Ecological Assessment of Selenium in the Aquatic Environment: Summary of a SETAC Pellston Workshop

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Abstract

This booklet summarizes the results of the Pellston Workshop on "Ecological Assessment of Selenium in the Aquatic Environment." The workshop was sponsored by the Society of Environmental Toxicology and Chemistry (SETAC) and held 22–28 February 2009 in Pensacola, Florida, USA. The full technical proceedings of the workshop will be published separately by SETAC in 2010.

Selenium (Se) has become a contaminant of potential concern (COPC) in North America, Australia, and New Zealand, and is likely an unrecognized COPC in other parts of the world. Se in excess and in critical chemical species in the diet of egg-laying vertebrates (fish, waterbirds, amphibians, and reptiles) can cause reproductive failures or abnormalities. Guidance for assessing Se in the aquatic environment is summarized in this booklet and will be provided in detail in the forthcoming workshop proceedings.

This SETAC Pellston Workshop brought 46 key individuals from business, academia, government, and nongovernmental organizations together with students to develop consensus on a path forward for the assessment of Se in the aquatic environment. Participants were divided into five workgroups:

1) Problem Formulation (past and current problems, lessons learned),
2) Environmental Partitioning (Se sources, speciation, entry into the food chain),
3) Bioaccumulation and Trophic Transfer (from primary producers to herbivores to carnivores, influence and description of ecological factors),
4) Toxic Effects (body burdens and toxicity), and
5) Risk Characterization (risk synthesis, approaches).

Key findings were as follows:

Problem Formulation

- Se is a growing problem of global concern.
- Diet is the primary pathway of Se exposure for both invertebrates and vertebrates.
- Traditional methods for predicting toxicity on the basis of exposure to dissolved concentrations do not work for Se because the behavior and toxicity of Se in aquatic systems are highly dependent upon site-specific factors, including food web structure and hydrology.
- Se toxicity is primarily manifested as reproductive impairment due to maternal transfer, resulting in embryotoxicity and teratogenicity in egg-laying vertebrates.

Environmental Partitioning, Bioaccumulation, and Trophic Transfer

- Understanding Se speciation is critical to understanding its mobility, transformation, partitioning in the environment, and potential risk to aquatic ecosystems.

- Se uptake is facilitated across most biological membranes (a nonpassive, carrier-mediated process), making its partitioning unique among metalloid contaminants.
- The single largest step in the bioaccumulation of Se occurs at the base of food webs, characterized by an "enrichment function"; thermodynamic or equilibrium-based principles are not appropriate for predicting Se bioaccumulation at the base of food webs.
- Se bioaccumulation by primary producers and predators varies widely among species, based on both ecology and physiology (biodynamics); uptake by individual species and in steps of the food web can be described by a trophic transfer function.

Toxic Effects

- A key aspect of Se toxicity is the narrow range between dietary essentiality and toxicity.
- Differences in species sensitivities to Se may be related to differences in reproductive physiology, dynamics of Se transfer from diet or body tissues to eggs, and/or differences in capacity to metabolize organic Se to more reactive oxidized species.
- Protection of top predators may not guarantee protection of all biota situated lower in the food web.
- Aquatic-dependent mammals do not appear to be as sensitive as fish or birds to dietary organic Se exposure.
- The most sensitive toxicity endpoint in birds is embryo mortality.
- The most sensitive toxicity endpoints in fish larvae are teratogenic deformities such as skeletal, craniofacial, and fin deformities, and various forms of edema.
- Embryo mortality and severe development abnormalities can result in impaired recruitment of individuals into populations.

Risk Assessment

- Population-level effects from Se in natural ecosystems are difficult to detect. This difficulty reflects differences in species sensitivity as well as food web complexities and demographics where population-level effects are suspected. Se contamination of Belews Lake and of Hyco and Kesterson Reservoirs (USA) resulted in whole-ecosystem exposures that had significant adverse population-level impacts. Few such widespread impacts on populations have been definitively documented in other ecosystems; however, population-level effects have been suspected at several other sites, including San Francisco Bay (USA) and Lake Macquarie (Australia).
- Risk assessment starts with reviewing available data on Se concentrations in various media, but more certainty in assessment of potential adverse effects is realized when Se measurements are made in reproductive tissue.

- A single, universal, dissolved water quality value is inappropriate for predicting toxicity. The dissolved Se concentration benchmark that is necessary to protect one site may be either insufficiently protective or unnecessarily protective at another site.
- There is consensus that fish and bird eggs are the critical media in terms of assessing or predicting Se toxicity at a given location, and measured concentrations in these tissues are most strongly linked to adverse effects.
- The vulnerability of a species is the product of its sensitivity to Se in its eggs, its propensity to transfer Se from its body into its eggs, and its propensity to accumulate Se from its environment, as affected by its diet choices and intake rates, and by site-specific factors controlling the transfer of Se into and within the food web.
- For reliable prediction of effect thresholds across a range of sites, numeric benchmarks for egg concentrations provide the greatest certainty. The more distantly connected a possible measurement medium is to the egg concentrations, the less certainty that the associated numeric benchmark will be appropriate across sites.
- For site-specific assessment of Se risks to fish, the field collection of ripe females or newly laid embryos for laboratory examination of larval effects is a reliable indicator of Se risks when the effect measure is related to the egg Se concentration.
- Se requires site-specific risk assessments, including adequate quality assurance and quality control of chemical and biological analyses, to a much greater extent than many other contaminants.

Introduction

Background and Need for Workshop

Selenium (Se) is a metal-like element, a "metalloid," discovered in 1818 by the Swedish chemist Berzelius, and named after Selene, the Greek goddess of the moon. It is a naturally occurring substance and an essential element required for the health of humans, other animals, and some plants. Specifically, it is necessary for the proper functioning of structural proteins and cellular defenses against oxidative damage.

However, Se has become a contaminant of potential concern (COPC) in North America, Australia, and New Zealand, and is likely an unrecognized COPC in other parts of the world. Selenium is a COPC as a result of activities conducted by a wide variety of industrial sectors, including mining (coal, hard rock, uranium, phosphate) and power generation (coal-fired power plants, oil refineries); it is found in organic-rich shales that are source rocks for such activities. Selenium is also a COPC for agriculture due to discharge of subsurface irrigation drainage waters and due to animal husbandry additions of this often-deficient essential element.

Concentrations of Se are increasing in many areas of North America, Australia, New Zealand, and China due to increasing mining and power-generation activities, but guidance for assessing and managing its environmental effects generally does not reflect the current state-of-the-science. This SETAC Pellston Workshop was held to develop information to support such guidance on a global basis (i.e., establish the present state-of-the-science) and to determine major sources of uncertainty requiring further research. Information provided by this workshop applies equally to areas of the world that currently recognize Se as a COPC and to those that may do so in the future.

Workshop Purpose and Goals

The purpose of this workshop was to develop guidance for ecological assessment of Se in the aquatic environment. The workshop comprised five separate workgroups (WGs):

- Problem Formulation (past and current problems, lessons learned),
- Environmental Partitioning (Se sources, speciation, entry into the food chain),
- Bioaccumulation and Trophic Transfer (from primary producers to herbivores to carnivores, influence and description of ecological factors),
- Toxic Effects (body burdens and toxicity), and
- Risk Characterization (risk synthesis, approaches).

The Workshop Steering Committee (Appendix A) developed a series of questions for each WG as a means to initiate discussion. The individual WGs subsequently refined those questions before and during the workshop. The WGs were not required to answer each question; rather, they were presented with the following challenges, which composed the goals of the workshop:

1) Review the science underpinning ecological assessment of Se in the aquatic environment.
2) Propose alternatives or improvements.
3) Identify both areas of consensus and areas that require further research because of technically defensible scientific disagreements.

Participation and Format

A multidisciplinary and international group of 46 scientists, managers, policy makers, and students from Australia, Canada, China, France, and the United States with a common interest in assessment of Se in aquatic environments came together in Pensacola, Florida, USA.

During the first full day, four separate series of presentations were given in a plenary session:

1) Selenium Past, Present, and Future;
2) Bioaccumulation and Trophic Transfer;
3) Selenium Toxicity Considerations; and
4) Risk Characterization.

Each series of presentations included a discussion given by one of the participants, followed by individual commentaries from two other participants, and then a plenary discussion. The three individuals presenting each series of presentations were encouraged to interact before the workshop such that areas of scientific agreement could be established along with areas of scientific disagreement and reasons for those disagreements. However, it was made clear that the presentations were to be individual, representing each presenter's unique viewpoint. The four presentation topics provided the basis for subsequent plenary and WG discussions.

Following this initial plenary session, workshop participants moved into their five assigned WGs (Appendix A). WG 1 reviewed available information on past and current problems related to Se in aquatic environments, together with lessons learned, and developed a generalized conceptual model. WG 2 reviewed scientific information on sources, speciation, and environmental partitioning, in particular Se speciation leading to its entry into the food chain, and developed conceptual models specific to environmental partitioning. WG 3 reviewed scientific information on Se bioaccumulation and trophic transfer from the physical environment (i.e., water-column particulates) and primary producers to herbivores to carnivores, including the influence of modifying ecological factors. WG 4 reviewed scientific information on toxic effects from Se, in particular body burdens and their relationship to toxicity. WG 5 integrated information from the other four WGs in a risk assessment format to determine the state-of-the-science related to risk characterization.

Daily afternoon plenary meetings during the subsequent three days of the workshop provided the opportunity for WG progress review and "cross-fertilization." A final plenary on the last full day of the workshop provided for consensus on areas of agreement and on major uncertainties that require further clarifying research. Key findings are summarized herein.

Workgroup Findings

Workgroup 1

Problem formulation: Context for selenium risk assessment

Selenium is an essential element for animal nutrition. However, the behavior of Se is unusual in that it also acts to cause adverse effects on reproductive success (including developmental abnormalities), which have been linked to declines in vertebrate populations. In both environmental and biological systems, Se participates in many of the same reactions as does sulfur, but Se has different chemical properties. Selenium may occur in a variety of chemical forms, but certain organic Se species are primarily linked with efficient bioaccumulation, food web transfer, and toxicity.

Selenium is a global problem

Selenium is distributed globally but not uniformly in organic-rich marine sedimentary rocks (e.g., black shales, petroleum source rocks, phosphorites). Anthropogenic activities such as coal, phosphate, and metals mining can expose Se-rich strata to greatly enhanced leaching and subsequent transport. Alluvial fans affected by weathering and erosion from surrounding or underlying sedimentary shales that support agriculture can contribute Se through agricultural irrigation runoff and drainage to watersheds. Selenium is also associated with fossil fuels such as coal and oil. Coal combustion and oil refinery wastes may contain greatly concentrated Se, compared to the raw material. Wastewater from these processes potentially adds elevated Se concentrations to the aquatic environment. Thus, these and other human uses of Se-associated products can transport contamination far from its sources, potentially generating problems in areas distant from those sources.

Demand for coal, oil, and phosphate ore has been growing in past decades and will probably continue in the foreseeable future. In addition, certain new technologies that use Se, such as nanotechnology, may have unpredicted impacts. While local contamination receives much attention, it is clear that Se contamination is a global issue that is expected to increase in prominence in the future.

Case studies

Important case studies from the history of Se contamination include Kesterson Reservoir, San Joaquin Valley drainage management systems, Grassland Bypass Project, Belews Lake, Hyco Reservoir, areas of the Appalachians affected by mountaintop mining and valley fills, North San Francisco Bay (USA), and Lake Macquarie (Australia). Documentation of the sources of Se, fate and transport of Se within the environment, effects of Se in ecosystems, and lessons learned from research associated with each case history provide insight into how current and future ecosystems may or may not be affected.

Conceptual model

A unifying conceptual model links sources, transformation and uptake through media phases, and consumer transfer and dynamics to help elucidate the movement of Se through ecosystems (Figure 1). The model shows that diet is the dominant pathway of Se exposure for both invertebrates and vertebrates. Selenium moves from water to particulates, a collection of biotic and abiotic compartments that includes primary producers, detritus, and sediments, which form the base of aquatic food webs. The ratio of the Se concentration in particulates to the Se concentration in water (referred to in this document as the "enrichment function," EF) is the initial concentrating function at the base of the food web. The EF can vary by up to four orders of magnitude at different locations. Transfer from particulates to primary consumers is less variable; trophic transfer factors (TTFs, the ratio of Se concentration in consumers relative to diet) are species specific and generally vary within one order of magnitude in nature, but higher transfer has been measured in the laboratory.

These observations help explain why the behavior and toxicity of Se in ecological systems are highly dependent upon site-specific factors. Knowledge of the food web is one of the keys to determining which biological species or other ecological characteristics will be affected. Other important parameters include rates of input of Se into the system, hydraulic residence time, and Se speciation in water and particulates.

Chronic Se toxicity primarily manifests through reproductive impairment via maternal transfer, resulting in embryotoxicity and teratogenicity in egg-laying vertebrates. Other chronic effects include reductions in growth, tissue pathologies, induction of oxidative stress, and mortality. Acute toxicity has been reported rarely in the aquatic environment.

While much has been learned about bird and fish species, far less is known about toxicity in other non-human vertebrates. A notable knowledge gap exists

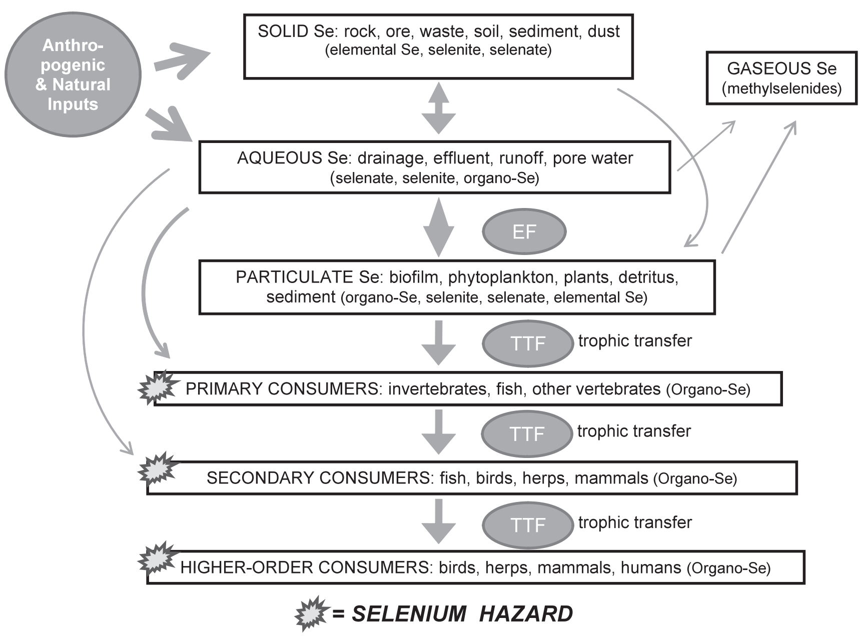

Figure 1: Conceptual model depicting Se dynamics and transfer in aquatic ecosystems (EF = enrichment function; TTF = trophic transfer function)

for egg-laying species of amphibians and reptiles, which include some of the most critically endangered vertebrate species.

Effects on other levels of biological organization are also relevant (Figure 2), and have been documented in selected sites at the population and community levels (e.g., fish populations at Belews Lake and resident bird populations at Kesterson Reservoir). There is, however, a paucity of information about other ecologically -relevant effects that may occur at the community or the ecosystem levels; one example might include changes in invertebrate community structure resulting from Se-induced loss of fish predators. Similarly, interactions with other factors (such as temperature or other natural and anthropogenic stressors) are poorly understood.

Estimates of risk are developed from knowledge of exposure and effects. Toxicity of Se varies among species, but the species that are most sensitive to Se are not always the most exposed to contamination in nature. Thus, species-specific feeding habits that result in high exposure levels can determine toxic effects.

It is difficult to generalize about recovery rates of systems when Se contamination is reduced or removed. Recovery is a function of the ecosystem and the ability to totally eliminate mass loading to the system. Experience at Belews Lake and Hyco Reservoir (USA) shows that once the source is removed, aquatic communities (although not always comprised of the same species) can return within a few years; however, Se in sediments can contribute to long (decadal) recovery times of tissue residues and potentially to associated adverse effects in consumers.

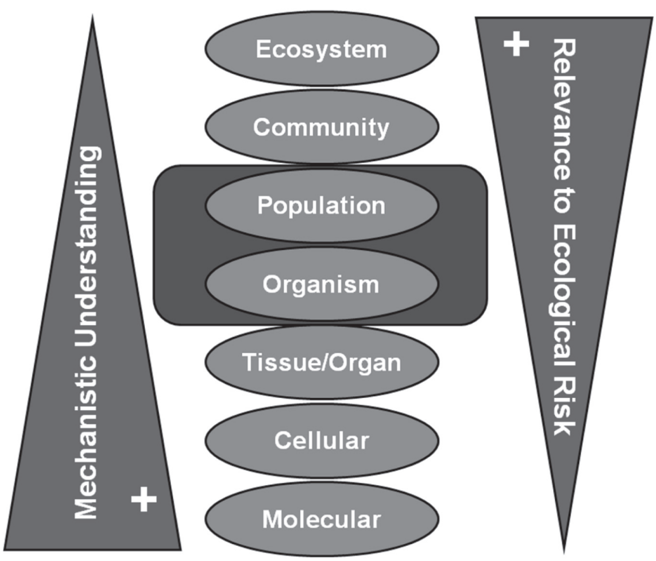

Figure 2: Hierarchy of effects across levels of biological organization

How to investigate a potential selenium problem

Key assessment endpoints and corresponding exposure and effects measures at increasing levels of biological organization are summarized in Table 1. Based on current knowledge, the endpoints most diagnostic of Se exposure occur at the tissue and organism levels. Depending on the level of prior knowledge of Se problems in the system, the most important exposure and effects endpoints may vary. For example, in a system where Se problems are suspected or known, collection of data on all of the key measures (in boldface type in Table 1) is recommended. For systems where less information exists, a useful starting point for assessments would be to measure Se concentrations in water, particulates, reproductive tissues from oviparous fish and wildlife, and tissues from primary consumers. Measurement of the organic carbon content of the sediment and speciation in water may also be useful. Important caveats regarding all of these measures are discussed in detail in the forthcoming workshop proceedings.

Recommendations

Selenium research has progressed in recent decades and has resulted in significant advances in our knowledge of Se dynamics and effects in aquatic systems. However, there are still important unknowns in the following areas:

- species sensitivity of other egg-laying vertebrates, including amphibians and reptiles;
- consistent methods for collection of particulate components;
- development of a comprehensive database of EF values;
- information on Se sensitivity of marine species;
- expansion of the information base for biodynamic modeling in freshwater systems;
- quantitative surrogates for reproductive endpoints;
- mechanisms of Se toxicity;
- indirect effects of Se exposure within ecosystems; and
- interactive effects of Se with other stressors (synergistic and antagonistic).

Workgroup 2

Environmental partitioning

Selenium exists in the natural environment in four oxidation states and forms a diverse and interchangeable array of inorganic and organic species through the action of physical, chemical, and biological processes. Selenium uptake is facilitated across most biological membranes, making its biogeochemical cycling unique among metalloid contaminants. While total Se concentration typically is used by resource managers and regulators for assessment and management, it is now recognized that understanding Se speciation is critical to understanding its

Table 1: Assessment endpoints and measures of exposure and effect for aquatic and aquatic-linked organisms (Key measures are shown in **boldface** type. Note: For measurements of solids, it is recommended that both dry weight and percent moisture be determined.)

Level of organization	Assessment endpoint	Measures of exposure	Measures of effect
Subcellular	Protection from oxidative stress Avoidance of changes in protein structure and function	Concentration of Se in subcellular fractions Subcellular shift in Se	Enzyme assays and gene expression Quantify Se substitution into specific amino acids within protein
Tissue	Normal tissue structure and function	Tissue concentrations of Se or selenomethionine and reactive oxyselenium species	Pathology of liver, kidney, eyes, gills, blood, gonad Relative organ weight
Organism	Survival, growth, and reproduction of aquatic organisms and egg-laying vertebrates	**Se in female reproductive tissue of oviparous vertebrates** [b] Se in whole-body or surrogate tissue	Survival Growth Body condition (mass wasting) **Edema**[a] **Embryo abnormalities** [a] **Embryo mortality** [b] **Egg hatchability** [b] Feather loss Immuno-competence Incidence of parasites or disease
Population	Population sustainability	Dietary Se concentration Difference in tissue Se across members of the population	**Reduced abundance** [c] **Population structure** [c] Change in genetic diversity
Community	Community structure and function	**Se concentration in water and particulates (EF)** [b] Se speciation in water and particulates **Se concentrations in primary consumers** Trophic transfer function Food web structure	**Presence or absence of sensitive species** Functional groups represented Taxa richness, diversity
Ecosystem	Ecosystem structure and function	Se loading and speciation in ecosystem Residence time of Se in ecosystem	Productivity

[a] Diagnostic for Se

[b] High sensitivity for Se and/or provides key information

[c] Difficult to implement (i.e., large sample size needed or specialized equipment required or extensive time and resources required)

EF = Enrichment Function

mobility, transformation, partitioning in the environment, and potential risk to aquatic ecosystems.

To understand Se processes and risks to aquatic ecosystems, development of site-specific conceptual models is a critical first step in quantifying source delivery, environmental partitioning, and potential for ecological effects. The conceptual models constructed by this working group provide a potential starting point (Figures 3, 4, and 5). Speciation measurements are also required to understand processes and assess risk in a given ecosystem.

There are a wide range of sources (Figure 3) that include natural (i.e., terrestrial geochemical weathering and mobilization, wildfires, volcanic activity) and anthropogenic activities (e.g., agriculture, mining, petroleum refining, coal burning, and municipal wastewater discharge). The relative flux of these sources to aquatic systems can vary spatially and temporally, including episodic or continual loading.

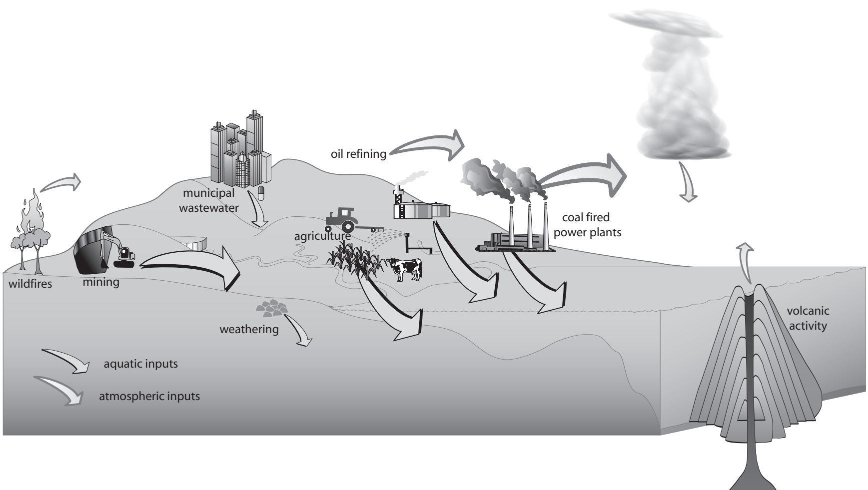

Figure 3: Potential sources of Se to aquatic systems (Symbols courtesy of the Integration and Application Network (ian.umces.edu/symbols/) University of Maryland Center for Environmental Science)

Multiple Se species are associated with three major processes in aquatic systems (Figure 4):

- deposition and resuspension (selenate, selenite, elemental Se, and Se-II);
- trophic transfer involving algae, plants, and animals (selenomethionine, selenocysteine, Se-II); and
- microbial processes (selenate, selenite, elemental Se, Se-II, and in gaseous form dimethylselenide and dimethyldiselenide).

With respect to environmental partitioning of Se in aquatic systems (Figure 5), the major redistribution between compartments can occur immediately on

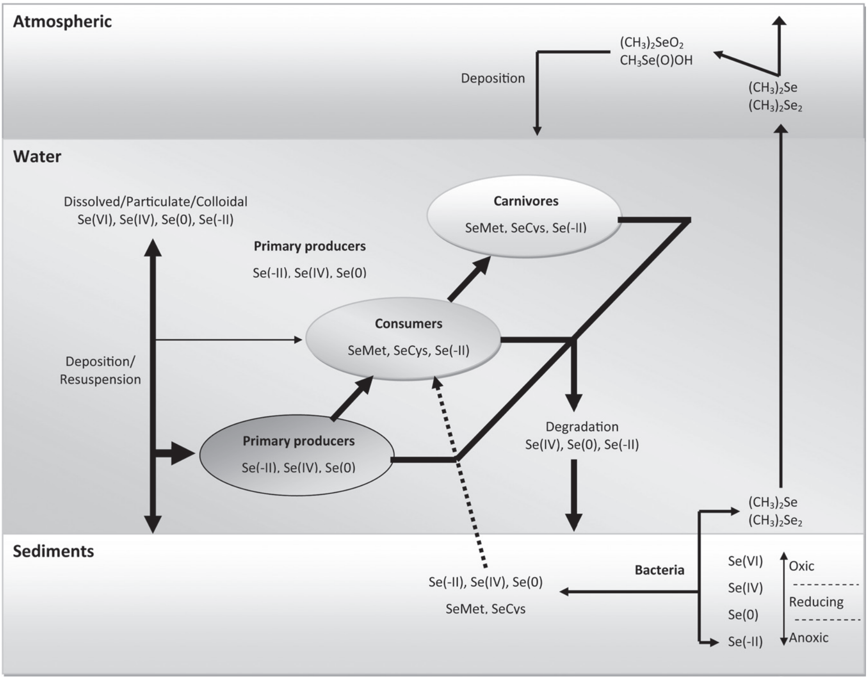

Figure 4: Selenium species associated with major processes in aquatic systems

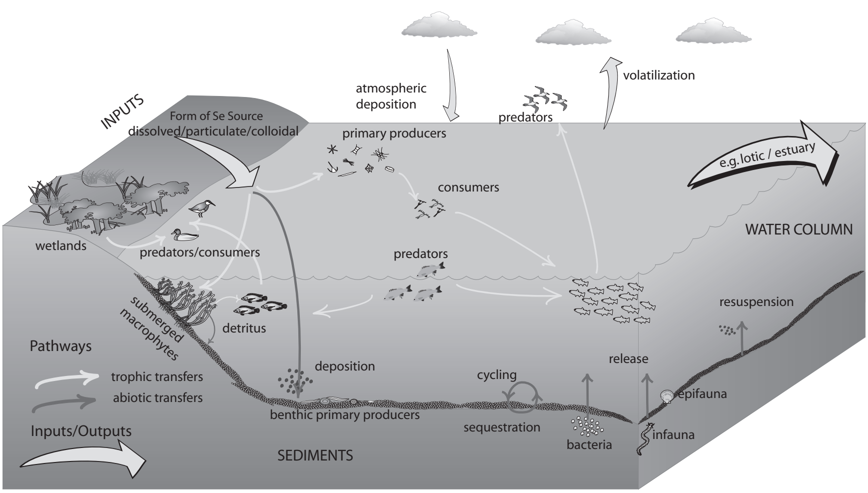

Figure 5: Partitioning of Se among environmental compartments in a typical aquatic system (Symbols courtesy of the Integration and Application Network (ian.umces.edu/symbols/) University of Maryland Center for Environmental Science)

delivery to the aquatic system (e.g., adsorption of Se on hydrated iron oxides, release of Se from particles). Selenium redistribution within the system is then dependent on the structure of the aquatic food web (e.g., detrital vs. phytoplankton-based food webs) and the hydraulic residence time.

A summary of major findings, knowledge gaps, and recommendations is provided below.

Major findings—Sources and loads:

- Selenium is being redistributed in the environment above natural background processes by human activities.
- Stochastic processes of volcanic activity and wildfires can periodically be regionally important Se sources.
- Selenium sources, species, and loads are spatially and temporally variable.
- There are multiple Se sources and sinks in the environment.
- Water is the most important vector for delivering Se to aquatic systems.

Major findings—Speciation:

- Knowledge of speciation is important to understand Se transport, partitioning, and biological effects.
- In certain cases, Se speciation including isotopic distribution, can be used to ascribe Se to a particular source.
- Development and application of Se speciation methods and models should be driven by effects.

Major findings—Environmental partitioning:

- Selenium uptake is facilitated across most biological membranes, making its partitioning in the environment unique among metalloid contaminants.
- To determine Se partitioning, it is necessary to understand the specific aquatic ecosystem under study (e.g., food web structure, hydrology).

Knowledge gaps:

- Microbial organisms are important in transforming Se in the environment; however, there are few flux estimates and few Se species mass-transfer rate data for contaminated systems, and thus their overall importance, while likely significant, is not well understood.
- A molecular-level understanding of the mechanism by which Se is transferred through food webs is needed.
- Variability in the measurement of Se concentrations and loads with respect to environmental dynamics such as diurnal, seasonal, climatic processes is not well understood.
- Selenium atmospheric re-deposition processes, global distillation, or regional deposition adjacent to source are not sufficiently understood in

the context of what is known about other contaminants such as mercury where diffuse global transport is significant.

- The atmosphere is least understood of all the Se compartments; extensive knowledge is available on water, sediment, and biota, but there are few data for the atmosphere.
- A major uncertainty in calculating Se budgets is Se volatilization; few data are available for this major loss and mobilization process.
- Data are lacking on how future approaches and technological advances in energy production will change Se loading to the environment.
- Due to the lack of identifiable quality control and quality assurance procedures in some published data, the reliability of those total Se and Se species measurements is of concern.

Recommendations—Priorities depend on site-specific challenges:

- Model loads with Se speciation, form, and phase to understand the potential for accumulation and risk in different environmental compartments.
- Calculate accurate mass balances, and thus loads, using knowledge of source discharges (pulse, press, non-point, point) and correct interpretation of the hydrologic discharge profile to understand Se partitioning.
- Include estimates of Se volatilization and aerosol deposition for investigations of the fate of Se and partitioning in aquatic ecosystems.
- Evaluate and include in risk assessment and risk management activities the technological advances in managing Se loads from industries such as power generation, mining, and agriculture and their impact on potentially changing Se loads.
- Continue development of reliable methods for measuring Se species in all environmental compartments and their sample matrices.
- Establish interlaboratory comparison studies and use suitable certified reference materials to ensure known-quality analytical data.
- Require that publications of Se data contain reference to quality control and quality assurance procedures and results.

In summary, Se remains a challenge for the future because global release through anthropogenic activities will continue to load aquatic ecosystems, thus increasing potential for risk. The complex interplay of Se species with the environment demands a thorough understanding of release, transport, and end-effect processes based on known-quality data collected through quality-assured processes.

Workgroup 3

Bioaccumulation and trophic transfer

Understanding bioaccumulation and trophic transfer is central to managing ecological risks from Se. The dietary route of exposure generally dominates bioaccumulation processes. This fact has practical implications because the traditional ways of predicting bioaccumulation in animals, on the basis of exposure to dissolved concentrations, do not work for Se. Further, the predominance of dietary Se exposure pathways mandates that we understand fundamental aspects of Se bioaccumulation in key components of the ecosystems we are trying to protect, from primary producers to top predators. Biodynamics provides a unifying basis for understanding and quantifying dietary uptake and the linkages among food web components.

The single largest step in the bioaccumulation of Se occurs at the base of food webs (Figure 6). Primary producers generally concentrate Se from 10^2- to 10^6-fold above ambient dissolved concentrations. We have termed this initial concentrating process the "enrichment function" (EF), because thermodynamic or equilibrium-based constants are not appropriate for describing Se bioaccumulation at the base of food webs. Concentration-dependent EFs are specific to each plant, microbe, or particulate material. Uptake of Se by phytoplankton is unlike uptake of trace metals (or organic contaminants). The fact that dead cells do not accumulate or appreciably sorb Se implies that Se bioaccumulation is a non-passive, carrier-mediated process.

Selenium bioaccumulation by primary producers, invertebrates, and predators varies widely among species. This variation, for animals, is a function of food

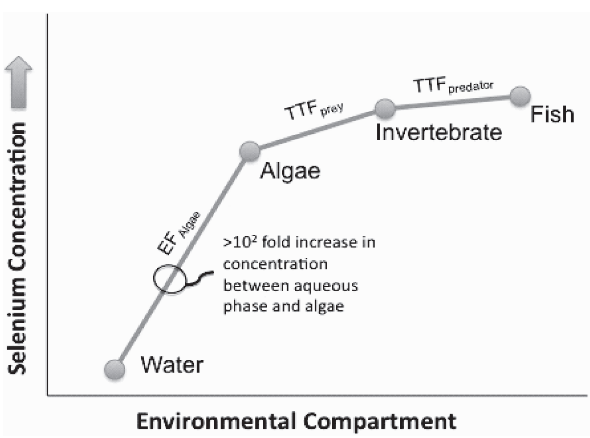

Figure 6: Selenium enrichment and trophic transfer in aquatic food webs. Enrichment function (EF_{algae}) represents the increase in Se concentration between water and the base of the aquatic food web (e.g., algae). Trophic transfer function represents the increase in Se concentration between algae and invertebrates (TTF_{prey}) and invertebrates and fish ($TTF_{predator}$).

choice and physiological processes, which can be fundamentally different among taxonomic groups (Figure 7). Selenium accumulated by consumer organisms is passed on efficiently to their predators. This finding implies that higher-trophic organisms could be at greater risk in Se-contaminated environments. However, relative to the initial large Se incorporation step at the base of the food web, subsequent transfers to higher trophic levels tend to be smaller. Depending on relative sensitivity to effects, protection of top predators may not guarantee protection of all biota situated lower in the food web.

Potential to bioaccumulate Se in consumer and predatory animals can be described by a trophic transfer function (TTF; Figure 7). TTFs can be derived from established laboratory experimental protocols (biodynamics) or, perhaps with more uncertainty, by using field data to calculate a ratio of the Se concentrations in an animal to Se concentrations in its assumed food. Further, it should be recognized that TTF can vary with the concentration of Se in the diet due to transport processes in the gastrointestinal tract. EFs vary widely among species and are useful for explaining and predicting bioaccumulation of Se from one step of a food web to another. TTFs can vary widely among species, depending on feeding rate and food choices.

In light of all of these factors, a single, universal dissolved Se water quality value cannot be derived to protect aquatic environments with any degree of certainty. Dissolved concentrations of Se that are considered protective in one system may not be protective or attainable in another.

The following knowledge gaps were identified:

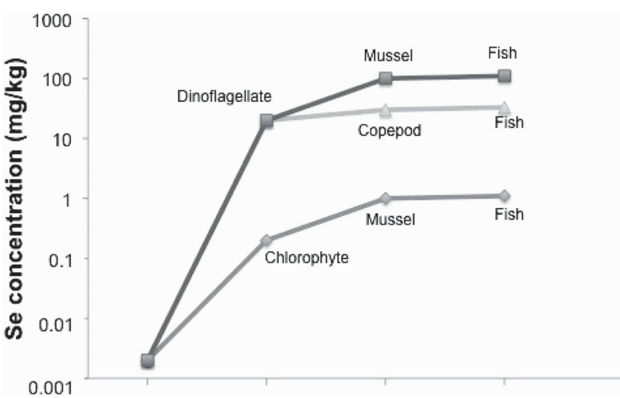

Figure 7: Selenium accumulation in different species of algae, invertebrates, and fish. TTFs are for a chlorophyte food web in fresh waters and a dinoflagellate food web in an estuary. Both food webs have a bivalve as the invertebrate, and use an average fish TTF of 1.1. The estuarine food web also illustrates the outcome for a copepod with a lower TTF from algae than a mussel.

- TTFs in freshwater environments have a relatively high degree of uncertainty because biodynamic parameters for invertebrates and fish are lacking. Therefore, the application of established experimental protocols for dominant freshwater groups (insects and fish) would be highly beneficial. Additionally, relatively little information is available for fish-to-fish TTFs in both freshwater and marine environments.
- The variability of TTFs as a function of taxonomy is unclear. Some trends have been identified in marine species, but no such understanding occurs for freshwater taxa. Additional data representing a broad taxonomic range from different ecosystems are required.
- We need to better understand enrichment at the base of food webs. Specific areas of weakness include our understanding of kinetic processes, particularly saturation kinetics at environmentally relevant concentrations in a wide variety of basal species. Additionally, data for Se uptake into and trophic transfer from bacteria are practically absent for both freshwater and marine systems.
- The bioavailability of selenate to freshwater primary producers deserves more study. In marine systems, the relative abundance of sulfate makes selenate uptake into primary producers relatively unimportant. In freshwaters, this may not be the case.
- Inter-organ transfers and thus distributions of Se in fish are obviously key mediators of toxicity, but interspecies differences in inter-organ distributions, their variability, and their relevance to reproductive toxicity, remain poorly understood.

Workgroup 4

Selenium toxicity to aquatic organisms

Selenium is an essential nutrient that is incorporated into functional and structural proteins as selenocysteine. Several of these proteins are enzymes that provide cellular antioxidant protection. A key aspect of the toxicity of Se is the extremely narrow range between dietary essentiality and toxicity. Another important aspect of Se toxicity is that, although it is involved in antioxidant processes at normal dietary levels, it can become involved in the generation of reactive oxidized species at higher exposures, resulting in oxidative stress. Toxicity results from dietary exposure to organic Se compounds, predominantly selenomethionine, and the subsequent production of reactive oxidized species.

In aquatic ecosystems, inorganic Se is rapidly and efficiently assimilated by primary producers (bacteria, fungi, algae, and plants) and transformed into organic Se species. These organic Se species are transferred throughout the food web via the diet to primary and secondary consumers (invertebrates and vertebrates). Oviparous (egg-laying) vertebrates such as fish and waterbirds are the most sensitive organisms to Se of those studied to date. Toxicity can result from maternal transfer of organic Se to eggs in oviparous vertebrates. Eggs are an important depuration pathway for fish but less so for birds. The most sensitive diagnostic indicators of Se toxicity in vertebrates occur when developing embryos metabolize organic Se that is present in egg albumen or yolk. Certain metabolites of organic Se can become involved in oxidation-–reduction cycling, generating reactive oxidized species that can cause oxidative stress and cellular dysfunction. Toxicity endpoints include embryo mortality (which is the most sensitive endpoint in birds), and a characteristic suite of teratogenic deformities (such as skeletal, craniofacial, and fin deformities, and various forms of edema) that are the most useful indicators of Se toxicity in fish larvae.

Relative species sensitivities are not well understood, but may be related to differences in reproductive physiology (e.g., the pattern of oogenesis or relative number of Se-containing amino acids in yolk), dynamics of Se transfer from diet or body tissues to eggs (i.e., dose), and/or differences in the capacity to metabolize Se to reactive forms (i.e., reactive oxidized species). Importantly, embryo mortality and severe malformations (developmental abnormalities) can result in impaired recruitment of individuals into populations, and have caused population reductions of sensitive fish and bird species. These established linkages between the molecular and cellular mechanism of toxicity (oxidative stress), effects on individuals (early life stage mortality and deformities), and negative effects on populations and community structure provide one of the clearest examples in ecotoxicology of cause–effect relationships between exposure and altered population dynamics.

Similar to other toxicants, many factors can modify the toxicological responses of organisms to Se. Selenium interacts with many other inorganic and organic compounds, both in the aquatic environment and in vivo, in a predominantly antagonistic fashion. Nutritional factors such as dietary protein and carbohydrate content can modify Se toxicity. Abiotic factors such as temperature also appear to be important modifying factors of Se toxicity in both poikilotherms and homeotherms. Differences among freshwater, estuarine, and marine environments in the toxicological responses of organisms to Se are important considerations but have not been studied in great detail. The ecology of a species, particularly its feeding niche, is a critical aspect related to its vulnerability to Se because of differential prey accumulation of organic Se and dietary exposure routes. Considerations of spatial and temporal variation in diet are important factors to consider when assessing potentially susceptible species; effects tend to be site specific.

Among taxa, there is a wide range of sensitivities to Se. Algae and plants are believed to be the least sensitive organisms. Very few studies have investigated the sensitivity of bacteria to Se, although they appear to be insensitive. Protozoans have also been understudied, and further investigation of Se toxicity in this taxon is needed. Most species of invertebrates, which are essential components of aquatic food webs and a key vector for transfer of organic Se to higher trophic levels, are also relatively insensitive to Se. Oviparous vertebrates appear to be the most sensitive organisms. Although fish and waterbird sensitivities are well documented, there are reasons to suspect that amphibians and reptiles with oviparous modes of reproductive strategy are also sensitive. Compared to oviparous vertebrates, aquatic-dependent mammals do not appear to be sensitive to dietary organic Se exposure, further illustrating the importance of oviparity in Se toxicity. Although there have been suggestions of tolerance to Se (physiological acclimation or genetic adaptation) in certain biota, it is not known whether this is an actual phenomenon.

Selenium enrichment of reservoir environments (e.g., Belews Lake, Hyco and Kesterson Reservoirs [USA]) provide classic examples of adverse effects that occur through different levels of biological organization, comprising integrated whole-ecosystem examples of trophic transfer resulting in population-level reductions of resident species. Recovery from adverse effects on fish populations occurred once Se sources were eliminated. However, population-level effects from Se in natural ecosystems are difficult to detect. This difficulty reflects differences in species sensitivity as well as food web complexities and demographics where population-level effects are suspected. Few such widespread impacts on populations as documented at Belews Lake and at Hyco and Kesterson Reservoirs have been definitively documented in other ecosystems; however, population-level effects have been suspected at several other sites, including San Francisco Bay, USA and Lake Macquarie, Australia.

Inability to observe population-level effects in the field can occur even when the species exposed in the field are the same or closely related to those for which adverse effects have been demonstrated in laboratory settings at lower Se tissue concentrations. In addition, several studies of aquatic ecosystems with naturally elevated Se concentrations have reported unaffected aquatic communities. These examples illustrate the critical importance of considering ecological and environmental factors when investigating potential Se toxicity in aquatic ecosystems.

Although we currently have a reasonably rich database on many aspects of Se ecotoxicology, there remain many unresolved uncertainties and needs for further research. Table 2 summarizes some key uncertainties and research needs in the area of aquatic ecotoxicology of Se.

Table 2: Uncertainties and recommendations for future research pertaining to toxicity of Se species

Aspect	Uncertainty	Recommendations for further research
Cellular mechanisms of Se toxicity	Effects of excessive dietary Se on immunocompetence Role of oxidative stress on toxicity	Investigate potential effects of Se on immune function and oxidative stress in the laboratory and the field.
Toxicokinetics and toxicodynamics	In egg-producing vertebrates, dependence of Se effects on reproductive strategy (e.g., oviparity vs. ovoviviparity, synchronous vs. asynchronous egg development) and on deposition of Se into eggs (i.e., amount and timing of Se deposition) Underlying reasons for large differences in transfer efficiencies from body tissues (e.g., liver, muscle) to eggs among species Disparate sensitivities among closely related species	Identify potentially susceptible species with different reproductive strategies, and evaluate relative Se bioaccumulation in eggs. Evaluate how different variables affect Se deposition into the eggs, such as timing of dietary Se exposure relative to vitellogenesis and number of spawns per season. Investigate mechanistic (physiological or ecological) basis for such differences in the laboratory and the field.
Factors modifying Se toxicity	Mechanisms and extent of antagonistic reactions between Se and other factors (e.g., other elements, biotic and abiotic stressors), with the exception of interactions between organic Se and methylmercury Occasional occurrences of synergistic rather than antagonistic interactions	Determine mechanisms, extent, and significance of antagonistic reactions between Se and other factors (chemical, biotic, and abiotic). Investigate mechanisms causing synergistic rather than antagonistic interactions.
Nutritional factors	Extent and significance of modification of Se toxicity by dietary factors, which can increase or reduce Se toxicity	Determine mechanisms, extent, and significance of dietary-based variations in Se toxicity.
Tolerance	Confirmation of apparent tolerance (acclimation, adaptation) by fish, waterbirds, and amphibians reported as lack of population-level impacts in highly Se-contaminated aquatic environments Energetic and other costs associated with such tolerance	Determine whether oviparous vertebrates can become tolerant such that organic Se toxicity is reduced or eliminated. Describe the types of tolerance possible among taxa (i.e., physiological or genetic). Model the ecological implications of such tolerance (including energetic or other costs) to populations exposed to increasing Se concentrations.
Comparative sensitivity (protozoans)	Potential toxicity of Se to protozoans, currently based on a very small database	Establish Se concentration thresholds for protozoans, with potential standardized endpoints relating to survival, behavior, growth, and reproduction.

Table 2: *continued*

Aspect	Uncertainty	Recommendations for further research
Comparative sensitivity (macroinverte-brates)	Potential for adverse effects among sensitive species within macroinvertebrate communities, despite apparent Se tolerance	Compare the composition of macroinvertebrate communities where Se concentrations are elevated and oviparous vertebrates are adversely affected to communities in reference areas, to determine whether adverse effects occur to sensitive species. Conduct controlled laboratory exposures to confirm effect concentrations and species-specific sensitivities.
Comparative sensitivity (fish)	Relative sensitivities among closely related species Relative sensitivity of fish based on diet-only juvenile exposures and maternal transfer exposures	Conduct laboratory and field studies to investigate differences in species sensitivity to Se. Investigate effects of diet-only Se exposures by juvenile and adult animals relative to maternal transfer laboratory studies.
Comparative sensitivity (amphibians and reptiles)	Relative sensitivity of understudied taxonomic groups	
Comparative sensitivity (birds)	Full extent and underlying mechanisms of inter-specific variation in sensitivity of developing bird embryos to maternal transfer of dietary Se Mechanisms underlying the relatively large variation among species in sensitivity to embryonic effects	Investigate egg hatchability and embryonic deformities in aquatic bird species not previously examined. Investigate mechanisms underlying inter-specific variation in embryonic sensitivity to Se.
Fish deformities	Ecological consequences of subtle deformities in vertebrates that survive and are recruited into the population	Incorporate protocol for evaluating and recording subtle morphological changes in catch-and-release field studies in areas with elevated Se concentrations to monitor those fish that show signs of subtle deformity.
Linkage between individual effects and impacts on populations	In different environments (e.g., freshwater, lotic vs. lentic, estuarine, marine) consequences of Se effects on individuals propagating to populations	Publish existing grey-literature studies in the primary literature. Conduct additional population-level studies at field sites with elevated Se concentrations.

Workgroup 5

Risk characterization

Characterizing risks from Se in the aquatic environment requires site-specific risk assessments to a much greater extent than many other contaminants. Selenium risk assessment is particularly challenging because of the complexity of Se chemistry and differences in dosages associated with effects, even among closely related species. Historical perspectives on Se-induced adverse effects, however, are an important consideration for the risk assessor. The magnitude and severity of adverse effects observed in unique high-exposure settings (e.g., Belews Lake, Kesterson and Hyco Reservoirs [USA]) were unexpected because of scientific unknowns concerning how Se could bioaccumulate and elicit toxic effects. Considerable advancements in the environmental chemistry and biogeochemical cycling, dose–response relationships, elucidation of vulnerable species and habitats, and toxic endpoints or benchmarks of Se have since been made, and will be summarized in the forthcoming workshop proceedings. The purpose of this summary is to identify the principal procedural steps and scientific knowledge required for a defensible risk characterization in settings where Se is known to occur, or has the potential to occur, above normal "background" levels. The primary goal is to ensure that historical, unintended adverse effects are not repeated.

Importance of problem formulation

The value of the risk characterization depends heavily on information developed in earlier phases of the risk assessment. The problem formulation is particularly important, because it must clearly define the issues or concerns to be addressed and lead to appropriate analyses of exposures and effects of Se on aquatic organisms and aquatic-dependent wildlife. Identification of assessment and measurement endpoints—as well as a conceptual model that considers sources, speciation, transport, and environmental partitioning—and ecological exposures are critical. Having this information available, the risk assessor can then consider the unique attributes and challenges associated with Se.

Risk characterization: Unique challenges concerning selenium

For the risk assessor, Se presents unique problems and considerations, including the following:

- Se is a natural metalloid (essential for metabolic function in vertebrates) and bioaccumulates in freshwater and marine environments.
- The range between nutritional requirements and the onset of adverse effects is comparatively narrow, leaving a small margin of error for risk characterization.
- Biogeochemical cycling and bioaccumulation dynamics can be complex and are highly site specific.

- The forms of Se released to the environment vary widely and the rates of transformation to organo-Se, the form that is most toxic, depend on site-specific factors; however, the risk assessment may not be concerned with the form of Se released, but with the forms to which the receptors (e.g., fish, waterbirds) are exposed.
- In contrast to cationic trace metals, the primary route of exposure that leads to adverse effects to aquatic organisms is dietary.

Unique features of Se biogeochemistry and ecotoxicology in the environment require refocusing of the conceptual risk characterization typically implemented for trace metals. Figure 8 shows the recommended conceptual model for conducting risk characterizations for Se. This figure depicts the relative certainty with which Se concentrations in compartments can be assessed concerning prediction or characterization of risk. The critical medium (i.e., the compartment that yields the greatest amount of certainty and insight into potential adverse effects) is biological tissue in the most vulnerable aquatic organisms. There is consensus that fish and bird reproduction are the critical assessment endpoints, and that larval or embryonic survival and Se concentrations in eggs are the appropriate measurement endpoints in terms of assessing or predicting a problem at a given location because measured levels in these tissues are often strongly linked to adverse effects. Information to date concerning Se effects on amphibians and reptiles suggests that, likewise, concentrations in eggs are reasonable predictors of toxicity. Because Se is bioaccumulative, the risk assessment should focus on longer-term average exposures via diet.

Other indicators of exposure such as Se concentrations in diet, particulate phases, and water or sediment, and their associated benchmarks, can be the starting point for an initial risk characterization. There is little confidence, however, in predicting risk on the basis of information for waterborne Se concentrations alone (see Figure 8).

For fish and birds, the weight of evidence indicates that reproductive effects occur at lower tissue Se concentrations than those associated with other adverse effects. Thus, benchmarks established for protection from reproductive effects should be the focus during a risk characterization. Benchmark concentrations, however, vary among species.

The vulnerability of a species depends on its propensity to bioaccumulate Se, the transfer rate into eggs, and the species' sensitivity to each unit of concentration in eggs. Risk characterization may start with Se concentrations in any environmental compartment, but uncertainty about potential adverse effects is lowest when the concentrations in reproductive tissue are known.

Selenium risk characterization may also involve direct measurement of reproductive effects. Results from such studies, when well conducted, may be the definitive indicator of the occurrence of effects in a target species at the study site. For conducting such studies with fish, standard protocols are essential.

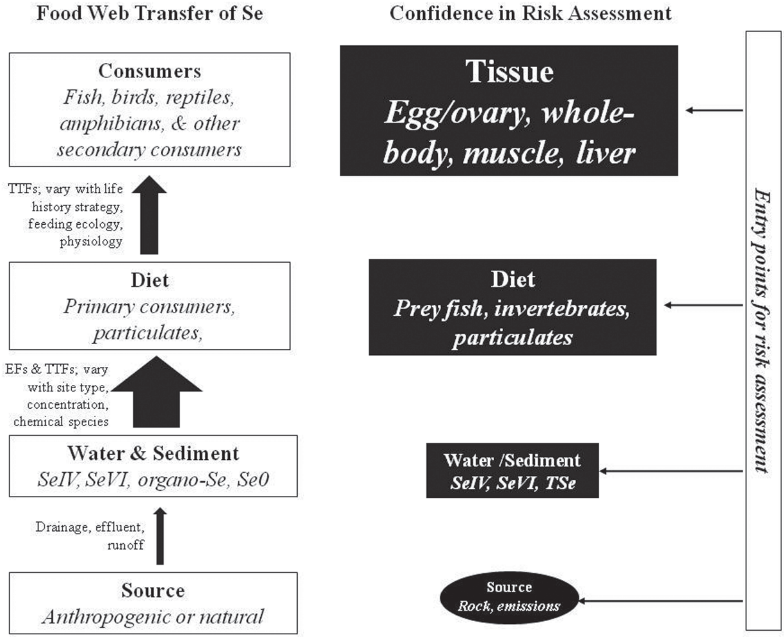

Figure 8: Conceptual pathway of Se transfer in aquatic ecosystems (left) and relative certainty with which Se concentrations in environmental compartments can be assessed in making accurate characterizations of risk. The size of the arrows in the left column indicates the relative rates of transfer and the size of the compartment in the right column indicates the relative confidence for deriving estimated risks.

Risk management

Risk management approaches for Se vary among countries, with differing terminologies, trigger values, guidelines or criteria, and resource management goals. Regardless, all risk management decisions should be based on the best available science. For Se, this means putting risk management activities into an ecosystem context. Best management practices and technologies that prevent entry of Se into aquatic systems should be considered as preferred management approaches. A good Se risk assessment that identifies the frequency and magnitude of potential effects, and the particular locations, receptors, and endpoints that would be affected, will provide the risk manager with a scientific foundation for selecting among pollution prevention or remedial options.

All regulatory approaches for managing risks of Se in water bodies would benefit by incorporating the key findings discussed throughout this workshop. Numerical benchmarks are typically used by all jurisdictions to trigger either further assessment of potential for risk or required pollution reduction actions. The efficacy of management decisions depends upon knowledge of how Se risks occur and what changes may be expected following source reduction. Thus, the scientific foundation laid out in this workshop is directly relevant to risk reduction and risk management under a wide range of regulatory jurisdictions, regardless of their location.

Uncertainties

Because Se is a naturally occurring substance and is essential for animal nutrition, assessing risks resulting from new or additional Se inputs is highly complex. There is a robust scientific literature about Se behavior in selected freshwater and marine systems, yet there remain significant uncertainties about its transport and environmental partitioning in different types of (relatively unstudied) ecosystems.

Knowledge about toxicity mechanisms is limited, and the relative sensitivity of organisms to Se exposures is limited to only a few animal groups. Whole taxonomic classes such as amphibians and aquatic reptiles are not well represented in the effects database, so the entire range of sensitivities remains elusive. Although it is now recognized that the most important step in Se becoming bioavailable is the initial uptake of waterborne Se into small organisms at the base of the food web, our ability to precisely predict when, where, and how much bioaccumulation will occur is limited.

A better understanding of the recycling of Se via sediments is needed because this recycling may influence recovery rates. Similarly, we know little about how adaptation or acclimation to Se can occur in chronic, high-exposure settings. Selenium enrichment often occurs in systems that are contaminated with other chemicals, but little is known about how these substances interact to either potentiate or ameliorate Se effects. Different types of ecosystems are known to be more or less vulnerable to the potential risks associated with Se, with slow-moving water having greater vulnerability than large, faster-moving streams or rivers. However, hydrological and ecological connectivity among systems, and differences in habitats within each type of system, make it difficult to generalize across systems with a high degree of accuracy. Because of these and other uncertainties, a major conclusion of the workshop is that Se requires site-specific risk assessments to a much greater extent than most other contaminants. For example, in large, complex ecosystems such as estuaries with multiple diffuse sources, risk characterization should divide the larger system into smaller units within which physicochemical characteristics are similar.

Overall Workshop Summary

This workshop on Ecological Assessment of Selenium in the Aquatic Environment synthesized and advanced the state-of-the-science regarding this unique metalloid, and established critical knowledge gaps. It is clear that results of studies and measurements appropriate for other metals and metalloids are not always appropriate for Se.

Participants, representing not only a broad cross-section of academia, government, business, and nongovernmental organizations but also different opinions regarding the seriousness of the Se issue, agreed to the following:

- Se is a growing problem of global concern for which mechanistic, biochemical understanding is required.
- Aquatic-dependent, egg-laying vertebrates are most at risk.
- The most sensitive toxicity endpoints are embryo mortality for waterbirds and larval deformities for fish.
- Aquatic-dependent mammals do not appear to be as sensitive as fish or birds to dietary organic Se exposure.
- Traditional methods for predicting effects based on direct exposure to dissolved concentrations do not work for Se.
- Site-specific factors are highly important in determining whether Se toxicity will occur.
- Se requires site-specific risk assessments to a much greater extent than most other contaminants.

There were a great many other areas of agreement as noted in this booklet and as will be detailed and explained in the forthcoming workshop proceedings.

The workshop participants reaffirmed that Se uptake is nonpassively facilitated across most biological membranes, making its partitioning unique among metalloids, and that to understand Se environmental partitioning and effects, knowledge of speciation is essential. Relating Se sources to risks to sensitive taxa requires measuring Se accumulation at the base of the food web and in key linkages through the food web (Figures 6 and 8). The greatest degree of site-to-site variation in bioaccumulation occurs at the base of the food web, but can be predicted by enrichment functions (EFs). Uptake by individual species and in steps of the food web can be described by a trophic transfer function (TTF).

The workshop participants also affirmed that Se concentrations in eggs are the best predictors of effects in sensitive egg-laying vertebrates. The vulnerability of a species is the product of its sensitivity to Se in its eggs, its propensity to transfer Se from its body into its eggs, and its propensity to accumulate Se from its environment, as affected by its diet and by site-specific factors controlling the transfer of Se into and within the food web.

Major uncertainties and key areas for reducing these uncertainties were also identified, including the fact that a notable knowledge gap exists for egg-laying species of amphibians and reptiles, which represent some of the most critically endangered vertebrates. Atmospheric partitioning and dispersion are not well characterized. A better understanding is needed of EFs at the base of food webs, and of inter-organ distributions of Se, both of which are key mediators of toxicity. Quantification of TTFs is required. Reasons for differential sensitivities among species, even within the same genus, remain to be elucidated, as do the possibility and significance of tolerance (acclimation and/or adaptation). Other uncertainties are noted in this booklet and will be detailed and explained in the forthcoming workshop proceedings.

The findings of this SETAC Pellston Workshop will benefit both scientists and managers. It is hoped that these findings will assist in preventing future environmental damage from Se and in providing focused management efforts that are based on good science.

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