new chemicals legislation in Europe 'Registration, Evaluation, Authorisation of CHemicals) to provide proof of safe production, use and disposal of a substance, IMOA has started a thorough review and categorisation of existing ecotoxicity data on molybdenum in freshwaters. Each identified study was thoroughly reviewed and categorised based on stringent criteria for reliability and relevancy.

Furthermore, based on the fact that very little data was categorised as high quality under the reliability and relevance criteria, IMOA has commissioned a series of ecotoxicity testing, to be conducted according to standard protocols. The intention is to build a high quality dataset to be used as a basis for developing an effect threshold level (based on the HC5 of a species sensitivity distribution).

These ecotoxicity tests are currently running in laboratories around the world (in Chile, the USA and Belgium).

We invite you to have a dialogue with IMOA during the course of the revision you intend to do on the molybdenum threshold level in freshwaters in Canada.

I look forward to further discussions with you. Please contact me on the number indicated below..

Yours sincerely,

Lidia Regoli
Consultant - Environmental Affairs
International Molybdenum Association

Rue Pere Eudore Devroye
1050 Bruxelles
BELGIUM
E-mail: lregoli@imoa.info
Telephone : +32 2 640 6104