



# **Report on the Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation**

September 1998

**REPORT ON THE  
PEER CONSULTATION WORKSHOP ON  
SELENIUM AQUATIC TOXICITY AND BIOACCUMULATION**

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Office of Water  
U.S. Environmental Protection Agency  
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## **NOTE**

This report was prepared by Eastern Research Group, Inc., a contractor to the U.S. Environmental Protection Agency (EPA), as a general record of discussion during the peer consultation workshop. As requested by EPA, this report captures the main points of scheduled presentations and discussions, and a summary of comments offered by observers attending the workshop; the report is not a complete record of all details discussed, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. This report will be used by EPA as an early scientific assessment of technical issues associated with selenium aquatic toxicology and bioaccumulation and will serve as a technical resource during EPA's review of freshwater selenium aquatic life criteria. The information in this document does not necessarily reflect the policy of the U.S. Environmental Protection Agency and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## **ACKNOWLEDGMENTS**

This document summarizes the proceedings and presentations made at a 2-day workshop sponsored by the U.S. Environmental Protection Agency (EPA) to discuss selenium aquatic toxicology and bioaccumulation. The meeting was chaired by Anne Fairbrother of ecological planning and toxicity, inc., who wrote the overall meeting summary section and led one of the discussion sessions. Other discussion leaders included William Adams (Kennecott Utah Copper Corporation), Steven Hamilton (U.S. Geological Survey) and William Van Derveer (Colorado Springs Utilities). Technical presentations were made by A. Dennis Lemly (Virginia Tech University) and George Bowie (Tetra Tech, Inc.). Keith Sappington of EPA's Office of Water served as the Work Assignment Manager for this task. Kate Schalk, Rebekah Lacey, Lauren Lariviere, and Beth O'Connor of Eastern Research Group provided support services to plan and coordinate the workshop and prepare a summary report for task 98-09 under EPA Contract No. 68-D5-0028.

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# CONTENTS

	<u>Page</u>
PREFACE .....	ii
I. INTRODUCTION .....	1
Background .....	1
Summary of Opening Remarks .....	2
Opening Presentations .....	3
Chair's Charge to the Experts and Highlights of Premeeting Comments .....	8
II. CHAIR'S SUMMARY OF WORKSHOP DISCUSSIONS .....	9
III. TECHNICAL DISCUSSION SESSIONS .....	14
DISCUSSION SESSION 1: Technical Issues Associated With a Water-Column-Based Chronic Criterion .....	14
DISCUSSION SESSION 2: Technical Issues Associated With a Tissue-Based Chronic Criterion .....	23
DISCUSSION SESSION 3: Technical Issues Associated With a Sediment-Based Chronic Criterion .....	31
DISCUSSION SESSION 4: Cross-Cutting Issues Associated With a Chronic Criterion .....	39
IV. OBSERVER COMMENTS .....	52
V. REFERENCES .....	55
APPENDIX A           Workshop Materials	
APPENDIX B           Technical Charge to Experts and Background Materials	
APPENDIX C           Premeeting Comments	
APPENDIX D           Additional References Provided by Experts	
APPENDIX E           Presentation Materials	
APPENDIX F           Observer Presentations	

## PREFACE

Under section 304(a) of the Clean Water Act, the U.S. Environmental Protection Agency (EPA) publishes ambient water quality criteria which serve as guidance to States and Tribes for setting enforceable water quality standards. Water quality standards form the basis for establishing pollutant discharge limits under the National Pollutant Discharge Elimination System (NPDES) and for setting Total Maximum Daily Loads (TMDLs). Given the importance of 304(a) criteria to the regulation of pollutant discharges to the Nation's waters, these criteria must be reviewed and revised periodically to reflect the latest scientific information.

Selenium is one chemical for which 304(a) aquatic life criteria have been derived, but which is currently undergoing review by EPA. Selenium exhibits a number of chemical and toxicological properties that complicate the derivation of numeric aquatic life criteria. Among these are: (1) its existence in at least four different oxidation states in the aquatic environment, (2) its propensity to bioaccumulate in aquatic food webs, and (3) its ability to convert between different chemical forms.

On May 27 and 28, 1998, EPA sponsored a workshop entitled: *Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation*. The goal of this peer consultation was to obtain early assessment of the state of the science on various technical issues associated with deriving aquatic life criteria for selenium. This document presents the proceedings from this workshop and is considered by EPA to be a valuable technical resource for future refinement of EPA's aquatic life criteria for selenium.

## **I. INTRODUCTION**

### **Background**

Selenium, a metalloid that is released to water from both natural and anthropogenic sources, can be highly toxic to aquatic life at relatively low concentrations. Selenium is also an essential trace nutrient for many aquatic and terrestrial species. Derivation of aquatic life criteria for selenium is complicated by its complex biogeochemistry in the aquatic environment. Specifically, selenium can exist in several different oxidation states in water, each with varying toxicities, and can undergo biotransformations between inorganic and organic forms. The biotransformation of selenium can significantly alter its bioavailability and toxicity to aquatic organisms. Selenium also has been shown to bioaccumulate in aquatic food webs, which makes dietary exposures to selenium a significant exposure pathway for aquatic organisms.

The most recent aquatic criteria for selenium were derived by the U.S. Environmental Protection Agency (EPA) in 1987. At the time of their publication, these criteria could not be conveniently adjusted to account for the combined toxicities of different selenium forms. Since then, a substantial body of literature has accumulated on the aquatic toxicity of different selenium forms (in combination and in isolation). In response to this and other new information, EPA has initiated an effort to evaluate and revise acute and chronic aquatic life criteria and site-specific criteria guidelines for selenium.

As part of this effort, EPA sponsored a Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation on May 27-28, 1998. This workshop brought together nine experts on the aquatic chemistry and biology of selenium to discuss technical issues underlying the freshwater aquatic life chronic criterion. The discussion among the experts was guided by questions posed in a technical charge written by EPA. While focusing on issues related to the chronic criterion, the charge also touched on technical questions pertinent to acute criteria, wildlife criteria, and site-specific criteria guidelines. The output from this meeting (recommendations in response to the technical charge) will be considered by an EPA-established work group that will be responsible for revising freshwater selenium criteria and for developing guidance for site-specific criteria.

Before the workshop, the experts submitted individual responses to the questions in the technical charge. At the workshop, the experts heard presentations by two leading selenium researchers; they then collectively discussed the questions in the technical charge and related issues. This report presents the results of this peer consultation. Section II of this report presents the chair's summary of the overarching themes and recommendations that emerged from the workshop. Section III summarizes the discussions and specific conclusions concerning each question in the technical charge. Section IV summarizes comments presented by observers at the meeting. Section V lists the references cited in the report.

Workshop materials, including the agenda and lists of experts, presenters, and observers, are provided in Appendix A. Appendix B includes the technical charge to the experts and background materials. Appendix C presents the experts' premeeting comments. Additional references provided by experts, presentation materials, and observer presentations are included in Appendices D, E, and F respectively.

### **Summary of Opening Remarks**

Dr. Jeanette Wiltse, director of the Health and Ecological Criteria Division of EPA's Office of Water, opened the meeting and welcomed participants. She said that the peer consultation process allows EPA to

benefit from the knowledge and experience of experts in the field, obtaining better understanding of the problem and new perspectives. She thanked the experts for their time and effort.

Dr. Wiltse commented that metals present a technically complex problem when developing water criteria. One key issue is the balance between sufficiency and toxicity: Many metals (including selenium) are required by organisms in small amounts, but are toxic in larger amounts. She predicted that the experts would find the selenium discussion challenging and thanked them again for participating in the consultation.

Keith Sappington, also of the Health and Ecological Criteria Division, then presented an overview and background of the revision of EPA's freshwater aquatic life criteria for selenium. He said that the purpose of the consultation was to provide an early assessment of the science on a number of the technical issues associated with the criteria, and that EPA would use this information as a basis for moving forward through the criteria revision process. He explained that the impetus for EPA's review of the selenium criteria included:

- New data and concern over the level of protection (too high or too low?).
- Ecological importance (as selenium is both an essential trace nutrient and a toxicant).
- The need to address the toxicity and bioavailability of different selenium forms.
- The need for site-specific criteria modification procedures (taking into account bioaccumulation and food-web exposure).

He added that some fundamental issues EPA is facing in the development of the new criteria include determining in which environmental compartment to express the criteria, establishing the duration of the averaging period, and identifying the key factors affecting the toxicity and bioaccumulation of selenium.

Mr. Sappington emphasized that the focus of the peer consultation would be on technical issues underlying the freshwater aquatic life chronic criterion. He reminded the experts that discussion of risk management or policy decisions would not be appropriate to this forum. He discussed the key steps that EPA would undertake in its criteria review process and concluded by presenting a rough timeline for the development of the revised criteria. (See Appendices B and E for more detail.)

Dr. Anne Fairbrother, the workshop chair, then discussed the workshop structure and objectives, reminding experts again to focus only on reviewing the state of the science; she added that waterbirds would not be considered in the discussion. (See Appendix E for presentation materials.)

## **Opening Presentations**

### *Belews Lake: Lessons Learned*

Dr. A. Dennis Lemly of the Department of Fisheries and Wildlife at Virginia Tech University gave a presentation entitled "Belews Lake: Lessons Learned." (See Appendix E for presentation materials.) Belews Lake is a reservoir in the northwestern Piedmont area of North Carolina. The reservoir is hydrologically divided by a highway crossing into a main lake and the "158-Arm." The main lake received selenium input from disposal of waste ash from a coal-fired power plant. Inputs occurred over a 10-year

period, stopping in 1985. The combination of a period of ongoing inputs and a period of declining selenium concentrations has allowed researchers to obtain a great deal of information on tissue residue levels and effects. Dr. Lemly's summary of the key information gained from research at Belews Lake is as follows:

#### Main Lake Studies:

A concentration of ~10 µg/L dissolved selenium (about 80-90% selenite as it entered the lake) can bioaccumulate in aquatic food chains and cause massive reproductive failure in warm-water fish. Centrarchids (e.g., largemouth bass, bluegill, crappie, sunfish) are among the most sensitive to elevated selenium; forage species such as red shiners, fathead minnows, and mosquitofish are relatively tolerant (Cumbie and Van Horn, 1978; Lemly, 1985).

Once ecosystem equilibration to ~10 µg/L has occurred in this type of a reservoir setting, natural removal/cleansing processes operate very slowly. Elevated residues and toxic (teratogenic) effects in fish were evident 10 years after selenium inputs stopped and waterborne concentrations dropped below 1 µg/L (Lemly, 1997); consumption advisories are still in effect because of public health concerns. Complete recovery can be on the order of decades.

Dietary selenium was the most important source leading to effects in fish. Across years, the sediment/detrital route of exposure delivered the most consistent dose to fish (i.e., residues in benthos were consistently high). However, within a given year, residues in the waterborne/planktonic route of exposure were occasionally as high as in the benthic pathway (70-90 µg/g dry weight, especially in summer). Thus, each route of exposure delivered a toxic dose to fish. Planktivores, omnivores, insectivores, and piscivores were all similarly affected.

#### 158-Arm Studies:

Concentrations of 0.2-4 µg/L dissolved selenium in the 158-Arm bioaccumulated to levels that caused teratogenic deformities and chronic selenosis (pathological lesions) in sensitive fish species (e.g., bluegill and green sunfish) (Sorensen et al., 1984; Lemly, 1993a, 1997).

Concentrations of 0.2-4 µg/L dissolved selenium bioaccumulated to >25 µg/g dry weight in aquatic food-chain organisms. This concentration is over five times the chronic dietary toxicity threshold for freshwater fish and aquatic birds, as determined in laboratory studies (i.e., 3-5 µg/g; Lemly 1993b).

Selenium concentrations in fish (especially bluegill) reached levels equal to or greater than those that caused reproductive failure in artificial crosses of bluegill from a sister lake (Hyco Reservoir; 38-54 µg/g dry weight whole body concentrations in fish; Cumbie and Van Horn, 1978; Holland, 1979; Gillespie and Baumann, 1986), and reproductive failure in laboratory feeding experiments with bluegill (13 and 33 µg/g dry weight in fish diets; Woock et al., 1987; Coyle et al., 1993).

#### Related Laboratory Studies:

Exposure to waterborne (only) selenium (selenite) at concentrations of 10 µg/L does not affect survival of juvenile bluegill. Although some bioconcentration occurs, residues in tissues do not reach the toxic threshold (Lemly, 1982).

Conditions mimicking those in the Belews 158-Arm (4-5 µg/L dissolved selenium; 5 µg/g dry weight dietary selenium) can induce physiological and metabolic stress in young centrarchids, resulting in



significant mortality during cold weather due to Winter Stress Syndrome (Lemly, 1993c, 1996). Thus, time of year may be an important factor in the toxicity process when concentrations are near the current EPA criterion for chronic exposure (5 µg/L).

#### Conclusions:

Because of the extensive and rapid collapse of fish populations, the main body of Belews Lake has received most of the research focus and notoriety. However, the 158-Arm provides valuable information on selenium bioaccumulation and effects when waterborne concentrations are below the EPA national criterion for chronic exposure (5 µg/L).

Historic and current reference to the 158-Arm as “unaffected” (e.g., EPA 1998 Draft Field Study Summary) are incorrect. Multiple lines of evidence from this field site, (diagnostic residues, tissue pathology, teratogenic deformities) as well as associated laboratory studies (simultaneous water/diet exposures), indicate that selenium can become toxic to fish when waterborne concentrations are 4 µg/L or less. The affected taxa include widely distributed, economically and recreationally important species such as largemouth bass and bluegill. In this type of field setting, the threshold for detrimental impacts is well below 5 µg/L.

The most sensitive biological endpoint for detecting toxicity in fish (that has demonstrated impacts at a population and community level) is reproductive failure ( i.e., teratogenic deformities and associated embryomortality that occur shortly after hatching). Winter Stress Syndrome may be a more sensitive indicator but it has not been confirmed in field studies.

From a toxicity perspective, the point of effect is the fish’s reproductive tissue ( i.e., eggs). The toxic threshold for selenium in eggs (10 µg/g dry weight) is consistent regardless of the source or chemical form of selenium in an aquatic system. Pairing water and egg concentrations gives a direct source-fate, cause-effect linkage that integrates all aspects of the selenium cycle. The existing national field database suggests that a single water-tissue method for setting criteria can be applied equally to both selenate and selenite dominated systems.

The practice of allowing exceedances in meeting water quality criteria is not supported by field evidence of effects. For example, current EPA guidelines allow up to 20 µg/L as an ambient (lake-wide) concentration once every 3 years. The concentration of waterborne selenium in Belews Lake reached this level only once in 10 years, yet 17 species of fish were eliminated.

In response to a question on the origin of the 4 µg/L of selenium in the uplake arm, Dr. Lemly replied that it must have come from backflow from the main lake, because he doubted that there was significant contribution from atmospheric deposition. Dr. Teresa Fan asked whether it had actually been determined that selenium was incorporated into proteins in the species with which Dr. Lemly was working. Dr. Lemly said there had been some speciation work done, but that he did not know if there were differences between mosquitofish and bluegill in terms of selenium incorporation into protein. He said that this was one possible explanation for why mosquitofish accumulate higher tissue levels of selenium than bluegills yet show fewer effects. Dr. Steven Hamilton asked about Dr. Lemly’s statement that 10 µg/g of selenium in fish eggs is correlated with 5 µg/g in the food chain and 2 µg/L in the water column. Dr. Lemly replied that this statement was based on both data from the Belews recovery period and data from other lakes.

#### *Modeling Selenium in Aquatic Ecosystems*

Dr. George Bowie of TetraTech gave a presentation entitled “Modeling Selenium in Aquatic Ecosystems,” and referred to the paper “Assessing Selenium Cycling and Accumulation in Aquatic Ecosystems” (Bowie et al., 1996). (See Appendix E for presentation materials.) The model was sponsored by the Electric Power Research Institute (EPRI) and was developed in conjunction with a major research program. The research had two major components: toxicology and biogeochemical processes. Dr. Bowie’s presentation focused on three of the five major components of the model: cycling processes in the water column and in the sediments, and accumulation in tissues of organisms.

For each of these areas, Dr. Bowie described the processes in the model, discussed areas of uncertainty or limitations in our understanding of these processes, and showed the results for an example application to Hyco Lake to illustrate which processes are most important. He used these results plus some of his experimental results to discuss the response times of aquatic organisms to changes in selenium exposure and the effects of water quality variables on selenium uptake. Since the model description, Hyco application, and conclusions are covered in the paper, Dr. Bowie listed the main points concerning uncertainty, pharmacokinetics, and water quality effects on uptake that are not included in the paper.

#### Water-Column Uncertainty:

Organic selenides represent a lumped selenium pool that includes many different selenium compounds which are poorly understood and most of which cannot be measured with current analytical techniques. Some, such as selenomethionine, may be very biologically reactive while others may be much more refractory. Most of the organic selenide pool is not selenomethionine since the high uptake rates measured in the lab are not consistent with accumulation levels and organic selenide turnover times observed in the field.

#### Sediment Uncertainty:

Sediment selenium accumulation depends on settling of particulate selenium (plankton, suspended organic detritus, elemental selenium, selenite adsorbed on clays), diffusion of water column inorganic selenium into sediment porewaters followed by rapid reduction to elemental selenium in anaerobic sediments, and decomposition of organic detrital selenium in the sediments. In lakes where sediments are usually anaerobic below a thin oxidized microzone, diffusion of inorganic selenium and subsequent reduction to elemental selenium is one of the most important processes. However, in other types of systems where the sediments are aerobic or anaerobic at much greater depths, other accumulation processes would be more important. Selenium speciation data in other types of systems are currently lacking, which limits an assessment of accumulation mechanisms in these systems. Sediment selenium concentrations depend not only on the selenium fluxes into the sediments, but also on the sediment deposition rates (and sediment transport rates in flowing systems). This makes sediment selenium concentrations very dependent on site-specific conditions.

#### Food Web Accumulation Uncertainty:

Most research on selenium accumulation in aquatic organisms has focused on planktonic food webs. Benthic invertebrates can be an important source of selenium accumulation in fish, and since the sediments contain most of the historical selenium loadings in aquatic ecosystems, detrital and sediment pathways to benthic organisms could be extremely important. Bacteria accumulate selenium to levels several times higher than algae, so sediment bacteria associated with organic detritus could be an important source of selenium accumulation in benthos. Much of the sediment selenium in lakes is elemental selenium, which was recently shown to be bioavailable to benthos (though organic selenium assimilation efficiencies are several times higher). The selenium

concentrations in organic detrital particles, associated bacteria, and the amount of elemental selenium ingested during feeding are what determine selenium accumulation in benthos, not the selenium concentrations in the bulk sediments. Systems with high sediment deposition rates or high sediment transport rates could dilute selenium concentrations in bulk sediments, even though the selenium content of the organic food particles remained the same.

#### Response Rates of Organism Tissue Concentrations to Changes in Exposure:

Uptake and depuration experiments, as well as other studies in the literature, indicate that the time it takes to reach equilibrium starting from no previous selenium exposure is on the order of a few days to a week for algae and bacteria, 1 week for microzooplankton, 1 to 2 weeks for zooplankton and benthic invertebrates, and 3 to 10 months for fish. Since most fish experiments are conducted with small fish in the laboratory, larger fish in the field could respond more slowly. Food is generally the primary route of selenium accumulation in consumer organisms, and since the sediments respond much more slowly to changes in selenium loadings than the water column, the benthic food web can continue to provide exposure to fish long after the planktonic food web levels drop.

#### Water Quality Effects on Selenium Accumulation:

Since most selenium accumulation occurs at the bottom of the food web and then moves to higher trophic components through food exposure, water quality factors that influence accumulation in primary producers can be very important. In experimental research with phytoplankton, three water quality variables had a significant effect on selenium uptake rates (Riedel and Sanders, 1996). Low pH and low phosphate increased selenite uptake by a factor of about 4 or 5, and low sulphate increased selenate uptake by a factor of 2.

Dr. Fan asked Dr. Bowie if the elemental selenium data he was using for sediments involved analytical confirmation. Dr. Fan cautioned that her group could not confirm using extraction methods that the red amorphous material secreted from algae was elemental selenium; this material contained <10% Se and >90% carbonaceous material, possibly polysaccharides. She suggested a particular analytical technique that should be used for elemental selenium. Dr. Bowie replied that he was using results from Dr. Greg Cutter's work (Cutter, 1991), but that Dr. Terry Layton's work (not yet published) at the University of California at Berkeley used the analytical technique referred to by Dr. Fan and found that a significant portion of the sediment selenium was elemental selenium.

#### **Chair's Charge to the Experts and Highlights of Premeeting Comments**

Dr. Fairbrother summarized the technical charge given to the experts by EPA, and the experts' premeeting responses to the questions in the charge. (See Appendix E for presentation materials.) She noted that the leaders of each discussion session would present the premeeting comments in more detail.

Dr. Fairbrother repeated that the charge to the experts was to address and comment on technical issues. She asked the experts to identify the rationale behind their comments and conclusions, assess the level of confidence in data cited, and discuss data quality.

Dr. Fairbrother first addressed the question "What do we know about the relationship between water-column measurements of selenium and biological effects?" She said that the experts generally agreed that

looking at this relationship alone is not a good approach for a bioaccumulative compound like selenium. Many of the experts noted that the most sensitive fully aquatic species are fish species and that diet is the primary exposure route. Also, there seemed to be a need to discuss selenium chemistry.

Next, Dr. Fairbrother discussed the experts' comments on the relationship between tissue concentrations and either sediment or water concentrations. She said that there had been mixed responses on this issue. There was disagreement on the state of the science; some of the experts said that the science base was good, while others said that there was too little data. The experts also disagreed somewhat in what form of selenium to measure in which tissue. There was some agreement that water-tissue correlations are poor, and that diet-tissue-effects correlations are better.

Concerning the link between sediment concentrations and both water concentrations and effects, Dr. Fairbrother said that there had been disagreement on several aspects of this question. Experts disagreed about the ability to relate sediment concentrations to either water-column concentrations or effects in fish. Finally, Dr. Fairbrother said that some of the cross-cutting issues brought up included selenium geochemistry, selenium kinetics within and between ecosystem compartments, and the differences between lotic and lentic systems.

## II. CHAIR'S SUMMARY OF WORKSHOP DISCUSSIONS

The following summary was written by the Workshop Chair, Anne Fairbrother, based on the experts' discussion and premeeting comments. Details of the experts' discussions are provided in Section III.

The technical sessions initiated discussions among the experts by first reviewing the questions provided in the premeeting comments and then allowing conversation to develop around a general theme. General themes were: relationship of effects to water, sediment, or tissue concentrations and a session on cross-cutting issues to capture ideas on chemistry, system variability, and other topics brought forward by individual experts.

### **Water-Effects Relationships**

This session began with a discussion of the scientific validity of predicting chronic effects of selenium from water concentrations. The experts quickly agreed that waterborne exposure to selenium in all its various forms is less important than dietary exposure in determining the potential for chronic effects. Therefore, predictions of ecological effects cannot be based on studies that use water-only exposures. Factors that modify the relationship between water concentration and effects include the types of organisms constituting the food web, speciation and rates of transformation of selenium, and rates of exchange of selenium between water, sediment, and organisms. It was noted that selenium speciation may be sensitive to salinity, thus altering bioaccumulation potential, but this has not yet been proven.

There were differences of opinion about what to measure in the water column for assessing the level of selenium contamination of an aquatic system. However, it was agreed that, at a minimum, dissolved (i.e., in the water phase) versus particulate (i.e., attached to particles of inorganic substances or to bacteria or phytoplankton) selenium be differentiated and that selenate and selenite (two oxidation states of selenium) be determined in both fractions. Peptide- and protein-bound forms of selenium are critically related to the potential for occurrence of chronic effects. The protein-bound forms should be specifically included in the analysis of selenium in the particulate fraction, as this is the primary step for the major route of bioaccumulation. The current definition of the dissolved fraction is the portion of the sample that passes freely through a 0.4  $\mu\text{m}$  filter. One expert suggested that an 0.2  $\mu\text{m}$  filter might be more appropriate in order to catch the smaller phytoplankton and bacteria in the particulate fraction, as these organisms are very important in the first step of bioaccumulation of selenium.

Experts concluded that insufficient information exists to quantitatively correlate water quality characteristics (such as sulfate, pH, and TOC) with chronic toxicity. Finally, the experts emphatically agreed that toxicity relationships derived from acute toxicity studies cannot be used to predict chronic toxicity, as the dietary route of concentration and exposure is so important for selenium. This also implies that bioconcentration factors (i.e., concentration in tissues divided by concentration in water) are not appropriate for use with this compound. In summary, water concentrations are related to effects, but it is a nonlinear (and site-specific) relationship.

### **Tissue – Effects Relationships**

Discussion then turned to technical issues associated with a tissue-based criterion. The experts agreed that tissue integrates all exposures, whether from food or water. The best tissue in which to measure selenium is fish ovaries or eggs as concentrations have been linked to reproductive effects in some species. There

was some discussion, however, that pointed out the need to develop a larger data set encompassing interspecies variability in the ovary concentration – reproductive effects relationship. If fish ovaries are not available (i.e., sampling needs to be done during the wrong time of year), then larval stages are the next-best tissue to measure as older life-stages are less sensitive to selenium effects. Liver tissue was mentioned as a third tissue for possible monitoring of residue concentrations. Muscle-plug biopsy techniques have been suggested for use with endangered species, but do not seem to correlate well with effects.

It was also pointed out that concentrations of selenium in benthic invertebrates could be measured in order to determine the potential for effects to the lower order organisms as well as to establish potential dietary exposure values for fish. Discussion highlighted the need to standardize this method, in order to be sure that sediment is removed from the organisms guts prior to measurement. A discussion ensued about the ability of selenium to alter community relationships of phytoplankton with ramifications throughout the entire food web. However, it was agreed that fish are the most sensitive to the chronic effects of selenium and therefore fish tissue continues to be the choice for a tissue-based toxicological threshold.

Further discussion centered on the form of selenium that is most appropriate to measure in tissue. To date, nearly all of the studies have measured total selenium, but it was agreed that a more accurate representation of selenium-effect relationships could be obtained through measuring protein- or peptide-bound forms of organoselenium. The incorporation of selenium into protein is the trigger for biological effects.

Finally, it may be difficult to correlate water column concentrations with tissue concentrations. There are many examples of sites where water levels are low and tissue levels are high, as a result of previous sediment loading with current reductions in water-column selenium. Sediment (and subsequent dietary) concentrations will decline over time if water levels are kept low, but there is a considerable lag from the time when water concentrations are reduced to the time when sediment concentrations reach low levels. Therefore, if the history of a site is not known, a single measurement of water and tissue (or sediment) concentrations may provide a misleading picture and inconclusive relationships.

### **Sediment – Effects Relationships**

Sediment is the dominant sink for selenium, and sedimentary organic materials (detritus) are an important dietary resource for aquatic invertebrates. The literature relating sediment-based criteria is sparse; most participants relied on three key references in their comments. A positive relationship between sedimentary selenium concentrations and effects in fish or bioaccumulation in invertebrate larvae has been shown in a few studies. However, one expert cautioned that a no-effects determination in field studies must always be tempered with an assertion that the test was powerful enough to have detected effects if they were there, albeit at low levels.

An analysis of data focusing only on fish indicates that toxic effects may occur when total sedimentary selenium concentrations exceed 4  $\mu\text{g/g}$  (dry weight). Elemental and organic selenium forms predominate in sediments. The process is affected by redox conditions, and selenium tends to associate with the organic detritus. In streams, total sedimentary selenium is related to water-column concentrations through normalization to total organic carbon. It was suggested that sedimentary aluminum concentrations might be useful as a marker for inorganic sediment composition, in an effort to further separate the detrital-bound selenium from inorganic-bound forms. For accumulation in sediments of lentic systems (i.e., lakes and slow moving water), consideration of residence time and use of a mass balance approach could relate sediment selenium to waterborne selenium.

Because waterborne selenium concentrations tend to exhibit large temporal variations, the strength of the water-to-sediment correlation is affected by the averaging period selected. The issue of spatial heterogeneity of benthic invertebrates as well as selenium deposition and speciation is very important. Other parameters that might affect the relationship of sediment concentrations and ecological effects include water retention time, volatilization rates, the type of benthic phytoplankton community, and whether or not the system is at equilibrium. Habitat selection by different types of aquatic biota and preferential feeding habits of higher organisms also modifies selenium exposure. Various experts made the points that redox potential (i.e., amount of oxygen in the system) affects selenium speciation and that improved analytical methods for sediments are needed. Two experts advocated the expansion of the use of liquid chromatography for sediment selenium analysis.

### **Cross-Cutting Issues**

The cross-cutting session captured issues that did not fit neatly into one of the above themes, as well as other comments or ideas. Spatio-temporal variability was addressed again, as it applies to water column, sediments, and tissues, although in different scales for each. Water concentrations may change rapidly (within days), whereas fish-tissue residue and sediment concentrations take months or years to change. The rate-limiting step may be the rate of conversion of the inorganic form of selenium to the organic form, which is a function of the species of selenium in the water column and the types of microorganisms present in the sediment.

There was agreement that the type of ecosystem has a large effect on selenium cycling in the system. Lentic and lotic (fast-flowing) systems, ephemeral or perennial waterbodies, saline systems, and northern (cold) streams, may differ in response to selenium input. Retention time of carbon, rate of sediment accumulation, rates of conversion of inorganic to organic forms of selenium, and tolerances of local species all differ among these types of systems. Bacteria and phytoplankton species differ between the two ecosystem types, which may cause differences in bioaccumulation rates. Also, lentic systems have higher primary productivity. Open (rather than closed) fish populations in lotic systems make changes in recruitment more difficult to document. While there was argument about the relative importance of considering one or both of these types of systems, there was agreement that their interconnections are important.

Two methods using existing field data were suggested for differentiating non-affected sites, areas with definite effects, and sites requiring a site-specific determination of effects. The apparent effects threshold (AET) method categorizes previously studied areas based on sediment or water concentrations. The sediment/water concentration above which effects always occurred would be identified, as would the concentration below which effects never occurred. New sites with sediment/water concentrations that fall between these two values (where effects sometimes occurred or sometimes did not) would require a site-specific assessment; otherwise, the site would be categorized as affected or not. A second method is based on fish tissue concentrations as a function of water concentrations. The empirical data from field studies that exist in the literature would be used to develop the bioaccumulation correlation on a global basis. Sites where measured fish tissue concentrations were statistically significantly different from what would be predicted based on water concentrations and the global bioaccumulation factor, would require a site-specific assessment of potential effects.

It was suggested that the Aquatic Toxicity Model presented by George Bowie could be used to make *a priori* predictions of whether a concentration of selenium in water would result in effects to the fish. Site-specific input parameters include selenium input (amount, rate, and species), flow rates, water depth, and a

few other hydrological parameters as well as food-web species. The more site-specific data that are used in the model, the more likely it is to accurately predict effects.

Selenium has the potential to interact with other metals, causing either greater or lesser responses than predicted from selenium alone. Furthermore, exposure to selenium may reduce an organism's ability to respond to other environmental stresses, such as has been shown for fish similar to those found in Belews Lake that were exposed to cold temperatures during laboratory studies. These types of interactions might confound the global empirical data set relating effects to selenium concentrations in water, sediment, or food.

Selenium is a required micronutrient for both plants and animals. Therefore, there is an exposure concentration below which insufficiency effects are seen and a different concentration above which toxicity occurs. The area in between is the Optimal Effects Concentration. In general, there is at least a 10-fold difference between insufficient and toxic concentrations and, on a practical basis, it does not appear to be of particular concern in field situations. However, this issue may be important in laboratory studies where appropriate minimum concentrations of selenium must be provided to maintain colonies of test species.

Analytic methods for detection of selenium in water, sediment, or tissue are technically complex. However, due to their importance in carefully and critically describing the systems at risk, a significant amount of time was devoted to discussion of this issue. Desired minimum detection limits, sample preparation requirements, cost, and laboratory capability all affect the selection of which method to use. A detailed summary of available methods, as well as sample collection and retention procedures, is included in the report.

One expert stated that at the national level, median background concentrations of selenium in aquatic systems do not vary greatly, being at about  $0.1 \mu\text{g/L}$ . However, there was disagreement on this value and particularly on the variability in background, which is dependent upon the spatial scale of the analysis as well as on site-specific geology. Methods are being developed for differentiating between natural and anthropogenic inputs of selenium into aquatic systems, but there remains a great deal of uncertainty.

Observer comments reinforced the recommendation to develop methods for setting site-specific criteria, as a universal numeric chronic criterion for selenium is highly unlikely to be predictive of effects for any particular site.



### III. TECHNICAL DISCUSSION SESSIONS

Generally, discussion leaders organized the discussions according to the questions provided in the technical charge. Each leader opened the discussion on each question by presenting an overhead summarizing the relevant premeeting comments. The following discussion session summaries include the presentation of the premeeting comments, followed by an account of the discussion for each question of the technical charge. Overall conclusions, which were written by the discussion leaders and reviewed by the other experts, are presented at the end of the discussion summary for each session.

#### DISCUSSION SESSION 1:

##### Technical Issues Associated With a Water-Column-Based Criterion

**Question 1: Besides selenite and selenate, which other forms of selenium in water are toxicologically important with respect to causing adverse effects on freshwater aquatic organisms under environmentally realistic conditions?**

*Discussion leader's summary of premeeting comments:*

Dr. William Adams presented his summary of the experts' premeeting comments concerning this question as follows: Selenate, selenite, seleno-cyanate, and organo-forms (seleno-methionine) are the key forms of interest. Selenate and selenite are the predominant forms derived from mining, agricultural practices, fly ash, and natural shales. Organo-selenium compounds produced from these inorganic forms are of most ecological relevance on a chronic basis; seleno-methionine is thought to be a key chemical form. Little is known, however, about environmental exposures of organo-forms, especially seleno-methionine; there is a general lack of analytical procedures for measuring organo-forms. Dr. Adams then asked the experts for any comments concerning his summary or question 1.

*Discussion:*

Dr. Gregory Cutter, disagreeing with the statements concerning seleno-methionine, said that free seleno-methionine is not important in water and is easy to measure. Dr. Fan expressed skepticism about the measurement of seleno-methionine, because most methods do not involve structure confirmation. She also pointed out that seleno-methionine is abundant in macromolecules and emphasized that macromolecular seleno-methionine may be important, although this hypothesis has been neither disputed nor confirmed by the literature. Dr. Cutter agreed and also stated that, based on his analysis using acid hydrolysis and ligand-exchange chromatography, the vast majority of organic selenium in unpolluted waters is peptide-bound.

Dr. Fan mentioned the possibility of the selenonium form, a cation, being present, as shown by Cooke and Bruland (1987). She added that, based on her work, salinity can drive speciation; she has found that one phytoplankton accumulates dimethyl selenonium propionate in a euryhaline environment. Dr. Cutter agreed that selenonium can be present in highly contaminated systems.

Returning to the discussion of seleno-methionine, Dr. Chapman asked whether laboratory tests using seleno-methionine are irrelevant to environmental exposures, given the small amounts of free seleno-methionine found in water. Other experts agreed that water-only exposures to seleno-methionine are of questionable relevance, but seleno-methionine may be important in food-chain transfer of selenium.

**Question 2: Which form (or combination of forms) of selenium in water are most closely correlated with chronic effects on aquatic life in the field? (In other words, given current or emerging analytical techniques, which forms of selenium in water would you measure for correlating exposure with adverse effects in the field?) Note: Your response should include consideration of operationally defined measurements of selenium (e.g., dissolved and total recoverable selenium), in addition to individual selenium species.**

*Discussion leader's summary of premeeting comments:*

Dr. Adams summarized the experts' premeeting comments for this question as follows: Total recoverable selenium is a useful form to measure. This would include all forms of selenium in the water except a limited amount of non-bioavailable selenium that might be tied up in the crystalline structure of suspended solids. There are no identified actual correlations between selenium forms and chronic effects. Future efforts should focus on proteinaceous forms (especially seleno-methionine). Dr. Adams then asked for the other experts' reactions to this question.

*Discussion:*

Dr. Fan asked for the other experts' opinions on making correlations between waterborne particulate selenium and accumulation of selenium in the food chain. She said that she had seen a couple of papers that indicated that there was a correlation (e.g., Saiki et al., 1993). Dr. Gerhardt Riedel replied that he thought that gathering data from multiple lakes would result in a correlation that was positive but would have large confidence limits.

Dr. Cutter advocated separating total recoverable selenium into the dissolved and particulate fractions, because those pools are available to different organisms. He said that this should be done by filtration using as small a pore size as possible, preferably 0.2 microns. Dr. Riedel and Dr. Adams agreed that separating the dissolved and particulate fractions is useful.

Dr. Gary Chapman raised the issue of the operational definition of dissolved selenium, which Dr. Cutter had mentioned in his premeeting comments. He asked Dr. Cutter to discuss this issue. Dr. Cutter replied that there is some work on colloidal selenium in estuaries, including a paper by Takayanagi and Wong (1984). He thinks that, based on these papers and his work, in most systems colloidal selenium represents a small fraction of "dissolved" ( $\leq 0.4 \mu\text{m}$ ) selenium. Thus, in his opinion, 0.4 microns is not a bad filter pore size for most systems, but he advocates 0.2 microns to ensure that the smaller phytoplankton and bacteria are included in the particulate fraction. Although Dr. Riedel suggested that cross-flow filtration could be used to get down to very small size ranges, Dr. Cutter replied that this technique is laborious. Dr. Cutter and Dr. Riedel agreed that the very small size range is not that important for selenium, although it is important for some other metals. Dr. Adams concluded this discussion by pointing out that the operational definition of "dissolved" is a topic currently under debate, particularly in respect to data collection by the United States Geological Survey (USGS).

Dr. Adams asked whether the experts thought it accurate to state that no forms of selenium in water have been correlated with chronic effects; he added that the science is uncertain, but it is probably a polypeptide/protein-bound form of selenium.

Dr. Chapman asked how much of particulate selenium is actually organic and how much is bound up in a

mineral matrix. Dr. Fan agreed that this was an important question for thinking about bioavailability. Dr. Cutter agreed and listed the possible forms of particulate selenium: adsorbed selenate or selenite (probably on clays), elemental selenium, and organic forms. He said that Luoma et al. (1992) have looked at the speciation of selenium on particles. Dr. Fairbrother responded that the separation of organic from mineralized selenium needs further research. Dr. Fan suggested that standard biochemical procedures could be used to determine what fraction of particulate selenium is bound to proteins. Dr. Adams observed that most of the previous discussion related to possible areas of future research, rather than currently practical techniques.

Dr. Joseph Skorupa asked the biochemists present if they felt that any form of selenium was toxicologically unimportant. Dr. Fan and Dr. Cutter responded that they did not, because all forms of selenium may eventually interconvert.

**Question 3A: In priority order, which water quality characteristics (e.g., pH, TOC, sulfate, interactions with other metals such as mercury) are most important in affecting the chronic toxicity and bioaccumulation of selenium to freshwater aquatic life under environmentally realistic exposure conditions?**

*Discussion leader's summary of premeeting comments:*

Dr. Adams summarized the experts' premeeting comments for this question as follows: It is not possible to rank these water quality characteristics with reasonable certainty due to insufficient information on their effects on expression of chronic toxicity. Overall, the Eh (oxidative/reductive) state of an ecosystem is most important in determining the potential for chronic toxicity to occur, because it significantly influences the formation of organo-forms of selenium. One could predict that, at the extremes and as a function of Eh, pH would be important due to speciation changes, but chronic data are not available to assess this. pH would be expected to have the most impact on selenite across typical environmental pH values. Sulfate appears unimportant in terms of the expression of chronic toxicity except potentially for primary producers. Arsenic and molybdenum are also mobilized under similar conditions as selenium and appear to be additive with selenate.

*Discussion:*

Dr. Cutter agreed that redox state is important for precipitating elemental selenium and removing dissolved selenium. He argued, however, that photosynthesis has more influence on the formation of organo-selenium. Dr. Adams and Dr. Fan pointed out that non-photosynthetic microbial processes are also important, particularly in sediments; these processes are somewhat coupled to redox state.

Dr. Fan added that the presence of sulfate or nitrate in a reducing environment encourages a certain type of microbial community (sulfate or nitrate reducers), which would have a major impact on selenium speciation. She cited evidence of hydrogen selenide and methaneselenol release into the marine atmosphere via phytoplankton activities (Amoroux and Donard, 1996). Dr. Cutter expressed skepticism about this possibility. Dr. Fan, Dr. Cutter, and Dr. Adams did agree, however, that the microbial loop is very important and that the presence of sulfate and nitrate reducers would affect selenium speciation, resulting primarily in the reduction of selenium to the elemental form.

Dr. Cutter commented that arsenic and molybdenum behave differently from selenium; in a reducing

environment, arsenic is mobilized while selenium is immobilized.

**Question 3B: Of these, which have been (or can be) quantitatively related to selenium chronic toxicity or bioaccumulation in aquatic organisms? How strong and robust are these relationships?**

*Discussion leader's summary of premeeting comments:*

Dr. Adams summarized the experts' premeeting comments for this question as follows: Insufficient information exists to quantitatively correlate water quality characteristics with chronic toxicity across multiple species and trophic levels. Sulfate, phosphate, and temperature have been shown to correlate with selenate for some species (i.e., primary producers).

*Discussion:*

Dr. Riedel amended Dr. Adams's comment by saying that, for primary producers, phosphate does not affect selenate uptake, but rather high phosphate concentrations appear to suppress selenite uptake.

**Question 3C: How certain are applications of toxicity relationships derived from acute toxicity and water quality characteristics to chronic toxicity situations in the field?**

*Discussion leader's summary of premeeting comments:*

Dr. Adams summarized the experts' premeeting comments for this question as follows: The applications of relationships derived from acute toxicity and water quality characteristics do not apply to chronic toxicity for most aquatic life (an exception to this might be the relationship between selenate and sulfate for algae). The primary reason for this is that acute toxicity is most often the result of water exposures, whereas chronic effects are the result of selenium being incorporated into the diet where the predominant form of selenium is no longer an inorganic form.

*Discussion:*

None of the experts had any objections to this summation.

**General Comments:**

*Discussion leader's summary of premeeting comments:*

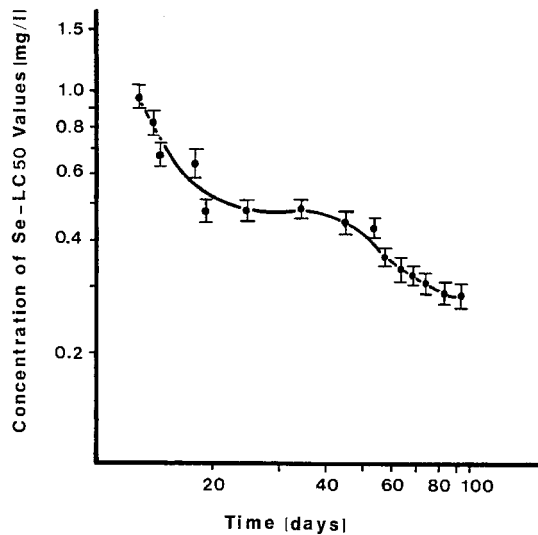
Dr. Adams offered for discussion the following statements taken from various premeeting comments: 1) Laboratory studies provide reasonable estimates of acute toxicity. 2) It seems imperative that chronic criteria include consideration of tissue residue and dietary route of uptake. 3) Fish eggs may represent a reasonably sensitive tissue to use as an endpoint for assessing the potential for species-level risk. 4) A useful approach might be to develop a generic criterion which also allows for site-specific approaches. Toxicity and bioconcentration factors (BCFs) are a function of time and exposure level. 5) Organic forms are thought to be produced in response to inorganic selenium enrichment and probably represent a net

reduction in potential for toxicity.

### Discussion:

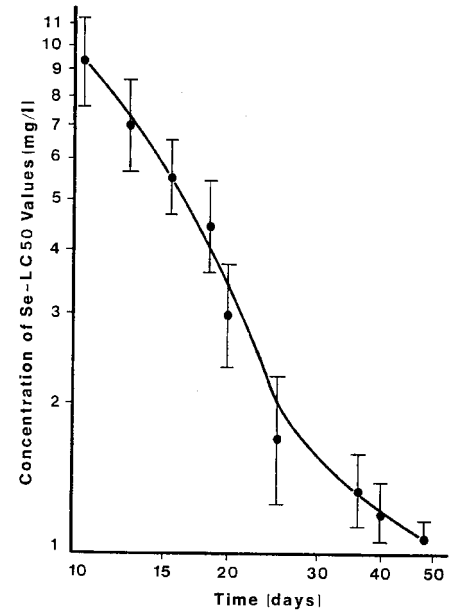
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**Figure 2.** The effect of time on the toxicity of sodium selenite to fingerling rainbow trout. The line was fitted by eye. (Adams, 1976.)

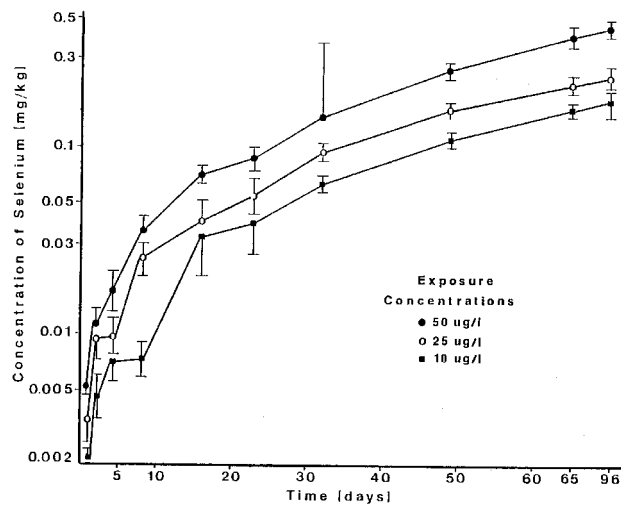
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**Figure 3.** The effect of time on the toxicity of sodium selenite to juvenile fathead minnows. The line was fitted by eye. (Adams, 1976.)

e rates are slow), he postulated that the 96-hour assay may not be the right test for acute toxicity. Dr. Cutter questioned the relevance of a water-only exposure. Dr. Skorupa pointed out that a short-term spike in selenium may have long-lasting food-chain implications, as shown by a paper by Maier et al. (1998). In this paper, a short-term 10 µg/L spike in a Sierra Nevada stream resulted in a concentration of 4 µg/g in the food chain for over a year. Dr. Chapman replied that a tissue-based criterion would require modeling with rate and fate functions and that in such a situation there would be no reason to draw an arbitrary timeline to separate acute dosings from chronic effects. Dr. Fairbrother said that that issue would be addressed in the discussion of averaging times during the cross-cutting session.

Dr. Adams then initiated point, concerning organic pointed out that toxic and can volatilize out they can also Cutter stated that a paper showed that dissolved less bioavailable to primary forms, such as selenite. distinction between essentially nontoxic to selenate, which is agreed that concentrations real waters are probably selenate. Dr. Fan pointed organic forms may be organisms such as small ingest them; Dr. Cutter agreed. Overall, however, Dr. Riedel and Dr. Cutter both stated that dissolved (not particulate) organic selenium in most waters is probably fairly persistent and refractory, and not very bioavailable. (It is taken up poorly and broken down slowly.) Dr. Cutter referred to a paper his group has published, which looks at the lifetime of dissolved organic selenium in the North Atlantic (Cutter and



**Figure 4.** The accumulation of selenium in the muscle of adult fathead minnows. (Adams, 1976.)

discussion on the last selenium forms. Dr. Fan methylated forms are less of the system, but that bioaccumulate. Dr. by Gobler et al. (1997) organic selenium was producers than inorganic Dr. Riedel made the selenite, which is phytoplankton, and moderately toxic. He of organic selenium in less toxic to algae than out that particulate more bioavailable to protozoans, which can

Cutter, 1998).

Dr. Adams directed the experts' attention to the comment concerning bioconcentration factors, which he defined as not including diet. (Bioaccumulation factors would include diet.) He showed a graph of bioconcentration factors observed at various intervals for fathead minnows exposed to four concentrations of selenium (Figure 4). Dr. Adams argued that, because there is a body of literature showing (as did his data) that BCF is inversely related to water concentration for selenium and many other metals, reporting a BCF for a given species at a given site is of questionable value. Dr. Chapman replied that he thought the experts could agree that BCFs were not relevant for selenium, as food chain is the key; Dr. Cutter agreed and said that this point should be emphasized.

Dr. Fan remarked that the emphasis on water-column concentration has led mitigators to focus on driving down those concentrations, which is not in fact the aspect of the system that is directly correlated with ecosystem effects. Dr. Fairbrother replied that EPA is struggling with this issue, because water quality criteria have been set using water column numbers. Dr. Adams postulated that the mass of selenium in the sediments may be more important than the concentration of selenium in the water. Dr. Cutter replied that water concentrations are related to effects but that it is a nonlinear relationship. Dr. Fan gave an example of two agricultural drainage ponds she has studied. Water concentrations of selenium differ by an order of magnitude between the two ponds, but sediment concentrations are similar. Dr. Adams speculated that one site might have more volatilization, and Dr. Fan agreed. Some of the experts discussed volatilization. Dr. Adams said he had seen papers that found that volatilization increases in reservoirs which have alternating

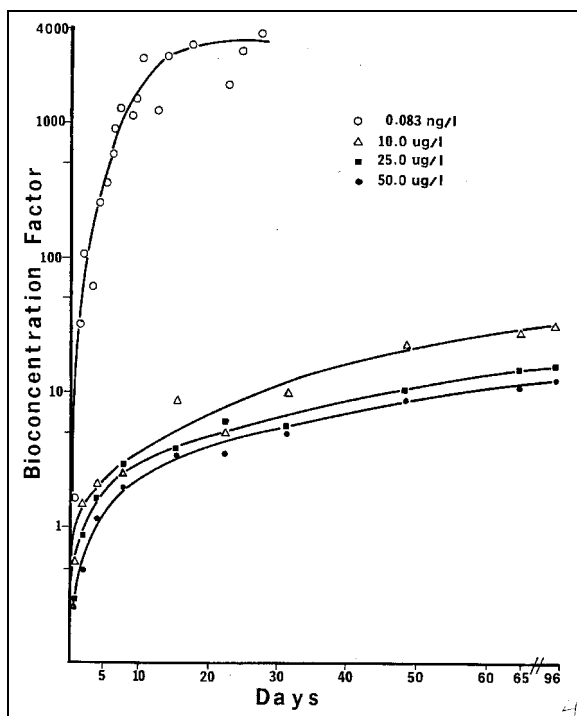
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Peptide/protein-bound forms are important. Free seleno-methionine is typically nonexistent or at low levels.



**Figure 5.** A comparison of the bioconcentration factors observed at various intervals for fathead minnows exposed to four concentrations of selenium. (Adams, 1976.)

own and refill cycles (Hansen et al., 1998; nberger and Karlson, 1994). The experts sed the residence time of volatilized selenium in atmosphere; Dr. Cutter said that it lasts a day or most, although Dr. Fan said it could be longer if selenium attaches to particles and/or aerosols.

Skorupa asked if the apparent lack of correlation en water and sediment selenium concentrations in Fan's evaporation ponds could be due to sediment geneity and small sampling size. Dr. Fairbrother that this question could be discussed during the nt session.

## p-Up

Adams summarized the discussion session as s: Dietary uptake is critical to determining chronic . The incorporation of waterborne selenium into diet is key; factors that should be taken into nt include transformations, rates of ormation, chemical species, and types of sms (e.g., microbes, invertebrates).

Dr. Adams asked what form(s) of selenium in water should be measured relative to assessing chronic toxicity and water quality standard compliance. Dr. Cutter said that, at a minimum, selenite, selenate, and total dissolved selenium should be measured. Another expert added that particulate should be measured as well. The experts discussed this question but did not come to agreement. Experts with opinions on this topic were asked to write summaries of their opinions.

Dr. Fan gave the following summary of her opinion regarding the significance of differentiating the protein-bound fraction of particulate selenium in the water column:

Particulate selenium can originate from live planktonic organisms, organismal debris/waste, and soil/sediment particles. The bioavailability of selenium associated with these different sources can vary. Presumably, selenium associated with organisms and biodebris represents a dietary route of exposure for aquatic consumers, and this fraction of selenium may be more concentrated and bioavailable. Since selenium bioaccumulation and toxic effects are mainly expressed through dietary exposure, it is important to distinguish the fraction of particulate selenium that is more representative of the consumers' diets. However, it would be a difficult task to speciate all of the selenium in particulate matter that is of biological origin. The fraction of biogenic selenium associated with soluble proteins may be convenient, because it may also be the most significant selenium sink in planktonic organisms exposed to environmentally relevant waterborne selenium concentrations. Major incorporations of selenium into bulk algal proteins have been documented for several categories of algae (Wrench, 1978; Fan et al., in press; Fan et al., 1998). Based on known selenium biochemistry (e.g., the propensity of selenium to substitute in sulfur amino acids), similar incorporations may well be applicable to other planktonic organisms. Therefore, monitoring protein-bound selenium in particulate matter may provide a more representative linkage from water to aquatic consumers in terms of selenium exposure.

Dr. Adams gave the following summary of his opinion regarding total recoverable selenium measurements:

Total recoverable selenium is recommended as one of several measurements that could be made to correlate with adverse effects in the field. This measurement includes all of the forms of selenium present in a water sample (both dissolved and particulate) except those tied-up in the crystalline structure of suspended solids. This recommendation is based on the need to identify a measurement that can be performed routinely and reliably across multiple laboratories. Additionally, many of the existing relationships between water, sediment and tissue have been developed around either total recoverable selenium or dissolved selenium. Ultimately, what form(s) of selenium should be measured depends upon the use of the data.

Dr. Cutter gave the following summary of his opinion regarding selenium measurements:

Additional measurements that are recommended for water include dissolved (defined as  $\leq 0.4 \mu\text{m}$ ) and particulate selenium. Dissolved measurements would be measured as total dissolved selenium, selenate, and selenite.  $\text{Se}^{-2}$  (selenides) would be determined by subtracting  $\text{Se}^{+4} + \text{Se}^{+6}$  from total dissolved selenium (Cutter 1982). Particulate selenium (defined as selenium associated with particles  $>0.4 \mu\text{m}$ ) could be measured as total selenium as well as  $\text{Se}^{+4}$  and  $\text{Se}^{+6}$ . Elemental selenium would be determined separately by direct analysis for  $\text{Se}^0$  (Velinsky and Cutter 1990).  $\text{Se}^{-2}$  would be determined by difference (i.e., subtracting [elemental +  $\text{Se}^{+4} + \text{Se}^{+6}$ ] from total particulate selenium). As an approach to reduce costs one could consider speciating samples, especially the particulate



fraction, only on a periodic basis.

**Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.**

1. Waterborne exposure to selenium in all its various forms is much less important than dietary exposure in determining the potential for chronic effects in aquatic organisms in general and for fish in particular.
2. The relationship between selenium in water and sediment relative to the aquatic organisms that live in these compartments and constitute the diet of fishes is key to understanding the food chain transfer of selenium. Factors that are important in understanding these relationships include rates of transformation and speciation of selenium, rates of exchange of selenium between sediment and water and organism tissues, and types of organisms constituting the food web.
3. Peptide- and protein-bound forms of selenium in the diet of aquatic organisms are emerging as critical factors in assessing the potential for chronic effects in aquatic organisms. Free selenomethionine appears to exist only at very low levels in tissues and in water.
4. Bioconcentration and bioaccumulation factors are inversely related to water exposure levels, which complicates their use in developing water quality criteria.
5. To evaluate selenium in the water compartment of aquatic ecosystems it is recommended that at a minimum dissolved versus particulate selenium be differentiated and that selenate and selenite be determined in the dissolved fraction. Additionally, it appears useful to determine selenite, selenate, and protein-bound and total selenium in the particulate fraction of natural surface waters. The latter may be of less importance for industrial discharges.

## **DISCUSSION SESSION 2:**

### **Technical Issues Associated With a Tissue-Based Chronic Criterion**

Dr. Hamilton opened the session by remarking that tissues integrate all exposures an organism experiences and represent the biological effects that water quality criteria are intended to prevent.

**Question 4: Which forms of selenium in tissues are toxicologically important with respect to causing adverse effects on freshwater aquatic organisms under environmentally realistic conditions and why?**

*Discussion leader's summary of premeeting comments:*

Dr. Hamilton presented a brief summary of each individual's comments on this question. He said there was general agreement that the form of selenium of concern in tissues was an organic, or protein-bound, form. He asked for any comments or concerns.

Dr. Chapman asked whether this question included organisms fed on by fish, pointing out that, if so, it would be important to think about the issue of gut contents and to specify whether organisms should be

depurated. Dr. Fairbrother asked the other experts to clarify whether fish were the only organisms in which effects were to be discussed, or whether anyone would say that selenium affects other organisms. Dr. Fan replied that, based on her review of the literature, there are not mortality or direct toxic effects on phytoplankton or invertebrates, but there may be community change. Dr. Riedel agreed. Dr. Fan and Dr. Riedel submitted additional comments on this point.

Dr. Fan submitted the following comments on the potential effect of selenium on community structure:

It is clear that selenium, regardless of the form, is less toxic to lower trophic organisms including primary and secondary producers, zooplankton, and benthic invertebrates. Selenium contamination, however, can have an effect on the competitiveness of different components of a given community, leading to an alteration of the community structure. For example, in San Francisco Bay in the 1980s, a shift from a diatom-dominated to a green algal community occurred. This shift preceded an explosive growth of the Asian clam, *Potamocorbula amurensis*, which is an extremely efficient accumulator of selenium (Brown and Luoma, 1995). It is unclear whether selenium contamination contributed to the change in the algal community, nor can we draw conclusions about the role of selenium in the abundance of the Asian clam. However, selenium is interacting with this new trophic system, and a selenium bioaccumulation factor of over 100,000 from water to the clam has been observed. In addition, the Asian clam is an important food source for the indigenous sturgeon. There is some evidence that the sturgeon population in the Bay is not actively reproducing and that field-collected sturgeon eggs exhibit high parts per million (ppm) selenium concentrations, particularly in certain protein fractions (Kroll and Doroshov, 1991). Unfortunately, the relationship between high selenium egg content and sturgeon reproduction problems has not been clearly established. It remains a real possibility, however, that selenium plays an important role in the impact of altered lower trophic community structure on fish reproduction.

Dr. Riedel submitted the following comments on selenium toxicity and algal communities:

Although most of the discussion of selenium toxicity has focused on fish reproductive effects, selenium toxicity can exert other effects on aquatic ecosystems. In some cases, environmental concentrations of selenium can also exceed the acute toxicity thresholds for a variety of algal species. The toxicity of selenium to algae is dependent both on the species of algae and the form of selenium. Of the two predominant forms of inorganic selenium in water, selenate has been generally observed to be more toxic to algae than selenite. For example, selenate concentrations from 50 to greater than >10,000 µg Se/L have been observed to inhibit growth of three species of phytoplankton from three different taxa. A diatom, *Cyclotella meneghiniana*, was observed to be the most sensitive ( $EC_{50} \approx 200$  µg/L). A green alga, *Chlamydomonas reinhardtii*, was the next most sensitive ( $EC_{50} \approx 2,000$  µg/L), while the cyanophyte *Anabaena flos-aquae* was the least sensitive, with an  $EC_{50}$  of >10,000 µg/L. None of these species were inhibited by concentrations of selenite up to 10,000 µg/L (Sanders et al., 1989). Similar toxicity results have been reported by Wheeler et al. (1982). Other authors, notably Kumar and Prakash (1971) and Moede et al. (1980), have observed that selenate and selenite have similar effects on several algal species. At least one green algae, *Ankistrodesmus falcatus*, may be unusually sensitive to selenite; Dr. Riedel has observed near complete growth inhibition in cultures spiked with 10 µg/L selenite, but not selenate (Riedel, unpublished observation).

Dr. Riedel has observed at least one “field” case of selenium toxicity at concentrations representative

of mildly contaminated sites. Riedel et al. (1996) made 10 µg/L additions of both selenate and selenite to natural phytoplankton cultures collected from Hyco Lake, as part of a biotransformation experiment. The selenate cultures showed a mild reduction in growth rate and maximum yield (~10%) compared to the control and selenite cultures. To verify the study, a series of selenate and selenite additions were made to another natural collection from the same site one month later; in this case, 10 µg/L selenate showed no inhibition, 20 µg/L decreased growth more than 10%, and inhibition was complete at 200 µg/L. Selenite did not show inhibition in these experiments either.

If selenium toxicity to a particular species or group of species were to occur in the field, it would be very difficult to observe from the existing community; the absence of some subset of possible species would not readily be detected (unlike the situation of fish in Belews where some 13 of 17 possible fish species were eliminated, there are hundreds of possible phytoplankton species, and rapid changes in species composition is the norm). Even a relatively small decrease in growth rate by an individual species could lead to a very rapid decline in its abundance relative to unaffected species. Nevertheless, the lack of these species could be significant in the food web, or as links in the chain of selenium bioaccumulation and biotransformation. If the sensitive species are truly randomly distributed among taxa, size classes, edibility to higher trophic levels, etc., differential selenium toxicity to phytoplankton is probably not a significant influence on aquatic ecosystems. It is unlikely, however, that the effects are truly random, and the net effect of selenium toxicity to phytoplankton may be to inhibit large cells to a greater extent than small cells (e.g., Munwar et al. 1987), diatoms to a greater extent than blue-greens (e.g., Sanders et al., 1989), and so on.

To return to the original question about toxicologically important selenium forms in tissue, Dr. Fan said that she did not believe that all selenium in tissue is in the protein-bound form. She cited a study of her group's, currently in press, which found that the percent allocation of selenium into protein in algae varies with varying selenium concentration (Fan et al., in press). Dr. Cutter, referencing his dissertation work (Cutter, 1982), said that the remaining selenium could be going into selenium esters, found in membranes. Dr. Hamilton asked the experts whether the bottom line of the discussion was still that incorporation of selenium into protein was the trigger for biological effects. The other experts agreed that this is at least "a" bottom line.

**Question 5: Which form (or combination of forms) of selenium in tissues are most closely correlated with chronic effects on aquatic life in the field? (In other words, given current or emerging analytical techniques, which forms of selenium in tissues would you measure for correlating exposure with adverse effects in the field?)**

*Discussion leader's summary of premeeting comments:*

Dr. Hamilton summarized the experts' premeeting comments for this question as follows: There were a variety of answers and agreement on some points. The experts agreed that there has been little speciation work in fish tissue. The forms suggested for measurement were largely total selenium or protein-bound selenium. William Van Derveer said that he would measure total selenium only if the exposure was a field exposure.

*Discussion:*

Dr. Hamilton asked Mr. Van Derveer to elaborate on his premeeting comments. Mr. Van Derveer replied that his concern is that, in laboratory studies, when diets are dosed with a specific selenium form, the residues that accumulate in the tissues may differ from the full biogeochemical spectrum that is found in the field. Dr. Hamilton replied that he had done a study in which fish were fed diets either spiked with seleno-methionine or made up of selenium-contaminated organisms from the field. He found mirror-image effects between the two diets (Hamilton et al., 1990). He added that there has been at least one other study that indicated that seleno-methionine is a good model for selenium present in the food chain (Bryson et al., 1985). Dr. Skorupa said that there is fairly strong consensus in the scientific literature that food-chain selenium, even though it is derived from different forms in water, exerts the same toxicity on a gram per gram basis. Besser et al. (1993) showed that seleno-methionine, selenate, and selenite bioaccumulate to different levels, but exert the same toxicity at the same levels. However, the various forms will move differently from water into the food chain; for example, compare Chevron Marsh to Kesterson (Skorupa, 1998). Dr. Cutter pointed out that the Bryson et al. study related to water exposure, not selenium added to the diet.

Dr. Hamilton summarized that the form of selenium in the tissue most closely associated with biological effects is an organic form. Dr. Fairbrother reminded the other experts that the original question was what to measure in tissues. She added that, historically, total selenium is what has been measured in tissues to relate to effects, but that in the future more measurement of protein-bound selenium should be done. Dr. Hamilton agreed, but Dr. Riedel said that, from a monitoring perspective, total selenium is adequate for tissues. Dr. Fairbrother pointed out that the morning's discussion indicated that there is not always a good correlation between total concentrations and effects. She speculated that these differences could be related to different amounts, or different types, of protein-bound selenium. The experts discussed the implications of the variation in the correlation between tissue levels of selenium and effects. Some argued that this variation mostly results from individual and interspecies variation in metabolism and fitness, whereas others said it may result from different forms of selenium in the tissues. The latter group thus argued for improved speciation of selenium forms in tissue.

**Question 6: Which tissues (and in which species of aquatic organisms) are best correlated with overall chronic toxicological effect thresholds for selenium?**

*Discussion leader's summary of premeeting comments:*

Dr. Hamilton summarized the experts' premeeting comments as follows: Almost all of the experts said that reproductive tissue is best correlated with effect thresholds. Some suggested that whole-body residue measurements would also be acceptable; whole fish are easier to obtain and much of the data in the literature is on whole-body residues. Dr. Fairbrother and Dr. Chapman suggested sampling benthic invertebrates; Dr. Cutter recommended the cytosol fraction of prey organisms.

*Discussion:*

Dr. Hamilton asked the experts whether they could recommend the ovaries as the tissue of choice, even though ovaries are not available all year. After a brief discussion, the experts agreed that fish ovaries are the tissue of choice in which to measure selenium levels. This agreement, however, was followed by further discussion.

Dr. Adams said that there needs to be a great deal more data on the variability of thresholds of effect

among various species, habitat types, and environments. Dr. Hamilton agreed. Dr. Adams said that it would be important to characterize the distribution of sensitivity among organisms of interest, as is currently done for the water-column criteria. Dr. Fairbrother asked whether the variability is based mostly on species sensitivity, or whether the type of selenium measured and the problem of gut contents contribute to the variability. Dr. Hamilton said that a lot of the variability in the current data set is due to life stage, as older organisms are more resistant. He said that, if whole-body residues are used, larval fish should be sampled.

Dr. Fairbrother asked Dr. Skorupa to comment based on his experience with the agricultural drainwater study. He replied that that type of dataset would be useful for taking a probabilistic approach to the criterion. The National Irrigation Water Quality Program (NIWQP) dataset (Seiler, 1996) has a large amount of data relating water concentrations to fish tissue levels (almost exclusively whole-body). Dr. Skorupa said that this data could be used, along with good measures of tissue effect levels, to develop a water column number that was associated with a certain probability of exceedance of effect thresholds. He agreed that more work would need to be done on effect-level variability among species. Dr. Fairbrother said that, if this type of analysis were done, it would be important to look at all the relevant parameters, such as what type of selenium is measured, whether the gut content is included, etc.

Dr. Fan asked how endangered species could be sampled for regulatory purposes. Dr. Hamilton replied that a muscle-plug technique has been developed, in which a biopsy is analyzed by neutron activation. Unfortunately, muscle tissue does not seem to correlate well with effects, based on his research (Hamilton, unpublished). Dr. Fan asked if blood sampling is an option; Dr. Riedel replied that it is, although it is hard to get blood from the smaller fish. Dr. Hamilton said that he has seen sampling of gills, blood, heart, and liver, but that are few data on these tissues. Dr. Riedel responded that his group had sampled various tissues in fathead minnows. They found that selenium concentrations increased more slowly in muscle tissues than in other tissues. Selenium concentrations in livers, however, mirrored concentrations in ovaries (Dr. Denise Breitburg, unpublished research for the EPRI project). Dr. Riedel noted that, unlike ovaries, livers are available all year.

Dr. Adams said that he thinks gonadal tissue is by far the first choice, because it is where the most sensitive effect is expressed; it is worth waiting to sample this tissue when it is available. Other experts agreed, although it was pointed out that there are additional sampling difficulties; some fish bear their young live, and sometimes it is difficult to get gonadal tissue even during the reproductive season. Dr. Lemly said a good approach would be to target a sensitive species that is widespread, such as a salmonid or a centrarchid, depending on the water body. Other experts reiterated that assessing data sensitivity across species would be crucial to the establishment of a tissue-based criterion.

**Question 7: How certain are we in relating water-column concentrations of selenium to tissue-residue concentrations in top trophic-level organisms such as fish? What are the primary sources of uncertainty in this extrapolation?**

*Discussion leader's summary of premeeting comments:*

Dr. Hamilton summarized the experts' premeeting comments as follows: Experts expressed that they were "not very certain" about making these correlations.

*Discussion:*

Dr. Hamilton made the point that there are many situations in which the water-column concentration of selenium is low but tissue levels are high (Hamilton et al., 1990; Schroeder et al., 1988; Skorupa and Ohlendorf, 1991; Zhang and Moore, 1996). Loading to tissue can come from the sediments and biota as well as from the water. Dr. Hamilton also asked whether it is possible that seleno-methionine is found in such low concentrations in the water column because it is highly bioavailable and taken up immediately when cells lyse. Dr. Cutter said that his group is working on this question.

The experts discussed using the NIWQP dataset to develop an empirical probabilistic approach to correlating water-column to tissue concentrations of selenium. Dr. Adams did not have great success in an initial attempt to make these correlations (Adams, unpublished), but he plans to redo his analysis. Dr. Hamilton said that better correlations could probably be achieved by taking site-specific factors into account. Dr. Adams agreed; he said that some of the published studies say that selenium transfer from the water to the food chain can be predicted well within a small site, but attempts to extrapolate to a regional or national scale fall apart.

Dr. Cutter raised the issue of detection limits, which he said are often not low enough for researchers to adequately make the correlations that are attempted. He recommends 0.01 ppb, because most uncontaminated waters are below 0.1 ppb total selenium. He and Dr. Skorupa discussed this issue. Dr. Skorupa questioned whether such a low detection limit is necessary if the effects threshold is much higher. Dr. Cutter responded that the lower the detection limit, the more useful the data will be for future uses and for looking at sublethal effects. Dr. Fairbrother agreed that a low detection limit was a good idea when trying to establish water-tissue correlations. Some experts objected to the characterization of the natural background concentration of selenium as 0.1 ppb, but this discussion was tabled until the cross-cutting session.

Dr. Hamilton then asked whether the other experts thought there would be more certainty in relating dietary concentrations to tissue residue in fish, and then in the two-step process of relating water to food organisms to fish. The experts agreed that there would be more certainty in these relationships, but that they still would be difficult to quantify. Many of the experts mentioned the difficulty caused by spatial and temporal variability in water-column selenium concentrations. Dr. Fan also questioned how to define diet. She mentioned Saiki's work in the San Joaquin River and San Luis drain (Saiki and Lowe, 1987; Saiki et al., 1993), which showed a good correlation between benthic invertebrates and detrital selenium. She emphasized, however, that it is crucial to determine what organisms are actually eating when trying to model food-chain transfer. Dr. Hamilton added that this point brought up the issue of sediments, which can be a source of loading to the food chain, and thus should potentially be included in correlation models. Dr. Fan said that migration of organisms in and out of the system poses another problem for correlations.

### **Wrap-Up:**

Dr. Hamilton summarized the discussion from this session. He said that he thought the experts had come to agreement that tissue integrates all exposures, whether different food types or water. Issues that had been raised included community change and variability in the sensitivity of the reproduction endpoint across fish species, and sometimes within species; there are limited data on both of these topics. He said that the group had not thoroughly discussed which endpoint was appropriate to examine (e.g., mortality, growth, deformities). Dr. Fan responded that this is why she thought the blood idea would be interesting. Selenium may reduce blood's oxygen-carrying capacity, and this endpoint would respond fairly quickly to ingestion of selenium. Dr. Hamilton replied that an important question to ask in considering an endpoint is whether

the effect is reversible. If so, the effect may not be truly adverse; it may not have effects at the population level.

Dr. Hamilton said that the experts had largely agreed that the ovary is the best tissue in which to measure residues; larval fish are a second choice if ovaries are not available. He reiterated that the issue of sensitive species is key. He said that information on linking sediments or water back to tissue is a data gap; too few data exist to build a good model. Dr. Adams said that he thinks the data exist, but that gathering sufficient data to encompass variability within and across sites would be a large task. He added that EPA should make a broad effort to compile these data sets. Dr. Fairbrother put in a cautionary note that the empirical approach of using large data sets to look at correlations is a useful starting point, but the real goal should be to understand mechanistically how selenium moves through the different compartments in different systems. Dr. Hamilton agreed, and said the data set should be built around reproductive studies in a series of fish species.

Dr. Hamilton said that some of the experts had suggested sampling benthic invertebrates because they are a key component of the food chain. He agreed that this is a good idea, and added that tissue concentrations in these organisms will be less variable than other components of the ecosystem. Dr. Riedel pointed out that selenium concentrations in benthic invertebrates are highly affected by gut contents, but other experts replied that this problem can be solved by depurating the organisms. Dr. Adams said that which compartment is most variable can be site-specific; sediments can be very heterogeneous and may therefore be highly variable. Other experts responded that this problem could be addressed by sampling in multiple locations.

Dr. Adams made the final point that, when looking at sensitive species, it is important to look at species that actually occur in the region under study. Dr. Hamilton agreed and added that, in the west, one may want to differentiate between native and introduced species.

**Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.**

There was an unexpected, readily reached agreement on the four issues concerning the possibility of a tissue-based chronic criterion. The experts agreed that the selenium form in tissue that is toxicologically important with respect to causing effects on freshwater aquatic organisms under environmentally realistic conditions is protein-bound selenium. By “protein-bound,” experts meant all organic selenium forms as a group. It was acknowledged that different forms of selenium can exist in tissue, but analysis of tissue selenium is typically as total selenium and not by speciated forms. In general, the organisms of concern were fish, which is the group usually emphasized in consideration of adverse effects on aquatic life. However, aquatic invertebrates were mentioned as another tissue of concern, because they represent an important link in food-chain transfer of selenium in the aquatic environment.

Protein-bound selenium, measured as total selenium, is the selenium form related to chronic toxicity. The major concern was organo-selenium forms bound by proteins rather than free organo-selenium or inorganic forms. One concern raised was that the form of selenium to which organisms are exposed might influence the resulting tissue residue; thus, emphasis should be on use of data from environmental field studies rather than laboratory studies in establishing a tissue-based criterion. The key tissues identified by experts were fish gonads, ovaries, or eggs. Due to the limited availability of ripe gonads/eggs, however, newly hatched larvae analyzed for whole-body residues were recognized as a possible alternative. Most data are on

whole-body fish, but for a variety of life stages rather than the preferred, sensitive larval life stage. The dataset for gonads, ovaries, and eggs are more limited. Liver tissue was mentioned as a third tissue for possible monitoring of residue concentrations.

Referring back to the dietary route for selenium, benthic invertebrates were recognized as a possible group of organisms to monitor in assessing adverse effects on aquatic environments, especially from the standpoint of shifts in the composition of a community and the resultant effects on higher trophic levels which might also shift in composition. One concern with benthic invertebrates was possible errors in residue concentrations due to gut contents.

Even though tissues were readily embraced as a possible component for establishing a criterion for selenium, the relation to water concentrations was questionable. Experts readily acknowledged that there was a lot of uncertainty in modeling the relation between concentrations in fish tissue and water. However, the level of uncertainty was less for the relation of selenium in water to that in aquatic invertebrates, and concomitantly, from selenium in dietary organisms to fish tissue.

Data gaps were identified including the limited number of fish reproductive studies where exposures included water and dietary routes using realistic water characteristics and food organisms and where meaningful endpoints were measured such as egg and larvae residues along with biological effects on offspring. These reproductive fish studies should include several representative families of fish.

### **DISCUSSION SESSION 3:**

#### **Technical Issues Associated With a Sediment-Based Chronic Criterion**

Mr. Van Derveer opened the session by making some general observations based on the premeeting comments. First, sediment is the dominant sink for selenium. Second, sedimentary organic materials (detritus) are an important dietary resource for aquatic invertebrates, and selenium tends to accumulate in detritus. He added that the literature applicable to sediment-based criteria is sparse; most participants relied on two to three references in their comments. Finally, he said that there was a range of opinions expressed in the comments regarding the potential merit of a sediment-based criterion.

#### **Question 8: Which forms of selenium in sediments are toxicologically important with respect to causing adverse effects on freshwater aquatic organisms under environmentally realistic conditions?**

*Discussion leader's summary of premeeting comments:*

Mr. Van Derveer presented a brief summary of each individual's comments on this question. Experts expressed a range of different opinions. Forms suggested included total selenium, elemental and organic selenium, and detrital selenium. Various experts made the points that redox affects speciation and that improved analytical methods are needed.

*Discussion:*

The issue of sediment heterogeneity was raised and discussed by some of the experts. They agreed that selenium can be distributed very heterogeneously in sediments, and that this should be considered in sampling and modeling. Dr. Skorupa added that the spatial heterogeneity of benthic invertebrate



distribution should also be noted. He said that this distribution often maps onto the spatial heterogeneity of selenium; both are found in areas of fine organic matter. In his opinion, sampling that does not concentrate on these areas misrepresents the toxicological risk. Dr. Riedel agreed and said that normalization to total organic carbon (TOC) is one way to solve this problem. Mr. Van Derveer said that he would later present some data showing that depositional zone selenium concentrations can fairly well predict concentrations in riffle-dwelling midges.

Mr. Van Derveer asked Dr. Adams to elaborate on his call for improved analytical methods for sedimentary selenium. Dr. Adams replied that he sees variability among analytical laboratories in determining sediment selenium speciation. Dr. Cutter responded that the techniques are established, but that better training may be needed. Dr. Skorupa said that he agreed with Dr. Adams, and added that it is important that all analytical data be evaluated. Dr. Riedel agreed that there is a problem with analysis for selenate. He and Dr. Fan advocated the expansion of the use of liquid chromatography for selenium analysis.

Mr. Van Derveer asked if there were any other issues related to question 8, recognizing that the literature relating sediment concentrations to toxicity is sparse. Dr. Cutter replied that, because of the lack of literature, the conclusion should be that the experts had low confidence in answering the question; Dr. Riedel agreed.

Mr. Van Derveer presented a graph using data from a publication of his (Van Derveer and Canton, 1997) (Figure 5). The graph showed the relationship between sedimentary selenium concentration and effects in fish, using data from a variety of sources, including NIWQP, Belews Lake, Hyco, and others. Mr. Van Derveer said that there appears to be a clear concentration-response ratio, but that more data are needed. Dr. Skorupa cautioned that the power of the study should be kept in mind when there is a finding of “no effect,” as many studies lack the necessary power to detect effects.

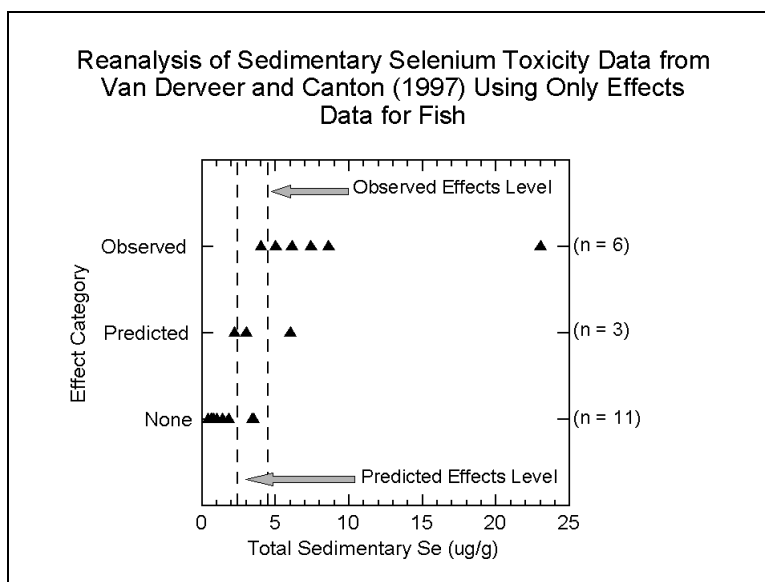
**Question 9: Which form (or combination of forms) in sediment are most closely correlated with chronic effects on aquatic life in the field? (In other words, given current or emerging analytical techniques, which of selenium in sediments would you measure for correlating exposure with adverse effects in the field?)**

*Discussion Summary*

Mr.

summary on this said to

mentioned his unpublished data indicating high sediment-to-benthos correlation in lotic (flowing-water) systems. Dr. Fairbrother said to measure total selenium and to consider lotic vs. lentic (standing water) differences. Dr. Adams said to measure total selenium, because individual species have not been correlated



**Figure 6.** Reanalysis of sedimentary selenium toxicity data using only effects data for fish. (Van Derveer and Canton, 1997.)

**Question 9: Which form (or combination of forms) in sediment are most closely correlated with chronic effects on aquatic life in the field? (In other words, given current or emerging analytical techniques, which of selenium in sediments would you measure for correlating exposure with adverse effects in the**

*Discussion Summary*

Van Derveer presented a brief summary of each individual's comments on this question as follows: He himself would measure total selenium and

with benthos. Dr. Fan said to measure proteinaceous selenium and seleno-methionine in benthos and detritus. Dr. Riedel said that better analytical methods are needed, and Dr. Skorupa said that a matched sediment and benthos study is needed.

### Discussion:

Dr. Adams clarified that the lack of correlation between selenium species and benthos results from the lack of data on the subject. Dr. Fan said that her recommendation to measure proteinaceous selenium was based on an educated guess that detrital selenium is probably concentrated in peptides or proteins. Dr. Cutter agreed that this is a reasonable assumption. Dr. Fan added that her group performed an experiment in which they compared detrital material captured in a sediment trap to cored sediments. The material that settled in the trap (rich in detritus) contained an order of magnitude more selenium than did the cored sediments (Fan, unpublished).

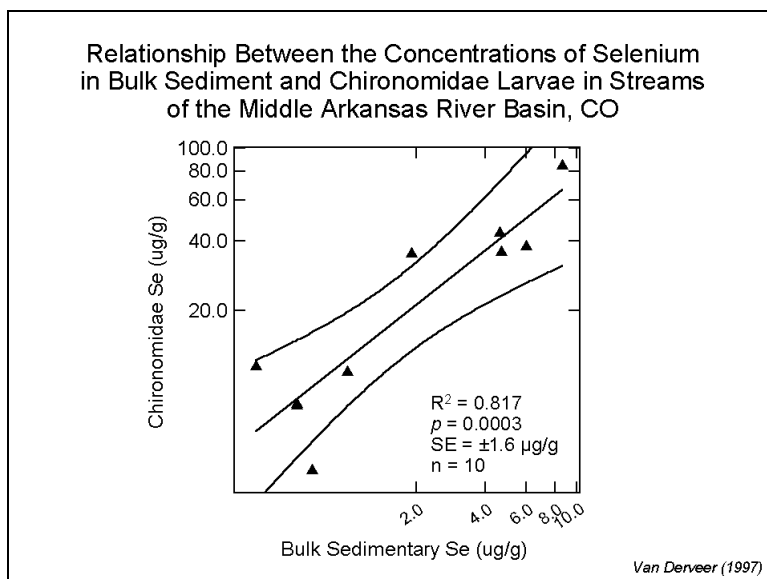
Mr. Van Derveer presented his unpublished data from a study in the Middle Arkansas River Basin in Colorado (Figure 6). The graph was a log-log plot relating sedimentary selenium to selenium concentrations in chironomids. He pointed out that there seemed to be a positive relationship. The experts discussed the possibility of relating this information to the effects information in the previous graph to estimate a threshold of dietary selenium associated with effects in fish. Mr. Van Derveer agreed that this was a useful direction for research, but he stressed that far more data would be needed. Dr. Skorupa added that, to perform such an analysis, it would be important to know what the fish were actually eating. The experts discussed the possibility of using assimilation efficiencies and protein-normalized selenium values in food-chain modeling. The variety of food chains present in different habitats was also discussed; not only do lotic and lentic systems differ, but lotic systems have high- and low-energy areas.

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mentioned TOC may be important, although Mr. Van Derveer pointed out that they all cited the same



**Figure 7.** The relationship between the concentrations of selenium in bulk sediment and chironomidae larvae in streams of the Middle Arkansas River Basin, CO. (Van Derveer, unpublished.)

**ion 10: In priority order, which ent quality characteristics (e.g., etc.) are most important in ng the chronic toxicity and umulation of selenium to water aquatic life under nmentally realistic conditions? these, which have been (or can quantitatively related to um chronic toxicity or umulation in aquatic isms?**

sion leader's summary of eting comments:

Van Derveer gave a brief summary each individual's comments on this on. He said there was a reasonable of agreement among those who ded. Everyone who responded

reference (Van Derveer and Canton, 1997). Other characteristics mentioned included Eh, pH, grain size, sulfate, sulfide, iron, and temperature. Dr. Fan mentioned detrital selenium and noted the issue of spatial variability. Dr. Cutter warned to beware of pseudocorrelation when looking at the effects of TOC.

*Discussion:*

Dr. Adams asked Dr. Cutter if the pseudocorrelation information was published. Dr. Cutter replied that it is (Velinsky and Cutter, 1991). He explained that high carbon deposition drives high microbial populations, which leads to anoxia in the sediment; elemental selenium is precipitated under anoxic conditions.

Dr. Fan said that there is a correlation between detrital selenium levels and accumulation of selenium by benthos. She cited Saiki's work in the San Joaquin River (Saiki and Lowe, 1987; Saiki et al, 1993), as well as the data presented by Mr. Van Derveer. She said that detritus should be looked at as a separate sediment component, using detrital selenium, rather than selenium in whole sediments, to relate back to the water column. In lentic systems, detritus may be sampled by putting out sediment traps; the top layer will be detrital. Dr. Adams responded that TOC was a good measure of detrital content. Dr. Fan replied that TOC is relevant, but that she was looking for a more direct correlation; the value of TOC can be false if the sediment contains a significant amount of carbon such as carbonate. Other experts brought up the difficulties in sampling detritus, but this discussion was deferred to the cross-cutting session.

**Question 11: How certain are we in relating water-column concentrations of selenium to sediment concentrations? What are the primary sources of uncertainty in this extrapolation?**

*Discussion leader's summary of premeeting comments:*

Mr. Van Derveer summarized each individual's comments on this question. He noted that many of the experts referenced his paper (Van Derveer and Canton 1997), which argued that TOC is a variable that may determine sedimentary selenium accumulation. He also cited lentic data obtained by Birkner (1978). Dr. Riedel said that we are very uncertain in relating sediments to water, and that this relationship is affected by many processes. Dr. Cutter said that water residence time determines sedimentary selenium accumulation, which can be calculated. Dr. Fan said that the sediment-water relationship is confounded by variability among sites, and she listed many possible sources of uncertainty.

*Discussion:*

Dr. Fan said that microbes may greatly affect the sediment-water relationship, because elemental selenium is an important sink, and also because microbes can volatilize a large amount of selenium. She noted that this has been shown under laboratory or microcosm conditions (Karlson and Frankenberger, 1990; Zhang and Moore, 1997). Dr. Cutter reiterated that in a lake system, the longer the residence time in the water, the more selenium accumulates in the sediments; this can be modeled relatively easily. He said that he did not know if this could be done for lotic systems. Mr. Van Derveer added that Birkner (1978) looked at selenium in many different compartments in lakes and reservoirs in Colorado and Wyoming and that this could be used to generate a lentic model similar to his lotic one. Dr. Fan brought up the problem of sediment heterogeneity and asked whether a selenium water/sediment ratio is a pseudocorrelation. Dr. Riedel responded that it is a real correlation, but a poor and confused one, affected by many poorly understood processes. Dr. Adams said that the relationship comes down to mass balance and rates of transfer. He said that he had only moderate success in attempting a sediment-water correlation for lentic

systems, using 204 water-sediment pairs from 15 water bodies (Adams, unpublished). The correlation coefficient was 0.66

Correlating water with fraction of sediments coefficient of 0.68; with grained fraction the 0.73. Dr. Riedel pointed fish, temporal variability correlation; because temporally variable and well buffered, it is not the correlation is poor.

Mr. Van Derveer graph from his work to conversation (Figure 7). showed the product of selenium and TOC on the x-axis and selenium on the y-axis.

at least in streams of the western United States, there is a fairly predictable relationship. Dr. Cutter suggested revisiting the data with a normalization to aluminum in the low-TOC range (i.e., normalize to “TOC *or* aluminum”). Other experts said that it is important to consider whether systems are at equilibrium or not. (For example, is there an ongoing input?)

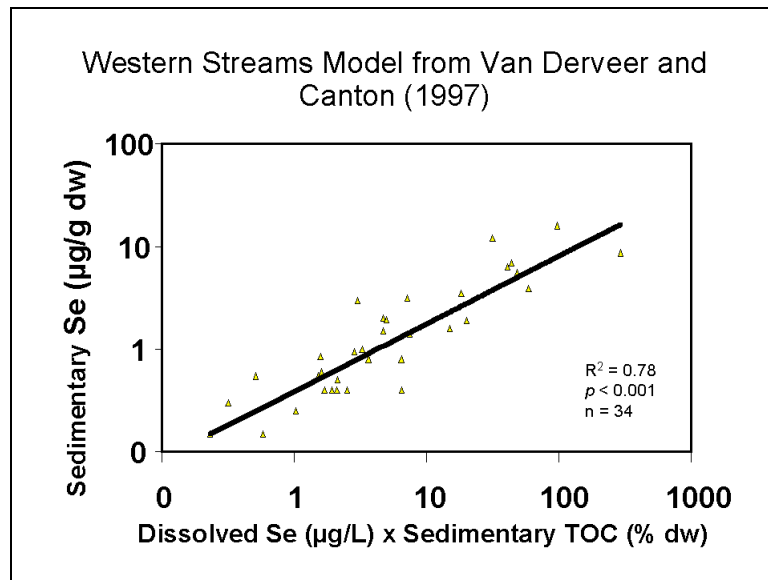


Figure 8. Western Streams Model. (Van Derveer and Canton, 1997.)

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## Research Needs

Dr. Fairbrother moved the conversation to the issue of research needs. Dr. Fan said there is a need to test the relationship among waterborne selenium, TOC, detrital selenium, total sediment selenium, and biota selenium for all abundant sediment species. Dr. Riedel said that it would be important to obtain the

assimilation coefficients for different benthic organisms and to examine how the different types of selenium in the food affect these coefficients. Mr. Van Derveer said that the issue of whether or not organisms are depurated should be addressed. Dr. Cutter said that a coupled examination of the ecosystem and the biogeochemical cycle should be performed at a site. Mr. Van Derveer said that he would like to see a more mechanistic understanding of what affects selenium accumulation in the sediments. Dr. Skorupa said he would like to see more data linking the biology of the most sensitive species to the heterogeneity of the sediments; some species may feed preferentially in high-selenium areas (because of other characteristics of these areas). Dr. Fan agreed that she would like to see if selenium accumulation by benthos can be correlated with selenium levels in organic-rich sediments. Dr. Hamilton mentioned the issue of differential accumulation of selenium by closely related species (e.g., flannelmouth vs. razorback suckers). Mr. Van Derveer said that it would be useful to do some controlled laboratory studies using field-collected sediments, perhaps running EPA's *Lumbriculus* bioaccumulation test. Dr. Adams said he would like to see examination of the sites that have relatively high levels of selenium but no effects seen; he said that these sites should help shed light on mechanistic understanding of processes. Dr. Fan said it is important to understand the mechanism of toxicity; she cited a review article from the biomedical field (Spallholz, 1994), which she urged the other experts to read.

## Wrap-Up

Mr. Van Derveer summarized the preceding discussion. After some further discussion, the experts agreed that the following was an accurate summary:

Elemental and organic selenium predominate in sediments. The process is somewhat redox driven, depending on the system type and the characteristics of the sediments. Selenium tends to be located in detritus. Total selenium may predict toxicity; there are some questions about relating selenium concentrations to TOC, the possibility of carbon-to-nitrogen (C:N) ratio normalization, normalization to proteins, and direct measurements of detritus vs. whole sediment. Spatial heterogeneity is an issue, as is preferential feeding (some species feeding in certain areas with high selenium concentrations). In addition, there are some issues with the power of biological assessments to detect effects. Concerning the question of what should be measured, there is some argument that total selenium in surficial sediments should be measured and it was also pointed out that multiple dietary pathways should be considered when they exist. Direct correlations of specific selenium forms to effects are lacking, but an overall causal relationship tends to exist, where high selenium in sediments tends to co-occur with effects at the population and community level. Some examples might be (1) effects seen in Belews Lake after the cessation of selenium input and (2) microbial community changes.

Which sediment characteristics appear to be most important? TOC seems to be important, but may be inappropriate for anoxic sediments where redox conditions are driving selenium accumulation; there may be some pseudocorrelation or a simple biogeochemical process moving selenium and sequestering it in sediment. Quantity of detritus may be important, and it may be important to measure that directly. In lentic systems, the residence time appears to be important; selenium accumulation can be calculated based on residence time and some other factors. Aluminum should be considered as a marker for inorganic sediment composition, to help differentiate detrital matter from inorganic material. Efflux from sediment to the water column is important. Sulfate may be important to sedimentary microbial communities, affecting selenium speciation. (Dr. Fairbrother noted that most items on this list reflect, not results reported in the literature, but things some or all of the experts think should be important, based on their understandings of the relevant processes.)

Finally, relating sediment to water, a TOC model exists for western streams. Residence time is important for both lentic and lotic systems. Whether the system is at equilibrium or not should be considered. Uncertainty is moderate overall for relating sediment to water, based on the small number of publications specifically addressing this relationship.

**Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.**

Sediment is the dominant sink for selenium in aquatic ecosystems. Elemental and organic selenium tend to predominate in sediment, with elemental selenium dominating under reducing conditions. Organic selenium is believed to be markedly more bioavailable than elemental selenium. Sedimentary organic materials (detritus) are an important dietary resource for aquatic invertebrates. Selenium tends to accumulate in detritus, thereby entering the benthic-detrital food web.

The literature regarding the toxicological effects of sedimentary selenium is sparse, and most workshop participants relied upon two to three publications for preparing their premeeting comments. Several participants cited a paper by Van Derveer and Canton (1997), which concluded that the total sedimentary selenium concentration is a reliable predictor of chronic toxicity in fish and birds. A reanalysis of those data (Van Derveer, premeeting comments), focusing only on fish, indicated that toxic effects may occur when total sedimentary selenium concentrations exceed  $4 \mu\text{g/g}$  (dry weight). The field data that were collected from Belews Lake after curtailment of fly ash input demonstrate the importance of sedimentary selenium in bioaccumulation and toxic effects on fish. Although waterborne selenium concentrations declined rapidly, Se concentrations in sediment and biota declined very slowly and teratogenic effects in fish populations persisted even 10 years later. Effects data for particular selenium forms in sediment are lacking in the literature; thus, preventing interpretation of sedimentary selenium speciation data.

The relationship between sedimentary selenium and toxicological effects may be affected by factors such as spatial heterogeneity in sedimentary selenium concentrations, habitat selection by different types of aquatic biota, and preferential feeding habits of aquatic biota. Moreover, efforts to relate toxicological effects to sedimentary selenium concentrations, or selenium concentrations in any environmental compartment, should consider the statistical power of the effects assessment. It was hypothesized that prediction of food web bioaccumulation and subsequent chronic effects on higher trophic levels might be improved by measuring detrital selenium, proteinaceous selenium in sediment, or seleno-methionine in sediment.

Unpublished data (Van Derveer, premeeting comments) were presented which indicate that a significant positive relationship exists between total selenium in surficial sediment (ca. 0-3 cm) and selenium accumulation in depurated Chironomidae larvae from streams of the middle Arkansas River basin, Colorado. These data suggest that, at least for some systems, total sedimentary selenium is well correlated with bioaccumulation in benthic organisms.

The following sediment quality characteristics were identified as potentially relevant to chronic selenium toxicity:

- Sedimentary TOC (possibly inappropriate for anoxic sediments where redox processes predominate);
- Quantity of sedimentary detritus present;
- Water residence time (longer residence time promotes greater sedimentary selenium accumulation);
- Normalization of sedimentary selenium to sedimentary carbon:nitrogen ratio;

- Normalization of sedimentary selenium to sedimentary protein content;
- Efflux of selenium from sediment to water; and
- Sulfate concentrations (may affect the composition of sedimentary microbial communities and thus the speciation of sedimentary selenium).

Sedimentary selenium can be related to waterborne selenium using two approaches, with a moderate degree of uncertainty. For streams of the western United States, a TOC-based model can be applied (Van Derveer and Canton, 1997). Sedimentary selenium accumulation in lentic and lotic systems can be calculated by considering residence time and applying a mass balance approach (Cutter, 1991). Because waterborne selenium concentrations tend to exhibit large temporal variations, the strength of the water-to-sediment correlation is affected by the averaging period selected. It is also important to consider whether the regime of waterborne selenium input to a system is relatively consistent over time (e.g., a stream receiving selenium from surrounding geological sources) or recently altered (e.g., Belews Lake after curtailment of fly ash input).

The following research issues were identified as being relevant to developing a more complete understanding of the role of sediment in chronic selenium toxicity:

- Assessing the relationship between detrital selenium and food web bioaccumulation;
- Understanding factors that may cause variability in selenium accumulation in benthic invertebrates, such as interspecific differences, assimilation rates, and effect of sedimentary selenium speciation;
- Evaluating the potential merit of depurating specimens prior to correlation with sediment, or any other environmental compartment;
- Correlating sedimentary selenium concentrations at preferred feeding sites with particular species of interest (e.g., endangered fish);
- Defining the mechanisms of selenium accumulation in sediment; and
- Performing laboratory studies of sedimentary selenium accumulation by invertebrates.

## **DISCUSSION SESSION 4:**

### **Cross-Cutting Issues Associated With a Chronic Criterion**

Dr. Fairbrother explained that the cross-cutting session was intended to capture issues that did not fit neatly in one compartment, as well as any other comments or ideas that any of the experts had not yet had a chance to raise. She listed the following issues to be discussed during the session: spatio-temporal variability and averaging times; ecosystem type (including lentic vs. lotic); site-specific approaches; analytical methods; sufficiency vs. toxicity; natural background; and interactions with other stressors.

**Question 12: How does time variability in ambient concentrations affect the bioaccumulation of selenium in aquatic food webs and, in particular, how rapidly do residues in fish respond to increases and decreases in water concentrations?**

*Discussion leader's summary of premeeting comments:*

Dr. Fairbrother summarized the experts' premeeting comments on this question as follows: Water concentrations can change by ten-fold in 1 month. Bioaccumulation in fish tissues changes over months. Phytoplankton and bacteria accumulate selenium rapidly (5-6 days), with turnover in 2 weeks. The rate-

limiting step is the conversion of the inorganic form to the organic form. The  $t_{1/2}$  for sediments depends on the form of selenium.

*Discussion:*

Dr. Cutter suggested that averaging time should be a function of retention time (the physics of the system), which varies greatly between lentic and lotic systems. Dr. Fan said that the biological component of a system can also have an effect on averaging time. Dr. Skorupa again raised the issue that a short-term spike can have long-term food-chain implications, based on the Maier et al. (1998) study. Dr. Fairbrother summarized that, in addition to the physics of the system, the biology of the system has to be considered, because organisms will have different effects on the residence time of selenium in the various compartments. Both physics and biology should be looked at when examining the relationship of water fluxes to responses or to fish tissue changes.

**Question 13: To what extent would the type of ecosystem (e.g., lentic, lotic) affect the chronic toxicity of selenium?**

*Discussion leader's summary of premeeting comments:*

Dr. Fairbrother summarized the experts' premeeting comments on this question as follows: There was general agreement that the type of ecosystem has a large effect on selenium cycling in the system. Lotic systems have a slower rate of conversion of inorganic to organic selenium, shorter retention time of carbon and decreased storage potential, and less accumulation of selenium in sediments. The modeling approach differs between lotic and lentic systems. Bacteria and phytoplankton species differ between the two ecosystem types, which may cause differences in bioaccumulation factors. Also, lentic systems have higher primary productivity. Open (rather than closed) fish populations make changes in recruitment more difficult to document.

*Discussion:*

Dr. Riedel added that lotic systems have a larger contribution of terrigenous detritus, which tends to dilute the selenium concentration. Dr. Fan replied that if the allochthonous input is through seleniferous soils, the reverse could be true. Dr. Skorupa said that another way in which lotic and lentic systems differ is that lotic systems are more likely to provide the source water for lentic rather than vice versa. Dr. Fairbrother replied that the reverse could also be true. Dr. Riedel said that the key point is not to consider parts of systems in isolation. Dr. Hamilton agreed that the interconnection of lentic and lotic systems is important. He cited a study by Radtke et al. (1988) on the Lower Colorado River, which showed that selenium in the backwaters was coming from the river's main stem. Conversely, a study by Engberg (currently in review) showed that only 18 percent of the selenium entering Lake Powell stays in the lake.

Dr. Adams said that there are other ecosystem types that should be considered, such as the Great Salt Lake, saline streams, ephemeral streams, and cold northern streams. He added that indigenous biology in each of the different environments should be taken into account.

Dr. Fairbrother questioned the statement that modeling approaches vary for different systems. She said that, in her opinion, the major components of the model are conceptually the same for different systems and that what varies are the rate processes. She asked for comments from the other experts. Dr. Fan replied



that components other than rates vary (e.g., food-web composition). Dr. Cutter replied that food-web composition is taken into account by Dr. Bowie's model. Dr. Bowie agreed.

Dr. Fan asked Dr. Bowie what was the minimum amount of information required to use his model for a site. Dr. Bowie said that one can use very little information and make guesses, but that the more actual data that are included, the better the model is. He said that the hydrology of the system and the selenium loadings would be the most important information, followed by the food web structure and some information on sediments. Dr. Fan replied that it is difficult to get a good mass balance for a dynamic system. She mentioned volatilization as an important component that is difficult to measure. Dr. Bowie replied that he didn't think volatilization was a major factor in most systems; further, the model takes into account factors which affect volatilization, such as the volatile fractions of bacterial and algal excretions. During the discussion, it was also clarified that the main purpose of the model is to be able to tie biological effects to water concentrations resulting from loadings, and possibly predict outcomes in hypothetical future situations.

### **Site-Specific Approaches:**

Dr. Fairbrother summarized suggestions Dr. Adams made about different approaches for doing site-specific assessments. These were: (1) Empirical database of fish tissue concentration as a function of water concentrations (develop for a variety of species and couple with reproductive effect concentrations); (2) Apparent Effects Threshold (AET -- use it to identify areas where site-specific effects measurements should be done); and (3) Modeling approach (parameterize for the ecosystem of concern).

### *Discussion:*

Dr. Adams elaborated further on the AET approach. He explained that it is the approach shown in the graph Mr. Van Derveer presented earlier (Figure 5). For multiple sites, concentrations of selenium in various compartments are coupled with information on the presence or absence of biological effects at the site. This approach identifies three ranges of concentrations: a range in which effects were never seen, one in which effects were sometimes seen, and one in which effects were always seen. This approach helps to establish rough effect thresholds and to identify sites for which more site-specific data are needed (i.e., those in the middle range). The AET approach has been articulated for marine sediments (Barrick et al., 1989). Dr. Bowie said that, for such an approach, using total selenium measurements might not be desirable for sediments, because detrital selenium is what gets into the food web. Dr. Fairbrother agreed that, in the sediments discussion session, there had been suggestions to normalize to TOC or protein. Dr. Fairbrother emphasized that, for the AET approach, it would be crucial to consider whether the studies used had adequate power to detect effects.

Dr. Fairbrother then asked Dr. Adams to discuss the idea of an empirical database. Dr. Adams said that this idea was based on various papers (e.g., Skorupa and Ohlendorf, 1991; Ohlendorf and Santolo, 1994). He said that, basically, this approach would again use information from multiple sites. Relationships between, for example, water concentrations and levels in fish reproductive tissue could be graphed and used to create a regression line. The strength of the regression's predictive power could be evaluated; in addition, as with the AET approach, sites with strong site-specific influences could be identified.

Dr. Riedel asked Dr. Adams how he would modify the water-to-fish regression if it did not fit well. Dr. Adams replied that his first step would be to remove sites like Belews Lake, in which there is not an

ongoing selenium discharge. Dr. Skorupa said that it should not be too hard to separate out the sites causing the “noise” in the data, based on knowledge of site-specific factors. He expressed optimism that it would be possible to create a good global relationship between water-column and fish-tissue selenium. Dr. Cutter added that another factor to consider would be the amount each site is elevated above background for its region.

Dr. Fairbrother said that the experts seemed to be contradicting their conclusions from the previous day, in which most of them had said that water concentrations could not be used to predict fish tissue concentrations. Dr. Adams said that part of the reason for that conclusion was that, to date, efforts to build global models had not been very successful. Dr. Skorupa said that two different scales of analysis were being discussed. During the water session, the experts addressed the question of what confidence they would have in predicting fish-tissue selenium concentrations from water selenium concentrations. He said that that was a different question from the current issue, which was looking globally at relationships between water and fish and trying to identify sites that are over or under the regression line. Dr. Cutter agreed. Dr. Adams said that, even if tissue levels are considered to have the best predictive power of effects, they still must be related back to water concentrations, or the tissue-based approach leads only to site-specific assessments for every site. Dr. Fan added that picking apart the variables that make some sites deviate from the global relationship would lead to a better understanding of the relationship between tissue concentrations and water concentrations.

Dr. Fairbrother commented that what the two approaches under discussion would mainly show is which sites need site-specific studies. Dr. Riedel asked whether a “site-specific study” means anything beyond analyzing selenium in the discharge and the receiving body. Dr. Skorupa replied that, in his opinion, site-specific analysis usually boils down to developing rigorous effects data to assess whether effects are occurring at a particular site.

### **Analytical Methods:**

Dr. Cutter presented the following remarks:

#### *The Chemical Forms of Selenium in Natural Waters*

##### DISSOLVED

Se(VI)	Selenate ( $\text{SeO}_4^{2-}$ )
Se(IV)	Selenite ( $\text{HSeO}_3^- + \text{SeO}_3^{2-}$ )
Se(0)	Elemental selenium (insoluble, but may be colloidal and pass through a 0.4 $\mu\text{m}$ filter)
Se(-II)	Selenide, primarily in the form of organic selenides such as seleno- amino acids (e.g., seleno-methionine, $\text{CH}_3\text{Se}(\text{CH}_2)_2\text{CH}(\text{NH}_3)\text{CO}_2\text{H}$ ) in dissolved peptides, and dimethyl selenide ( $(\text{CH}_3)_2\text{Se}$ )

##### PARTICULATE

Se (IV+VI)	Adsorbed to mineral or biogenic phases
Se(VI)	Selenate esters in membranes
Se(0)	Elemental Se precipitated from water column or produced in sediments

Se(0/-II)	Metal selenides (pyrite-like compounds)
Se(-II)	Organic selenides (primarily seleno- amino acids in proteins)

*Factors to Consider for Selecting Appropriate Analytical Methods for Determining Selenium in Natural Waters*

1. Accuracy. For obvious reasons, systematic errors must be eliminated. Standard additions method of calibration should be used and appropriate (i.e., same matrix type) standard reference materials should be analyzed (although only limited speciation data for these are available).
2. Precision. The analytical precision must be much less than the environmental variability in order to discern it.
3. Low detection limits. Natural concentrations of dissolved selenium can be as low as 2 ng Se/L, necessitating low detection limits. In this respect, for determining loadings, etc. a lack of data (i.e., below detection limits) should be avoided. Moreover, low detection limits allow potential interferences to be minimized via dilution. As a general rule, the detection limits should be approximately 10x lower than the expected concentrations.
4. Ability to determine dissolved and particulate speciation. The speciation of selenium in both the dissolved and particulate phases has been shown to affect its bioavailability and/or toxicity.

*Analytical Techniques for Selenium Determinations in Natural Waters*

Method	Speciation		Interferences	Detection Limit	Relative Cost
	Dissolved	Particulate			
SHG AAS	yes	yes	few	2 pptr	\$
SHG ICP-MS	yes	yes	few	<2 pptr	\$\$\$\$
Deriv.-fluorimetry	yes	no	many	5 pptr	\$
Deriv.-GC	yes	no	few	5 pptr	\$\$
IC	yes	no	many	1 ppb	\$
IC-ICP-MS	yes	no	many	<2 pptr	\$\$\$\$

SHG = selective hydride generation  
AAS = atomic absorption spectrometry  
ICP = inductively coupled plasma

What can we do now?

Dissolved: IV, IV + VI, total, selected or operationally defined organics

VI = (IV + VI) - IV

organic Se (-II) = Total - (IV + VI)

Particulate: IV, IV + VI, total, Se(0), pyrite-Se

organic Se (-II) = Total - (IV + VI) - Se(0) - pyrite-Se

Organic Se: The big problem. HPLC, etc. require knowledge about specific compounds. Can get at specific compounds or compound classes. For example: Copper-chelex gets primary amine Se; cation resin gets the selenonium cation.

Dr. Fan pointed out that the cost of disposal has to be factored into the cost of analysis using selective hydride generation, because a very acidic waste is generated for which disposal can be expensive. She added that her laboratory has had problems with their nebulizer becoming clogged. Dr. Cutter replied that a nebulizer is not necessary for his AA-hydride method.

Dr. Fan noted that selenium can be analyzed for by spiking whole water with base and analyzing the resulting head space. She asked Dr. Cutter if he had tried using the copper chelex method to analyze for seleno-methionine in sediments, and he replied that he had not. Dr. Riedel said that his group, after dosing algae with selenium-75, had detected small amounts of free seleno-methionine in water (in the parts per trillion range) using copper chelex. Dr. Skorupa asked Dr. Cutter to comment on neutron activation. Dr. Cutter replied that this method does not do speciation and that special attention must be paid to sample preparation.

Dr. Cutter presented further remarks:

#### *Water-Column Sampling*

Sample

--> 0.4 um filter (immediate)

--> "dissolved" (pH <2 with HCl, borosilicate glass)

--> suspended particles (freeze; dry at low temp)

Why? Dissolved and particulate represent different "pools" available to different parts of food web.

#### *Sediment Sampling*

Box core (or equivalent)

--> "squeeze" and filter

--> dissolved

--> particulate (dry at low temp)

Why? Dissolved and particulate availability; fluxes; selenium changes with depth; preserve flocculent matter at surface.

References for sediment sampling: Bender et al., 1987; Blomqvist, 1985; Blomqvist, 1991; Jahnke, 1988; Zhang et al., 1998.

For determination of selenium in sediments, Dr. Fan brought up benchtop x-ray fluorescence spectrometry. She said that it has the advantage of not requiring digestion, which minimizes sample handling and thus the potential for technician error. Dr. Cutter replied that the detection limits for this method are very high. Dr. Fan agreed, saying they are currently around 2 ppm, but she said the method could be useful for more highly contaminated sediments. She added that this technique determines other metals at the same time, which can be useful for looking at interactions. Dr. Cutter replied that it is an expensive instrument. Dr. Fan responded that it is not more expensive than other instruments he had referred to and that it results in large savings in labor costs.

Dr. Adams commented that Dr. Cutter's chart of analytical methods was a summary of the state of the art, rather than the methods commonly used. He said he thought a detection limit of 2 ppb was a stretch for some of the methods and was certainly a stretch for contract laboratories. Most contract laboratories, he added, are struggling to do a good quantitative analysis at the 2 ppb level. Dr. Riedel replied that EPA is currently publishing and validating a method for arsenic and that the selenium method will come in time. Dr. Cutter replied that, in his opinion, it is crucial that detection limits be ten times below the concentrations being analyzed. He added, however, that he understands the situation faced by a contract or utility lab analyzing large quantities of samples in short time periods. He said that, with EPRI funding, he had developed a methods "cookbook" currently used by many utility labs. He said that the approach he recommends for these labs is to analyze for total selenium, making sure that their method is accurate and precise, and to speciate a subset of samples.

### **Sufficiency vs. Toxicity:**

Dr. Fairbrother introduced this topic by saying that selenium is a required micronutrient; the question, then, is whether the range between sufficiency and toxicity levels is large enough that we need not worry about sufficiency. Dr. Riedel responded that there are regions, such as places on the Canadian Shield, in which selenium concentrations are so low (in the low ppb in the water column) that algae respond to selenium administration. Dr. Fan added that she found that she needed to add selenium to an algal culture in her laboratory that she had isolated from an evaporation pond. Algal growth had been diminished, but was ameliorated when she added 10 ppb of selenium to the culture. Dr. Fairbrother pointed out that these algae were adapted to a high-selenium environment. She reiterated the question of how wide the zone between sufficiency and toxicity is, and Dr. Riedel replied that for plants and algae it is quite wide.

For fish, Dr. Hamilton cited a study in which a selenite-spiked diet was fed to rainbow trout (Hilton et al., 1980). The researchers determined that between 0.15 and 0.38 µg/g dry weight selenium in the diet was the sufficiency level; they estimated that the toxicity level was about 3 µg/g. Dr. Hamilton pointed out that this was only a ten-fold difference, which is fairly narrow. Mr. Van Derveer said that spiking with selenite did not realistically mirror an environmental exposure.

Dr. Cutter said that, in his opinion, one would not have to worry about making a system too clean. He pointed out that low-selenium environments would have an assemblage of species that were adapted to the lack of selenium. Dr. Skorupa agreed; he said that, in 10 years of research, he has never found selenium levels in a waterbird egg in the wild that were below the level of selenium sufficiency determined for chickens.

Dr. Adams said that published papers have established a selenium requirement for daphnids in the range of 0.5 to 1 µg/L added to the algal culture that is fed to the daphnids. He also commented that European

researchers have started to develop sufficiency-toxicity curves for metals and said that this is interesting because it allows one to look at the gradations of effect. He added that, in the Netherlands, water criteria for metals are adjusted for natural background concentrations. Dr. Fairbrother then turned the discussion to the topic of natural background.

### **Natural Background:**

Dr. Fairbrother asked Dr. Cutter to elaborate on his assertion that 0.1 ppb is the natural background for selenium in U.S. freshwaters. He replied that the data he based this on were presented in a chapter he wrote on selenium in freshwater systems, which he had provided to the group (Cutter, 1989). He said that he only included data he considered to have been produced using sound analytical methods; he acknowledged that the western United States was not adequately represented. He also cited another reference he provided (Cutter and San Diego-McGlone, 1990), detailing variability in selenium concentrations over 2 years in the Sacramento and San Joaquin rivers. He added, however, that concentrations in the San Joaquin are affected by agricultural input, and that headwater data would be necessary to estimate natural background. Dr. Riedel said that using headwater data ignores the natural selenium inputs that occur as one moves downstream. Dr. Fan said that researchers had addressed this issue in the San Joaquin by looking at tracers; they determined that approximately 90% of the selenium inputs were agricultural. Dr. Fairbrother asked if this method could be used to determine natural background in systems with anthropogenic inputs. Dr. Fan replied that some researchers are trying to do this, but it is not yet a proven method. Dr. Adams questioned how one defines a number for “background,” since there is a range of values; he cited some examples of water bodies with natural selenium levels much higher than 0.1 ppb.

Dr. Cutter turned the discussion to the natural background selenium level for U.S. freshwater sediments, which he said is about 1 ppm. Dr. Adams agreed. Dr. Cutter said there is not much regional variation. Dr. Skorupa said that the USGS study of surficial soils in the United States found little regional variation in selenium soil levels. Dr. Fairbrother questioned how numbers were averaged in this study, agreeing with Dr. Adams’s comment that one must look at the distribution as well as the median. She summarized the discussion by saying that there is still debate about natural background and that more work must be done to allow good determinations to be made of whether sites’ selenium concentrations are at natural background or elevated.

### **Interactions with Other Stressors:**

Dr. Fairbrother raised the issue of the interaction of selenium with other stressors, asking the experts whether they had confidence that effects seen in the empirical data set are due just to selenium. Dr. Cutter said that he did not have confidence that this was the case, because when there is an excess of selenium, there is often an excess of something else. Dr. Hamilton said that the literature is fairly limited on many other elements. He cited an example from his research; in a study he did on the Green River, vanadium was somewhat elevated and may have been a confounding factor, but he could only find one relevant study about vanadium. Dr. Fairbrother and other experts pointed out the additional problem of extrapolating from the laboratory to the field. Dr. Fan said that, as broad element scans are becoming easier to do, she is hopeful that more field data will soon be available. Dr. Skorupa said that he feels there are sufficient data establishing that effects attributed to selenium are actually caused by selenium alone. His group has done studies in reservoirs that have a suite of pollutants excluding selenium, and they have not seen the effects typically associated with selenium.

## **Clarification Requested by EPA:**

At this point, Mr. Sappington asked the experts to clarify a couple of issues. First, he pointed out that, during the cross-cutting session, experts had discussed possible global approaches in relating tissue concentrations to water concentrations; however, during the water-column issues session the day before, experts had expressed skepticism about performing water-to-tissue correlations. He asked them to clarify this, and also to state some of the factors that they think might make the correlation poor. He asked whether the experts considered loading from sediments and spatio-temporal variability in the water column to be important factors.

Dr. Fan replied that the problem might be more complex than that and cited an example of an irrigation pond in California in which large changes in selenium load in bird eggs were observed with only a minor dilution of waterborne selenium concentrations, for unknown reasons. Dr. Fairbrother asked the experts to also clarify whether the form of selenium that is discharged to receiving waters changes the temporal or magnitudinal dynamics of what happens in the food chain. Dr. Cutter replied that it does; for example, the uptake rate of selenate is slow compared to selenite. Dr. Fairbrother said that part of the problem in trying to establish relationships is that the systems under study are generally non-equilibrium, dynamic systems.

Dr. Adams responded to Mr. Sappington's original question by agreeing that both mass in the sediments and spatio-temporal variability in the water column are important. He added that fish behavior is also important, including what fish feed on and where they forage.

Mr. Sappington asked whether the experts would expect tissue residue effect levels to differ between the laboratory and the field, or whether laboratory data are in fact useful for generating effect-level information. Dr. Hamilton replied that when he did laboratory studies, with both water-only and dietary exposure to selenium, he found the residue effect level to be very similar between the two; in other words, how the selenium got into the tissue did not affect the effect level. Dr. Riedel agreed that this is probably generally true, but that there are exceptions. He pointed out that there are many unknowns in the field, while organisms in the laboratory are kept under optimal conditions. Dr. Hamilton agreed.

**Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.**

### *1. Spatio-temporal variability*

There is a large amount of variability in selenium concentrations within compartments of an ecosystem (e.g., water, sediment, biota) across both time and space. The relationships between the compartments are not linear, however. Water concentrations may change rapidly (within days) whereas sediment concentrations take months or years to change, particularly in lentic systems. Fish tissue residues integrate all compartments and theoretically may change in response to alterations in any of them although food-chain exposures tend to dominate. Therefore, fish tissue residues also change over a period of months, and do not reflect the faster fluctuations of water.

The major factors influencing spatio-temporal variability are water residence time and biological processing (i.e., the type of organisms in the food web). The rate-limiting step may be the rate of conversion of

inorganic form to organic form, which is a function of the form of selenium and species of microorganisms in the sediment.

## 2. *Ecosystem type*

Ecosystems can be divided into lentic or lotic systems. Further subdivisions include ephemeral or perennial, highly saline, and northern (cold) streams. Differences in these systems that may lead to different responses to similar selenium input include retention time of carbon, rate of sediment accumulation, rates of conversion of inorganic to organic forms of selenium, and tolerance of local species. In addition, rates of allochthonous inputs (i.e., input of selenium materials from outside the aquatic system) versus autochthonous inputs (i.e., from within the system) differ. Most lotic systems are biologically open systems which makes it more difficult to measure ecologically-relevant effects on fish species that may move through the system, rather than being resident.

## 3. *Site-specific approaches*

Three approaches to site-specific assessments were proposed:

- Apparent effects threshold: This method would use existing field data to categorize systems as affected or not affected relative to selenium concentrations in sediment or water. The sediment/water concentration above which effects always occurred would be identified, as would the concentration below which effects never occurred. The concentrations in-between (where effects sometimes occurred or sometimes did not) would identify sites where a site-specific assessment would be needed.
- Fish tissue concentrations as a function of water concentrations: The empirical data from field studies that exist in the literature would be used to develop this bioaccumulation correlation on a global basis. Sites where measured fish tissue concentrations were different from the predicted concentrations, based on the amount of selenium in the water, would require a site-specific approach. If fish tissue – effects relationships are known for the species of concern, then sites could be further characterized as those with potentially higher than predicted effects or those with potentially lower effects.
- Modeling approach: The Aquatic Toxicity Model presented by George Bowie could be used to make *a priori* predictions of whether a concentration of selenium in water would result in effects to the fish. Site-specific input parameters include selenium input (amount, rate, and species), flow rates, water depth, and a few other hydrological parameters as well as food web species. The more site-specific data that are used in the model, the more likely is it to accurately predict effects.

## 4. *Analytical methods*

There are several methods for analyzing selenium in water, sediment, or tissue. No one method is the best for all media. Important considerations are desired minimum detection limits (ideally, should be ten-fold lower than the concentrations of interest), sample preparation requirements, and laboratory capabilities. Cost may be a factor as well. While methods are available that can achieve very low detection limits, many (if not most) contract laboratories are not set up to conduct these methods with appropriate accuracy or precision.

In addition to analytical methodology, appropriate sample collection and storage are required. Water samples should be acidified (with HCl) and kept cool; solid matrices should be kept frozen. Selenium may volatilize when a sample is heated and provide an incorrectly low value. Box core samplers are preferred



for sediment sampling as they preserve the depth structure of the sediment, allowing measurements to be made on the upper flocculent (organic) material versus the lower inorganic portions.

#### *5. Sufficiency versus toxicity*

Since selenium is a required micronutrient for both plants and animals, there is an exposure concentration below which insufficiency effects are seen and a different concentration above which toxicity occurs. The area in-between is the Optimal Effects Concentration. For algae, there is a wide sufficiency zone and the required amount may differ depending on the amount of selenium in the system from which the test colony was derived (due to adaptation to a higher selenium environment). Fish have at least a ten-fold difference between required and toxic amounts. In general, there does not appear to be any naturally deficient systems, with the exception of some lakes in the Laurentian Shield area in Canada that may be deficient for algae. Furthermore, on a practical basis, it does not appear that source reduction of site remediation would result in systems with insufficient selenium concentrations. However, this issue may be important in laboratory studies where appropriate minimum concentrations of selenium must be provided to maintain colonies of test species.

#### *6. Natural background*

On the national level, the median background concentration of selenium in aquatic systems is about 0.1 µg/L. However, there is disagreement about this value and about the variability and range of natural background concentrations. Areas of highly seleniferous soils in the western U.S. may have naturally higher background concentrations either through movement of soils into waterbodies or into groundwater. Methods are being developed for differentiating between natural and anthropogenic inputs of selenium into an aquatic system, but there remains a great deal of uncertainty in the follow-on calculation of what a resulting natural background concentration would be.

#### *7. Interactions with other stressors*

Selenium has the potential to interact with other metals, causing either greater or lesser responses than predicted from selenium alone. Furthermore, exposure to selenium may reduce an organisms' ability to respond to other environmental stresses, such as has been shown for fish similar to those found in Belews Lake that were exposed to cold temperatures during laboratory studies (Lemly, 1993c, 1996). These types of interactions might confound the global empirical dataset relating effects to selenium concentrations in water, sediment, or food. Examples where this may have occurred include interactions between vanadium and selenium in a field study of fish reproduction. On the other hand, another study showed that effects were correlated only with the selenium concentration in the food, and that additional elements had no discernible effects. The endpoint of interest also may affect the potential for interactive effects to occur.

#### IV. OBSERVER COMMENTS

At the end of each day of the meeting, Dr. Fairbrother opened the floor to comments from observers. These comments are summarized below. In addition, observer presentation materials may be found in Appendix F.

##### Peter Chapman, EVS Consultants

This observer (speaking on the first day of the meeting) noted that discussions to date had mostly focused on standing-water systems. In contrast, his interest is flowing cold-water streams, particularly in Alaska and southeast British Columbia, with inputs of selenium from hard-rock mining and coal mining. He pointed out that these systems are quite different in many aspects from the systems under discussion by the experts. To date, his group's studies have found no adverse effects in streams in British Columbia with concentrations of selenium as high as 65 µg/L. He urged the experts and EPA to consider three key points:

- Flowing-water systems are very different from standing-water systems; much higher concentrations can be tolerated without adverse effects.
- Site-specific factors are incredibly important.
- Not all waters or biota require the same level of protection.

##### Philip Dorn, Shell Development Company

This observer questioned the need for a revision of the national freshwater chronic water quality criterion for selenium. He argued that no compelling field effects have been demonstrated in waters with selenium levels below the existing 5 µg/L chronic criterion. In addition, analytical methods for compliance testing are limited below 10 µg/L. Finally, there is large uncertainty in making correlations at the national scale between water-column selenium concentrations, selenium concentrations in the food chain, and selenium concentrations in bird eggs. He urged EPA to move toward developing site-specific residue- or effects-based criteria. He also noted that the cost per pound to remove selenium from discharge is quite high and that the removal process generates a large volume of sludge which must be disposed of. He asked EPA to ensure that future regulations are developed upon fact-based science.

##### Rob Reash, American Electric Power

This observer made comments on behalf of the Utility Water Act Group (UWAG), an association of electric utility companies and trade associations. UWAG is interested in EPA's reevaluation of the freshwater chronic aquatic life criterion for selenium because selenium is a natural trace element in coal and

many of UWAG's members use coal as the primary fuel for electrical generation. The observer said that UWAG views a universal numeric chronic criterion for selenium as inappropriate. He urged EPA to consider the following issues:

- Stratification by waterbody type;
- Accurate accounting of site-specific factors affecting selenium toxicity; and
- Development of site-specific criteria technical guidance.

In addition, he offered the opinion that fish liver is a good tissue in which to measure residues if ovaries are unavailable; in his work, he has found that fish liver tissue mirrors water-column selenium concentrations.

Walter Kuit, Cominco, Ltd.

Speaking on behalf of Cominco Alaska, this observer said that selenium is a key issue at his company's Red Dog Mine in northern Alaska. An impending NPDES permit will lower the mine's selenium discharge limit to a level that the company cannot meet. He said that flowing streams should be considered separately from standing water and urged EPA to move quickly in developing site-specific guidance. He also asked EPA to provide preliminary guidance on possible changes in sampling procedures (e.g., implementation of fish ovary sampling), so that affected parties can start gathering relevant data as soon as possible.

William Wright, Montgomery Watson

This observer, an ecologist, is managing the Southeast Idaho Phosphate Resource Area Selenium Project. This project involves the evaluation of a 1,200-square-mile area containing 14 mines, where selenium is leaching from interburden waste shales. Receiving waters are typically intermittent tributaries of montane trout streams and are generally sulfate rich. Sampling to date has found water-column concentrations of selenium ranging from below detection limits to 2,000 ppb. Ninety percent of the selenium is in the selenate form. His group does not have definitive results yet, but has seen no adverse effects so far. Healthy populations have been found in areas with high concentrations of selenium. He echoed Peter Chapman's comments, saying that site-specificity is important, and beneficial use should be taken into account.

Chris Stanford, JD Consulting

This observer expressed the opinion that we have a long way to go in regard to quantifying the behavior and effects of selenium in the environment. He added that although revising the chronic criterion is a good goal, we do not yet have enough information to be able to develop a new nationwide criterion that is a definite improvement over the existing one. The solution to this in the short term, he said, is to develop site-specific standards, including guidance on sampling and data analysis and interpretations. In addition, he asked EPA to establish standards that can serve as guidance to contract laboratories.

John Goodrich-Mahoney, EPRI Environment Division

This observer said that EPRI will be coming out with their Selenium Aquatic Toxicity Model this fall. He invited experts and observers to be beta testers for the model. He can be contacted at <jmahoney@epri.com>. He added that EPRI encourages EPA to develop site-specific guidance and is willing to offer any assistance it can.

Judith Schofield, DynCorp

This observer stated that DynCorp has been providing support to EPA in the development of 1600-series analytical methods; she updated the attendees on the status of the two methods that apply to selenium. EPA Draft Method 1638 is an ICP-MS method with an estimated detection limit of 0.45 µg/L. EPA Draft Method 1639 is a gas furnace-AA method with an estimated detection limit of 0.3 µg/L. The methods and their detection limits will be tested in upcoming interlaboratory validation studies. Formal proposal of the methods will probably occur in early 1999. She added that EPA is also working on a streamlining rule, which is a performance-based measurement system approach to analytical methods.

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