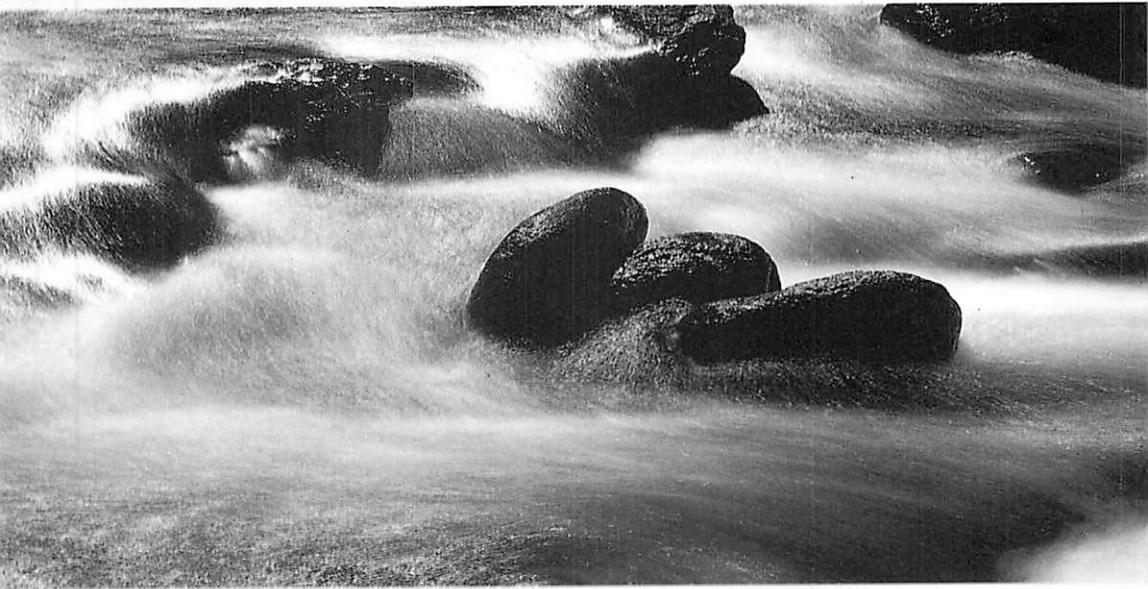


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SILVER

IN THE ENVIRONMENT:

TRANSPORT, FATE, AND EFFECTS



RESEARCH FINDINGS OF THE ARGENTUM INTERNATIONAL CONFERENCE SERIES 1993-2000

Edited by
Anders W. Andren
Thomas W. Bober

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Research Findings of the Argentum International Conference Series, 1993-2000

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Environmental Quality Through Science®

Silver in the Environment: Transport, Fate, and Effects

Research Findings of the Argentum International Conference Series, 1993-2000

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Introduction

Significant advances have been made in the understanding of the environmental chemistry, toxicology, and biological behavior of silver, Ag(I), over the past decade. The scientific community has learned much new information about sources, concentration levels in natural waters and biota, physico-chemical forms, adsorption and desorption reactions, toxicology, bioaccumulation, influence of ligands, and transport and fate characteristics of silver.

A rather unique example of multidisciplinary collaboration between academia, government, industry, and private research institutions has taken place during this time. The most recent findings from these international collaborative efforts now promise to revolutionize scientific thinking not only with regard to silver behavior, but for other metals in the environment as well.

Concerning silver, these advances have, in large measure, been stimulated by two factors. The first is the application of "clean techniques" to the analytical determination of silver in natural waters. The second is the infusion of research funding to academia since 1989 from a coalition of companies in the photographic industry. Data have been shared and discussed using an interdisciplinary approach via several government-sponsored and private meeting formats, as well as the Argentum series of six conferences. Results have been made public through individually published peer-reviewed papers and the Argentum proceedings. This book presents a synthesis of the progress on silver research in terms of its environmental chemistry, effects to biota, biological processes, risk analyses, and current regulatory practices.

It is now well established that one important source of silver to the environment is effluent from publicly owned treatment works (POTWs). Prior to recent work, available data indicated that effluent-receiving waters might be near the provisional silver criteria (based on ionic silver, Ag⁺) for protection of aquatic life. This would mean that POTWs might have to refuse acceptance of effluents from photo processors and other commercial users of silver, even though most had already implemented advanced recovery processes that were approaching the upper practical limit of technical capability. In addition, information became available that indicated errors in previously reported ambient silver concentration data in natural waters. New data also became available on the physico-chemical forms of silver in effluents from POTWs. Because of the new data and additional uncertainties, questions also arose on the interpretation of bioassay data for silver. Consequently, one practical aspect of the new data on the levels and the forms of silver in natural waters was that many researchers and government regulators began to reevaluate the basis upon which federal and state effluent criteria had been established. Faced with these and other challenges, additional research was needed to further improve our understanding of silver in the environment, including the elucidation of biochemical and chemical mechanisms.

The Expanded Silver Research Program

Some silver toxicological research had been performed during the latter half of the 20th century by universities, government agencies, and private institutions, but compared to other metals was rather limited. With increased focus on environmental fate of metals in the mid-1980s by government agencies, expanded research efforts began to be sponsored by industry. Initially these academic contracts and grants were funded in the late 1980s by Eastman Kodak Company, then later through the Photographic and Imaging Manufacturer's Association (PIMA), previously known as the National Association of Photographic Manufacturers (NAPM), now known as I3A (International Imaging Industry Association).

As the environmental research program on silver acquired more momentum and it became clear that the new research efforts were breaking previously untrodden ground, additional funding sources surfaced. At the University of Wisconsin-Madison, WI, USA, industrial funding for silver was used to expand ongoing efforts with other metals within the Wisconsin Department of Natural Resources. The U.S. Environmental Protection Agency (USEPA) laboratories at Duluth, MN, USA, and Narragansett, RI, USA, as well as the Florida Department of Environmental Protection, supported new studies on silver. The Canadian government provided matching funds to Canadian universities that had received research grants from industrial sources. Independently funded research in areas ranging from biogeochemistry to fish physiology to plant uptake also has been performed by numerous universities, industries, and research institutions ranging from Scandinavia to the UK to Central Europe to North America. Most recently funding has been awarded to Canadian and U.S. researchers through the Water Environment Research Foundation (WERF).

The Argentum Conference Series

The Argentum series of international silver conferences was first conceived in 1992 when the editors of this publication traveled together to Scandinavia, to discuss the implications of silver speciation in regard to some proposed silver discharge rules being considered by the governments of Sweden and Norway. Recent research findings at that time had shown that discrepancies clearly existed between many prior laboratory toxicological studies on silver and similar studies performed in the field under natural conditions. Data on the association of Ag(I) with natural and synthetic sediments indicated conclusively that the metal was highly reactive with respect to solids and numerous ligands. In other words, preliminary data indicated that Ag(I) in natural waters could not exist as Ag^+ in significant concentrations. As data from the work on other metals have clearly shown, the physico-chemical form being tested makes a huge difference in the observed uptake and toxicity. For example, complexed silver species apparently could be more than 3 to 4 orders of magnitude less acutely toxic to aquatic organisms than fully dissociated ionic silver. At the same time, there were observed

instances of bioaccumulation of silver in some biota. Thus, silver exhibited some quite unique characteristics, yet had not been as extensively studied as other metals such as copper, zinc, or mercury.

Following the discussions in Scandinavia, it was agreed that the questions raised and the interesting possibilities for basic silver research should be brought together in a multidisciplinary conference format, which was titled "Argentum." With the agreement of sponsors, it was decided that the Argentum project would be headquartered at the University of Wisconsin-Madison, WI, USA, where the concept of cooperative research between various scientific disciplines has been an established, long-standing principle, particularly in the area of environmental studies. The conferences would be held once a year initially, with the findings of independent participating scientists reported in official conference proceedings. The University of Wisconsin Sea Grant Institute would be the sponsoring campus unit.

Five conferences (Argentum I through V) were held in Madison; Washington, DC, USA; and Hamilton, ON, Canada during the years 1993-1997 using a conventional format of platform and poster presentations for scientific papers, followed by limited panel discussions of the findings at the end of each conference. Proceedings were published following each meeting (see www.seagrants.wisc.edu/argentum/index.html). These sessions were invaluable in presenting the varied findings of the participants from the perspectives of their own disciplines. They generated many useful comments and observations from other participants that helped plan and streamline future studies. In particular, certain variables that might have been considered unimportant within one discipline, and therefore neglected, were shown by scientists in other disciplines to be important and thus were included in the studies. This minimized the chances that results might be adversely criticized as lacking in scope, thereby saving considerable time, money, and effort.

The Argentum VI Workshop

By 1998, much new information had evolved. The application of clean sampling techniques and the ability to determine several physico-chemical forms of silver in natural waters had enabled the scientific community to reexamine the environmental chemistry of silver, especially with respect to complexation. It was evident the composite findings should be critically examined and summarized, and certain issues needed to be put in proper perspective with respect to their scientific and regulatory implications.

The previous Argentum panel discussions had been useful, but many participants agreed that insufficient time had been available to fully debate all issues. A different format such as a workshop was needed where salient points could be discussed and argued by experts until consensus was reached. Following the

excellent suggestion of Professor James R. Kramer of McMaster University, it was decided to use a modified version of the format successfully employed by the Dahlem Konferenzen in Germany. In this format, invited scientists who were experts in their fields would be assigned to small groups wherein one scientific discipline would predominate but representatives of other disciplines were also present. All groups would be guided by several overarching questions. Each group would originally be given specific questions as guidelines for debate; however, each group was free to develop its own agenda and questions as appropriate.

It was decided to form five groups of scientists. Each group would include a moderator to serve as presiding officer, keeping the group focused and on schedule and issuing writing assignments to group members, and a rapporteur serving as scribe to take notes during the discussions and collate the written output into final form. Periodically, the moderators would present their group's interim findings to the entire assembly, for the edification of all and to receive suggestions and criticisms. The first four groups would meet individually and also jointly once (due to limited time) with each of the other three groups to debate points of common interest, in a "round-robin" arrangement. Members of the fifth group would be free to circulate as observers among the first four groups.

The overarching questions presented to all participants in advance of the workshop, held in Madison in August 1999, were as follows:

- 1) What chemical, biological, and biochemical information is available (and needed) to establish the risk and to determine logical regulations for silver?
- 2) What level of toxicity or environmental risk is associated with environmentally realistic levels and forms of silver in natural waters?
- 3) What is (free) ionic silver, and how can (free) ionic silver test data be used in the development of regulations and regulatory policy for silver in natural waters?

The groups were chosen and assigned lead-off questions as follows:

Group A: Environmental chemistry

In the environment, what are the predominant forms of silver and their concentrations? How do these forms and concentrations vary in different media (i.e., marine, freshwater, sediment) and location (i.e., urban, rural, POTW)? How does one analyze for these forms in different media? What analytical techniques should be specified for regulatory use? What is the change in bound and complexed silver when an effluent is diluted with receiving water? Is oxidation of sediments (containing silver sulfide) a significant source of dissolved silver? Are there sediment biogeochemical processes that would mobilize silver? How important is chloride in the speciation of silver in marine waters?

Group B: Biological effects

What are the acute and chronic levels and the effects of silver in marine/freshwater aquatic and sediment-dwelling organisms, and aquatic/terrestrial plants and animals? What are the more sensitive biological species? What are the data distributions from various studies and how do they correlate? Do laboratory experiments support and agree with field studies? How do these data relate to the chemical and physical species of silver? Consider among other factors (a) background, (b) historic deposition sites, and (c) POTWs.

Group C: Biological processes

What are the routes of Ag exposure for different organisms in the food chain? How are the forms (and their concentrations) of silver involved in and/or modified in the exposure processes (i.e., enzyme transport, depuration, etc.)? What solid chemical forms of Ag(I) should be used in bioassay studies (i.e., AgNO₃, AgCl, AgDOC, etc.)? Does bioaccumulation occur, and what is the ultimate fate of silver in these processes? Are there biogeochemical processes that would mobilize bound silver?

Group D: Risk analysis

What is the risk process related to silver? How widely applicable is the Biotic Ligand Model (BLM) for risk assessment? What other models and processes need to be considered? What are the risk aspects of colloidal silver and other silver species? What are the pathways to proceed from this risk analysis to regulation scenarios?

Group E: Application to regulations

How can the information generated from these discussions be integrated into practical regulations? What are the present and proposed federal, foreign, and state regulations? Consider water, sediment, and sludge. What is the best approach to evaluate the large differences in the silver concentration and effects data to obtain optimum regulations? How are missing data and uncertainty handled? How should analytical protocols (i.e., "total recoverable" versus filtered (size) versus specific species) for silver be specified in the requirements, given the different physical and chemical species that need to be considered? Are the present protocols such as water-effect ratio and the use of partition coefficients (size?) adequate to establish site-specific regulations, given the ultra-trace concentrations and different forms of silver?

The following overall conclusions were reached at the workshop:

- Virtually all silver in oxidation state (I) in the aquatic environment is complexed, much of it to reduced sulfur. Given the protective effects of these and other ligands, and the relatively low acute toxicity of silver to fish, it is concluded that silver rarely presents an acute threat.

- Inorganic reduced sulfur forms appear to exist for extended periods of time in oxic as well as anoxic waters. This, coupled with the known existence of organic forms in anoxic waters, makes sulfide ubiquitous in nearly all natural waters.
- Field observations of chronic toxicity to bivalves (mussels) have been made at a few sites with elevated sedimentary silver concentrations. In-situ observations of chronic toxicity for other aquatic organisms are not well documented in terms of cause and effect.
- Chronic toxicity appears to occur via mechanisms that might not be apparent in short-term laboratory tests.
- Since chronic toxicity probably occurs via different mechanisms than those for acute toxicity, the acute-to-chronic ratio approach is invalid.
- Laboratory toxicological procedures which historically may have utilized free ionic silver (Ag^+), usually in the form of silver nitrate, and generally with sulfide and other complexing components such as dissolved organic matter (DOM) absent, need to be critically reexamined in terms of their relationship to the real-world natural environment. Measurement of silver and other chemical concentrations needs to be performed by actual chemical analysis during the course of these studies, rather than relying on calculated nominal concentrations based on the amounts originally added.

Following the conclusion of debate, each rapporteur collated the written product and generated the group's chapter with assistance from the moderators and additional author input. All authors then had opportunities to critique all chapters and supply missing information during several edits, via a password-protected Internet website. Editors, moderators, rapporteurs, and several senior authors reassembled in February 2000 to resolve remaining text questions, with the results again posted to the website for further editing. Peer review and final editing produced the following compilation.

Practical Results of the Research Program

As previously reported at five Argentum conferences (Argentum I through V, see www.seagrant.wisc.edu/argentum/index.html), and in papers published in *Environmental Toxicology and Chemistry* (Volume 17:537-649 and Volume 18:1-108) and other journals, the implementation of clean, sensitive field and laboratory analytical methods has shown that silver levels in natural waters are typically very low, in the ng/L or picomolar range. POTWs can provide efficient removal of silver, so that levels in effluents studied to date are typically 10 nanomolar or less, even in plants receiving high loadings of silver. Subsequently, levels in effluent-receiving streams rapidly fall to subnanomolar and then to picomolar ranges. The low levels reflect, in part, the reactivity of Ag^+ with particles and colloids, resulting in efficient particle scavenging. Significantly, the binding of the Ag^+ ion to dissolved organic carbon (DOC) and other ligands (reduced sulfur compounds in particular),

suggests that only a transient fraction of aqueous silver is likely to exist as the Ag^+ ion in the natural environment. Only recently has the potential influence of sulfide clusters on the chemistry of metals in natural waters begun to be appreciated.

Research on fish and other aquatic organisms has provided substantial information on Ag(I) toxicity, especially acute toxicity and the mechanisms of toxicity. This research has shown that binding to DOC and other waterborne ligands reduces the Ag(I) toxicity to fish, mainly because very little "free" Ag^+ can exist in the presence of particles, colloids, and ligands. It follows that any toxicity experiment must be done under carefully controlled conditions where Ag(I) speciation is monitored closely. The ratio of $\text{Ag}_{\text{tot}}/\text{Ag}^+$ also varies considerably in nature, making it very difficult to interpret "old" bioassay data. This protective effect must be interpreted cautiously, however, since new data indicate that subacute effects in lower organisms, such as planktonic herbivores, may occur at considerably lower levels than those that have been observed in fish.

As the reader will find, recent research has resolved several important questions, but additional challenges remain before reaching a full understanding of the environmental chemistry, toxicology, and behavior of silver. Environmental risk assessment for aquatic silver must acknowledge that most prior toxicological data most likely have been based on laboratory exposure of aquatic organisms to an excessive and often unknown quantity of the Ag^+ ion, which is shown to be almost nonexistent under practical natural conditions. Thus, the scientific community needs to be able to measure or model the speciation of silver, including its association with all environmentally important ligands. In particular, the factors and mechanisms controlling the binding of Ag^+ to natural organic ligands, colloids, and particles still are not well understood. Thermodynamic predictions of the physicochemical forms of silver in natural waters do not work well because of the existence of nonequilibrium conditions, noncharacterized ligands and colloids, and noncharacterized binding groups on particles. The effect of redox conditions on Ag(I) or Ag(0) speciation conversions also is not thoroughly studied. The recent findings that sulfide clusters may exist for extended periods in oxic as well as anoxic waters are particularly significant. Similarly, further dose-response information for aquatic organisms is needed, especially with respect to possible chronic or subacute effects. While important linkages between the speciation and toxicology of silver have been identified, information needed for quantitative risk assessments remains incomplete. It is expected that this will be a major area for future advances in the environmental chemistry and biological effects of silver as well as other metals.

Admirable strides have been made over the past decade in understanding not only the behavior of silver but metal speciation in general, particularly the ubiquitous influence of factors such as sulfide and DOM under typical environmental conditions, in oxic as well as anoxic waters. However, many past conclusions regarding metal behavior and toxicity, as well as laboratory toxicological practices, now need to be reexamined by the regulatory and research communities in light of these new

findings. The editors hope that the following synthesis will provide a rigorous update of available information on the behavior of silver in the environment and will serve to stimulate future research efforts.

—*Anders W. Andren, Thomas W. Bober*

CHAPTER 1

Environmental Chemistry of Silver

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Introduction

This chapter provides a summary of current knowledge on the abundance, distribution, speciation, potential bioaccumulation, and analysis of silver in natural waters and sediments. Of primary importance to the behavior and fate of silver is the recent discovery that significant amounts of dissolved reduced sulfur are very common in surface waters. Consequently, since it is very reactive with reduced sulfur, silver probably occurs almost entirely bound to sulfur species. Future research on the bioavailability and bioreactivity of silver needs to take into account this central fact.

Abundance of Silver in Aqueous Systems

Recent studies on the environmental chemistry of silver have provided consensus values for concentrations of silver in natural waters that are accepted as reliable by the trace element community (Table 1-1). This table includes virtually all known current data, and many environments and fractions remain unmeasured. Because of contamination artifacts, most—if not all—of the aqueous silver data published before the mid-1980s must be considered suspect. Application of new analytical detection technologies, clean techniques, and, most importantly, the recognition that silver levels in the environment could be much lower than previously thought have driven the recent advances.

Total levels in the water column

Many important conclusions can be drawn from the compilation of silver levels (shown in Table 1-1): (1) silver is found in the environment in aqueous solution at picomolar to low nanomolar levels (1 to low 100s of ng/L); (2) even in highly impacted systems, total silver levels rarely exceed a few nM; and (3) background levels of total silver in seawater range from 1 to 20 pM and in freshwater 5 to 50 pM.

Table 1-1 Concentrations of silver (pM) in different systems (multiply values by 0.1 for ng/L). Proximity to point source discharges is relative to other sites of the same type, e.g., coastal waters near a discharge point are close to such a point relative to other coastal waters.

System	Near point source discharge		Far from point source discharge	
	Filtered (0.2 - 0.45 μm)	Unfiltered	Filtered (0.2 - 0.45 μm)	Unfiltered
Open ocean (Depth < 1 km)			0.4 - 10 ^A	< 0.24 - 5.6 ^{B,C,D}
Open ocean (Depth > 1 km)			10 - 23 ^A	1.9 - 54 ^{B,C,D}
Estuaries and bays	3.2 - 307 ^{E,F,G,H}	8.1 - 1000 ^{D,E,F}		5.8 ^D
Coastal waters	39 ^G		3 - 11 ^{F,G}	4 - 17 ^G
Rivers	< 0.1 - 2900 ^{E,I,K,L,M}	< 0.1 - 4600 ^{E,I,K,L,M}	< 0.1 - 480 ^{I,O}	< 0.1 - 1400 ^{E,I}
Laurentian Great Lakes				2 - 5 ^J
Other lakes			40 ^L	67 ^L
A Martin et al. 1983	F Miller and Bruland 1995		J Shafer 1995	
B Murozumi 1981	G Saftudo-Wilhelmy and Flegal 1992		K Shafer et al. 1998	
C Flegal et al. 1995	H Benoit et al. 1994		L Kramer et al. 1999	
D Bloom and Crocelsius 1984	I Benoit 1994		M Benoit and Rozan 1999	
E Wen, Santschi et al. 1997				

Effluents from publicly owned treatment works (POTWs) are recognized as the principal source of silver in riverine systems. Silver in this effluent comes mainly from pretreated industrial wastes discharged to sanitary sewer systems (e.g., from the photographic industry). Silver levels in POTW effluents may be up to 100 nM, and low nM levels of silver can be observed in the mixing zone of POTW effluents. However, levels in the receiving stream are reduced rapidly by mixing and removal processes. Despite elevated levels of silver in many tributaries impacted by POTWs or other anthropogenic sources, atmospheric input is the dominant source of silver in the Great Lakes and the open ocean.

The very low natural background levels of silver make it a useful tracer for contamination (e.g., tracing POTW effluents). Amounts of silver in POTW effluents can be 3 orders of magnitude higher than background levels in streams.

Particulate silver

Silver is an extremely particle-reactive metal. Distribution coefficients (K_d) based on filtrate and particulate silver concentrations (Table 1-2) are typically $10^{4.5}$ to 10^6 , exceeded only by lead among the commonly measured metals. As a consequence, the residence time of silver in most aquatic systems is short compared with other metals. Silver is quickly scavenged from the water column and incorporated into sediments. Resuspension of sediments will, however, continue to resupply total silver to the overlying waters. Distribution coefficients shown in Table 1-2 are remarkably similar considering the differences in silver concentrations, chloride concentrations, and other variables among the systems represented. In most aqueous environments, especially rivers, particulate (> 0.4 μm) phases dominate

Table 1-2 Distribution coefficients and concentrations (on a per mass particle basis) for silver in different systems in the U.S.

System	Size cutoff (μm)	$\log K_d$ (L/kg)	Silver per mass particle (nmol/g)	Reference
Texas estuaries (Gulf Coast)	0.4	4 - 6	0.3 - 30	1
Galveston Bay, TX	0.45	4.6 - 5.5	0.5 - 2.8	2
Texas rivers	0.45	4.4 - 6.6	0.1 - 50	2
Texas rivers	0.1	4.5 - 6.6	N/A	2
Cedar Creek, WI	0.4	4.9 - 5.4	1 - 2.3	3
Quinnipiac River, CT	0.4	4.9 - 6.3	0.5 - 32	4
Naugatuck River, CT	0.4	5.1 - 6.3	0.5 - 23	4
Hammonasset River, CT	0.4	5.2 - 6.3	0.7 - 5.5	4
Pawcatuck River, CT	0.4	4.5 - 5.9	0.2 - 2.8	4

A Benoit et al. 1994

B Wen, Santschi et al. 1997

C Shafer et al. 1998

D Benoit and Rozan 1999

the size spectrum of total silver. Only in systems where particle concentrations tend to be very low, such as the open ocean, is relatively little silver associated with particles. Background levels of silver in suspended particles and sediments, expressed on a mass basis, fall in the range of 0.2 to 2 nmol/g. The few measurements of relatively clean phytoplankton show silver levels similar to those of suspended particles—possibly indicating a strong, nonspecific, passive association of silver with most surfaces.

Surface complexation of silver is not limited to macroparticles (i.e., $> 0.4 \mu\text{m}$). Much of the silver that passes through conventional filters is actually associated with colloids. Distribution coefficients of silver to colloids are surprisingly similar to those for macroparticles. Wen, Santschi et al. (1997) and Wen, Tang et al. (1997) showed that virtually all silver in ultrafiltration (UF) retentates from the Trinity River and Galveston Bay, TX, USA, eluted from a high-performance liquid chromatography (HPLC) column at the same time as an organic species containing a thiol functionality. The organic species and silver eluted in a hydrophobic fraction. These results point toward the potential existence of strong, thiol-based, colloidal silver complexers in natural waters.

The efficient removal of silver from waste streams in POTWs ($> 94\%$) is another outcome of the strong binding affinity of silver for particle and colloid surfaces (Shafer et al. 1998). An examination of a large suite of metals demonstrated that metal removal efficiency in POTWs is directly related to the K_d . Silver exhibited the highest K_d and removal efficiency in the group of 14 metals studied. Silver removal efficiency in POTWs would have been even greater were it not for the strong affinity of silver for colloids, which are abundant in the sewage.

Silver in sediments

Table 1-3 is a partial survey of the literature on silver in sediments. Note that many of these studies were conducted intentionally in areas known to be contaminated with silver, so results are biased toward higher concentration levels. In general, these studies show that silver levels in uncontaminated fine-grained sediments are typically similar to those for average crustal abundance (0.05 $\mu\text{g/g}$). (Lower concentrations can occur in sediments that contain significant amounts of sand.) Values can range upward from this baseline to tens or even hundreds of $\mu\text{g/g}$. Areal distribution patterns generally reflect proximity to known sources (mining sites, urban centers, sewage treatment plant outfalls). In many cases where variations were measured with depth, silver levels increased from near baseline values in the past to subsurface maxima, and have declined in recent years.

Table 1-3 Silver in sediments

Location	Environment	Ag ($\mu\text{g/g}$)	Reference
Maratoto (New Zealand)	stream	0.75 - 7.0	A
South Platte River (WY, NB, CO)	river	0.2 - 5.1	B
Hanover Pond (CT)	river impoundment	9.4 - 282	C
St. Lawrence River (Canada)	river - estuary	0.04 - 1.1	D
Quinnipiac River Marsh (CT)	salt marsh	0.1 - 31.6	E, F
Puget Sound (WA)	estuary	0.015 - 0.71	G
Long Island Sound (CT - NY)	estuary	0.006 - 25	H
San Francisco Bay (CA)	estuary	0.09 - 1.10	I
San Francisco Bay (CA)	estuary	0.04 - 0.83	J
Jordan Cove (CT)	estuary	0.06 - 1.62	K
New Haven Harbor (CT)	estuary	0.06 - 33.9	C
Brisbane River (Australia)	mangrove swamp	< 0.6 - 2.8	L
MA and Cape Cod Bays (USA)	coastal	0.03 - 6.7	M

A Ward et al. 1977

B Heiny and Tate 1997

C Benoit and Rozan (submitted)

D Gobeil 1999

E Rozan and Benoit 1999

F Rozan and Hunter 2001

G Bloom and Crecelius 1987

H Robertson et al. 1991

I Luoma et al. 1995

J Thompson et al. 1999

K Benoit et al. 1999

L Mackey and Hodgkinson 1995

M Ravizza and Bothner 1996

Speciation

General comments

Silver is a class B, soft metal¹ that preferentially forms complexes with sulfur(II-) containing ligands. Silver can be expected to bind most strongly to S-containing and I-containing ligands and more weakly to N-containing and O-containing ones. The importance of this is highlighted in Table 1-4, which lists the strength of silver complexes with sulfur and other common ligands.

Silver forms complexes with reduced sulfur about 10^{10} times stronger than with N or Cl. Furthermore, silver forms stronger complexes with reduced sulfur than any other common metal, with the possible exception of Hg.

Reduced sulfur compounds are now known to be kinetically metastable in oxic fresh and marine waters at nanomolar concentrations (Luther and Tsamakis 1989; Radford-Knoery and Cutter 1994; Benoit and Rozan 1997; Theberge et al. 1997; Kramer et al. 1999; Rozan et al. 1999)². Nanomolar amounts of reduced sulfur have been found in virtually all surface waters measured with suitably sensitive methods (see section: *Levels of sulfide in the water column*). The total number of sites tested is not large, but they represent a wide variety of water bodies. Furthermore, 3 independent analytical methods have been used: gas chromatography, voltammetry, and classical colorimetry (Cline 1969; Radford-Knoery and Cutter 1994; Rozan et al. 1999; Rozan, Lassman et al. 2000). The specific sets of reduced sulfur compounds measured by each of these methods are not identical, and true comparison of these methods on identical samples has not been performed. Nevertheless, the agreement of results derived from several independent analytical techniques carried out by separate investigators rules out the possibility

Table 1-4 Formation constants for silver complexes (Ag:L = 1:1) with various ligands

Silver Complexes	log K	Reference
Inorganic sulfides	14 – 21	A, B
Organic sulfides (thiols)	12 – 15	C, D
Thiosulfate	8.2	C
N (NH ₃ and amino)	3 – 6	C
Γ	6.6	C
Cl ⁻	3	C
O (carboxylates)	< 2	C

A Schwarzenbach and Widmer 1966

B Cloke 1963

C Smith and Martell 1997

D Adams and Kramer 1998a

Note: Constants are conditional, usually because of differences in ionic strength. Corrections to actual constants generally should result in less than one order of magnitude change. Complexes with reduced sulfur compounds are many orders of magnitude more stable than with other ligands, and no realistic background chemistry (pH and competing cations) would make silver-reduced sulfur complexes unimportant.

¹ Class A (hard) metals typically form higher (> +2) oxidation states and bind preferentially to oxygen and nitrogen. Class B (soft) metals typically form lower oxidation states (+1, +2) and bind preferentially to sulfur.

² The exact identity of the reduced sulfur forms present in oxic freshwaters remains a subject of investigation. Consequently, the nomenclature used to refer to these forms is similarly inexact. Terms like "S(II-)," "sulfides," and "reduced sulfur compounds" all refer in a general way to sulfur in the (II-) oxidation state occurring as an inorganic or organic metal cluster, sorbed on or in an organic or inorganic surface and/or mineral particle, or present as a functional group in DOM.

that the measured reduced sulfur compounds are a measurement artifact. Simple free sulfides (H_2S , HS^- , S^{2-}) in solution are normally oxidized rapidly in the presence of O_2 . The observed persistence of S(II-) in oxygenated waters may be due to stabilization of reduced sulfur by strong complexation to metals (e.g., Zn, see below). These S(II-) species appear to be stable for periods of up to a month, although some loss does occur during that time (Luther and Tsamakis 1989; Rozan et al. 1999).

Recent studies have examined the concentrations of reduced sulfur compounds that react to form methylene blue (e.g., that measured by the Cline test [Cline 1969]) in water samples in relation to silver concentrations (Table 1-5)³. Measurements show that, in waters ranging from pristine streams to POTW outfalls, the reactive S(II-) concentrations typically exceed silver by 3 orders of magnitude (Kramer et al. 1999; Manolopoulos 2000; Rozan and Luther 2001; Martin M. Shafer, University of Wisconsin, personal communication). Consequently, silver should always be bound to S(II-) , assuming no kinetic limitations and the unlikely occurrence of other high stability or very abundant ligands. This new finding contradicts the earlier belief that complexes with chloride (sea water) or natural organic matter (NOM) (fresh water) should dominate silver speciation. This result also strongly indicates that free ionic (hydrated) silver occurs in natural waters only at concentrations that are too low to be measured by even the most sensitive of current analytical techniques. Thus, if dissolved silver is available to aquatic organisms, it is necessary to consider forms other than Ag^+ as the one that are biologically available (see section: *Biological implications*). The extremely low levels of Ag^+ that are present as a tiny fraction of total dissolved silver can only be derived by calculation and serve no important function in the environment.

The virtual lack of free silver in the environment does not necessarily mean that aquatic organisms cannot bioaccumulate silver. Indeed, studies have documented silver uptake in several taxa, notably bivalves (Riedel et al. 1995; Wang et al. 1996). Reduced-sulfur-containing ligands are common in biological cells, and at least one laboratory study shows that thiols are capable of mobilizing silver from inorganic sulfides (Adams and Kramer 1998b). The kinetics of ligand exchange for Ag(I) are rapid and can occur within a time frame of milliseconds (Alberto et al. 1996; Bell and Kramer 1999). Therefore, it can be concluded that organic S(II-) ligands in cells may also be capable of extracting silver from silver- S(II-) species. Consideration of the speciation of the silver- S(II-) compounds will be critical to understanding bioaccumulation and chronic toxicity of silver to aquatic organisms.

Details of silver-sulfide interactions

The widespread occurrence of reduced sulfur in surface waters, and the extraordinarily high affinity of silver for reduced sulfur, imply that most aquatic silver

³ Since this reduced sulfur pool is operationally defined as that which can react after treatment with strong acid under the conditions employed in the Cline test, it is henceforth refer to as "acid-reactive sulfide" (ARS).

Table 1-5 Summary of dissolved silver and acid-reactive sulfide (ARS) measurements for a range of surface water bodies and POTWs (ARS is typically 3 orders of magnitude greater than silver)

Ag (nM)	MBMS (nM)	log ([MBMS]/[Ag])	Site	Comment	Reference
0.042	485	4.1	Cobalt, ON, Canada	river, maximum	A
< 0.003	18	> 3.8	Cobalt, ON, Canada	river, minimum	A
0.29	< 1	< 0.5	Cobalt, ON, Canada	river	A
< 0.003	< 1	—	Cobalt, ON, Canada	tailings	A
0.49	< 1	< 0.3	Cobalt, ON, Canada	seep, maximum	A
0.18	240	3.1	Cobalt, ON, Canada	seep, minimum	A
< 0.003	< 1 - 190	—	Cobalt, ON, Canada	background	A
0.79	250	2.5	Dundas, ON, Canada, POTW	secondary clarifier	B
0.78	230	2.5	Dundas, ON, Canada, POTW	effluent	B
0.51	155	2.5	Dundas, ON, Canada, POTW	250 m downstream	B
0.45	137	2.5	Dundas, ON, Canada, POTW	500 m downstream	B
—	10 - 20	—	Sava River, Slovenia		C
—	30 - 60	—	Fort Erie, ON, Canada		C
—	30 - 60	—	Niagara on the Lake, ON, Canada		C
0.024 - 0.057	34 - 78	3.2	Hammonasset River, CT, USA	no POTW	D
0.019 - 0.045	16 - 50	3.1	Naugatuck River, CT, USA	above POTW	D
0.21 - 0.80	59 - 104	2.3	Naugatuck River, CT, USA	below POTW	D
0.037 - 0.095	44 - 85	3.1	Quinnipiac River, CT, USA	above POTW	D
0.18 - 0.28	59 - 100	2.5	Quinnipiac River, CT, USA	below POTW	D
0.007 - 0.033	31 - 112	3.6	Pawcatuck River, RI, USA	below wetlands	D
0.005 - 0.02	1.2 - 6.1	2.4	9 lakes, WI, MN, MI, USA		E
0.005 - 0.04	1.1 - 19	2.7	9 rivers, WI, MN, MI, USA		E
0.4 - 2	51 - 110	1.9	6 POTWs, WI, USA		E

MBMS = methylene blue measurable sulfide

A Kramer et al. 1999

B Adams and Kramer 1999b

C Manolopoulos 2000

D Rozan and Hunter 2001

E Shafer unpublished data

occurs bound to sulfur. Nevertheless, specific silver-sulfur species have not been measured in nature, in part because silver generally occurs at such low levels that it is difficult to detect, let alone speciate. Laboratory studies conducted at elevated concentrations may shed light on Ag-S interactions in natural waters. In particular, there is growing evidence that several metals occur in nature as clusters with sulfide. Iron sulfide clusters are known to be important multipurpose biochemical building blocks (Beinert et al. 1997). Other metals, such as Mn(II, III), Cu(I, II), Ni(II), and even Ag(I) have been associated with these "bioclusters" in laboratory experiments.

Zn-S cluster

Recent work by Luther et al. (1999) shows that in the laboratory, zinc sulfide solutions form soluble zinc sulfide cluster molecules and ions at pH values ≥ 5 . Titrations of aqueous Zn(II) with bisulfide indicate that these sulfide clusters can be formed at concentrations of 20 μM (or less) of metal and bisulfide. Precipitation does not occur based on voltammetric measurements at the mercury electrode, UV-visible (UV-VIS) spectroscopic data, and mass spectrometric tests. UV-VIS data and filtration experiments indicate that the material passes through 1000 dalton ultrafilters, so it is truly dissolved, not colloidal. The complexes form rapidly ($k_f > 10^8 \text{ M}^{-1}\text{s}^{-1}$) and are kinetically inert to dissociation and thermodynamically strong. Evidence from a variety of measurement techniques and theoretical calculations support the likelihood that negatively charged $\text{Zn}_4\text{S}_4^{6-}$ is the major species (more specifically, the hydrated form $[\text{Zn}_4\text{S}_6(\text{H}_2\text{O})_4]^{4-}$). This cluster can be a precursor to mineral formation or alternatively can form as a consequence of mineral dissolution. It now has been shown that both ZnS and CuS clusters exist in natural freshwaters (Rozan et al. 2000), as well as in the laboratory. As has been demonstrated in kinetic studies (Vazquez et al. 1989), these clusters resist oxidation by O_2 , and they also may be less available than free Zn^{2+} for uptake by organisms. Voltammetric experiments indicate neutral and anionic clusters exist for zinc and agree with ion chromatographic results for zinc in the sulfidic waters of the Black Sea (Landing and Lewis 1991).

Relationship of zinc sulfide clusters with silver

Freshwater and open ocean environments that contain fully saturated oxygen levels (Theberge and Luther 1997; Theberge et al. 1997; Rozan et al. 1999) give similar metal sulfide data as discussed above for laboratory solutions; i.e., sulfide is complexed with metals like zinc and can be released with 1 M acid. Based on a limited number of studies covering a diversity of environments, the occurrence of sulfide in oxic waters appears to be very common (Luther and Tsamakis 1989; Kuwabara and Luther 1993; Radford-Knoery and Cutter 1994; Theberge et al. 1997; Kramer et al. 1999; Rozan et al. 1999), and Ag^+ is a candidate for the formation of inert sulfide complexes. Because of the difficulty of analyzing speciation at low concentrations, so far there have been no efforts to measure silver

sulfide clusters in nature, though direct evidence exists for real-world occurrence of S(II-) clusters with the more abundant Fe, Cu, and Zn. In a laboratory study, Rozan and Luther (2001) have found that the sulfide bound to Ag⁺ cannot be detected electrochemically. Acid-base titrations show that silver-sulfide is not protonated until a pH of approximately 2, indicating that the possible presence of silver sulfide clusters is, very likely, similar to Zn. In this case, they should not be readily toxic to organisms. From their titration data, Rozan and Luther also determined the thermodynamic stability constants for the following silver sulfide species:



Mass spectrometry (MS) has not been performed to date on silver sulfide solutions to determine the stoichiometry of these clusters more accurately.

In order to understand possible reactivity of zinc sulfide with silver, the complexes of similar stoichiometry were compared. The AgS⁻ constant (Rozan and Luther 2001) is log K = 22.8, and the ZnS constant (Luther et al. 1996) is log K = 11.7. These data demonstrate that Ag⁺ should replace Zn²⁺ in zinc sulfide complexes in solution. This has been shown to occur for Ag(I) replacing Fe(II), Zn(II), Pb(II), Cd(II), and As(III) in minerals (Phillips and Krauss 1965) and in clusters of Zn and Cu (Rozan and Luther 2001).

Although the kinetics for the Zn-S cluster replacement reaction are unknown for reaction with silver, they are expected to be fast because the sulfide in these clusters is 2-coordinate rather than 4-coordinate, as in many sulfide minerals. Thus, there are no steric factors that should significantly slow silver replacement of zinc. This replacement reaction can be monitored by (1) tracking the decrease in absorbance of the UV peaks for the zinc-sulfide clusters and (2) voltammetric measurement of the release of Zn²⁺ to solution. Calibrations based on standard solutions indicate that a 10 nM zinc-sulfide solution can be monitored in a 10 cm cell (corresponding to an absorbance approximately 0.001; Luther et al. 1999).

In spite of this compelling indirect evidence for the likely occurrence of silver sulfide clusters in nature, none have been measured directly. In natural waters, a significant fraction of total silver, and very likely sulfide, is colloidal and therefore larger than the size expected for clusters. To be consistent with the cluster hypothesis, this observation suggests that a significant fraction of the posited silver clusters occurs as ternary complexes with NOM or other colloids, or that additional, larger (colloidal) species occur. Indeed, as noted earlier, Wen, Santschi et al. (1997) and Wen, Tang et al. (1997) have found evidence that colloidal silver in Galveston Bay occurs in association with organic sulfur. Furthermore, reduced sulfur species in humic substances have been documented as important ligands for

mercury, and the same would be expected to hold true for silver. In summary, silver-sulfide clusters are likely to be an important form of this metal in nature, but other silver-S(II-) associations need to be considered as well.

Levels of sulfide in the water column

It can be stated with a reasonable degree of confidence across a range of aquatic environments that levels of acid-reactive sulfide (ARS) (Cline 1969) nearly always exceed those of filtrate silver (Table 1-5). The sulfide data set is relatively sparse, and in only a subset of those has silver been measured in parallel. Nevertheless, the consistency of the data and the magnitude of the difference support our general conclusion. In several studies spanning multiple lacustrine and riverine systems, levels of methylene blue measurable sulfide (MBMS) were at least an order of magnitude greater than silver levels, commonly 100 to 1000 times greater (Table 1-5).

For example, the Dundas POTW (Adams and Kramer 1999b) has about 80 ng/L (0.80 nM) silver in its effluent. By comparison, roughly 20,000 ng/L (200 nM) silver would be required to saturate all the available sulfide sites.

Macroparticles and colloids

Due to its particle reactivity, silver occurs in natural waters predominantly bound to macroparticles ($> 0.45 \mu\text{m}$) and colloids ($< 0.45 \mu\text{m}$) (Shafer et al. 1996; Wen, Santschi et al. 1997). Of the so-called "dissolved" silver species ($< 0.45 \mu\text{m}$), it is now believed that colloidal particles predominate over truly dissolved species in fresh waters (Wen, Tang et al. 1997; Kramer et al. 1999). The nature of the colloidal particles with which silver is associated, remains uncertain. Various researchers suggest that silver in river water is attached to organic matter (Krahforst and Wallace 1996; Wen, Santschi et al. 1997; Shafer et al. 1998; Pham and Garnier 1998). The importance of inorganic cluster compounds should not be overlooked. (see section: *Details of silver-sulfide interactions*), although the presence of silver or zinc sulfide clusters in Fe-rich and NOM-rich natural waters has not yet been demonstrated. The proportion of truly dissolved species and even the size distribution of the colloidal particles and associated metal phases remains uncertain due to analytical difficulties caused by losses on low cutoff ($< 3 \text{ kDa}$) membrane filters (Martin M. Shafer, University of Wisconsin, personal communication). Both inorganic and organic sulfur complexes of silver are able to form dissolved species as clusters, including rings and spirals (Bell and Kramer 1999). Larger accumulations form as chains and sheets, which may deform to form colloidal particles (Bell and Kramer 1999).

What can be said with some certainty is that silver is associated with particles and colloids over a range of sizes from $< 1 \text{ kDa}$ to $> 0.45 \mu\text{m}$. The biological relevance of particular size fractions is unknown and remains to be determined. Therefore, it is not recommended that UF be used in regulatory monitoring because it is not

presently known whether silver associated with 500 nm particles is any less bioavailable than that associated with 10 nm colloids. This conclusion should be reassessed once more information on the toxicity of silver sulfide compounds has been elucidated.

Biological Implications

Bioaccumulation of silver-sulfide complexes

Given the premise that most silver present in aquatic environments is strongly bound to reduced-sulfur ligands and that silver has been measured in numerous aquatic organisms, it is likely that there are pathways whereby silver originally associated with reduced sulfur can be bioaccumulated by many aquatic species. This section evaluates the prospects for silver accumulation strictly through interactions between Ag-S complexes and biomolecules and the implications of these interactions on toxicity. Other chapters discuss uptake route, membrane transport, and mechanisms of silver toxicity.

The most probable hypothesis for the transfer of Ag from Ag-S complexes to biomolecules is ligand exchange, given in the following equation:



where S_1 is an aquatic sulfide ligand and S_2 is a biomolecule containing a sulfhydryl group or groups. Ligand exchange would depend upon the relative binding of Ag(I) to S_1 and S_2 and the activities (concentrations) of S_1 and S_2 . Potential biomolecules include (1) the amino acids (cysteine); (2) the tripeptide, glutathione (GSH), and (3) sulfhydryl-rich proteins such as metallothionein (MT), phytochelatin, and cysteine-rich intestinal peptides (Table 1-6). Of these, GSH and MT are two of the most prominent ones. MTs are a class of sulfhydryl-rich proteins that are thought to play a major role in the metabolism or detoxification of metals, including silver. MT normally occurs in most tissues (e.g., brain, gill, gonad, kidney, liver) in trace amounts and has been found in mucus (Dang et al. 1999). However, exposure to certain metals induces synthesis of MT, and silver is known to be a direct inducer (Mayer et al. 1996). MT strongly binds silver, and the stoichiometry of this binding normally is 10 to 12 atoms of silver per molecule of MT (Mason and Jenkins 1995). Kägi and Schäffer (1988) note that the binding energy for Ag to MT should be higher than for Zn-MT, which they calculate to be $\log K = 11.7$.

The tripeptide GSH (gg-glutamylcysteinylglycine) is commonly found within the cells of most tissues at high concentrations, 5 to 10 μM . It is the most abundant nonprotein thiol in mammalian cells (Hwang et al. 1992). Silver can strongly bind GSH ($\log K = 12.3$; Adams and Kramer 1999a), and the stoichiometry of the Ag-

Table 1-6 Conditional stability constants and intracellular concentrations

	Log K	Biomolecule concentration (mM)
Ag-Metallothionein	> 11.7 ^A	0.0015 - 0.06 ^B
Ag-Glutathione	12.3 ^C	5 - 10 ^D
Ag-Cysteine	11.5 ^C	N/A

A 11.7 is the reported binding constant for Zn-MT

B Hogstrand et al. 1996

C Adams and Kramer 1999a

D Mason and Jenkins 1995

GSH complex is 1:1 (Russell A. Bell, McMaster University, personal communication). Unlike many of the essential metals, Ag(I) does not promote the oxidation of GSH to the disulfide (GSSG). This has toxicological significance since GSH is one of the principal redox buffers in the cell protecting against oxidative stress (e.g., from metal-catalyzed redox reactions). It has been hypothesized that GSH may kinetically buffer transient cellular fluctuations of metals and keep the level of bioreactive⁴ metal low until MT can exert its protection, which can take days in fish (Mason and Jenkins 1995).

Phytochelatin (PC) is a class III MT produced by plants and algae (Lauro and Plocke 1999) that is involved in metal metabolism or detoxication. While PCs have received less attention than other classes of MTs, they could play an important role in the transport and fate of silver in aquatic environments, especially given recent evidence that algae probably represent a major silver exposure pathway (Sanders and Abbe 1989; Schmittschmitt et al. 1996; Fisher and Hook 1997; Bielmyer and Klaine 1999; Reinfelder and Chang 1999). PCs are derived from GSH via enzymatic addition of a gg-glutamylcysteine moiety to glycine to yield (gg-glutamylcysteine)_n-glycine (n = 2 to 11). The enzyme responsible for this addition, phytochelatin synthase, is a dipeptidyl transferase and is thought to be constitutively expressed (Grill et al. 1989). Thus, PCs are not under direct transcriptional control and more closely resemble GSH in their production pathway. In studies of *Rubia tinctorum* L., PC synthesis increased following exposure to Ag(I) for 3 days (Maitani et al. 1999). The most abundant PC species was (gg-glutamylcysteine)₄-glycine, and (gg-glutamylcysteine)₅-glycine also was detected. These PCs would bind 4 and 5 atoms of Ag(I) per molecule, respectively.

Exchange reactions, as in Equation 1-3, occur very rapidly for Ag(I). Considering the magnitude of the stability constants for Ag-S species, one must question whether ligand exchange with biomolecules is favored. While there are no direct studies investigating this exchange, there is some indirect evidence that it can take place. For example, Adams and Kramer (1998b) investigated the effects of 3-mercaptopropionic acid (3-MPA), a prominent environmental thiol, on Ag₂S(s). In

⁴ Bioreactive is used here to mean the fraction of bioavailable metal (metal taken into an organism) that results in toxicity.

this experiment, silver equilibrated with FeS to form Ag_2S was spiked with 0.1 and 1 mM 3-MPA, and silver was remobilized from the solid phase to the dissolved phase presumably as silver thiolate complexes. While the stability constants for Ag-cysteine, Ag-GSH, and Ag-MT are greater than for Ag-(3-MPA), it remains to be demonstrated whether ligand exchange between Ag-S species and biomolecules is favorable in the cell. Since silver-sulfide solids exchange silver with 3-MPA, it is probable that silver in silver-sulfide clusters also is exchangeable (e.g., at the cell surface). Other cellular parameters that could influence this exchange include the redox state of the cell and compartmentalization within cells, which can change intracellular concentrations of biomolecules.

Toxicity of silver-sulfide complexes

Although Ag-S species apparently accumulate in aquatic organisms, little is known about their toxicity. In most laboratory waters used in the past for toxicity studies, it seems likely that sulfides were not present because they were removed unintentionally by chlorination, by carbon filtration, or by the use of reconstituted water. Thus, sulfides were not present in these test waters, and the toxicity of Ag-S complexes has not been tested. There has been one investigation of the toxicity of Ag-(3-MPA) complexes, whose stability constant is slightly lower than AgHS (Shaw et al. 1997). These tests were conducted in seawater using 3 marine fish species: inland silverside, topsmelt, and sheepshead minnow. For all species tested, thiols reduced acute silver toxicity. Mortality of the fish exposed to silver and equimolar concentrations of 3-MPA was similar to that of the control fish. Mortality of fish exposed to silver increased with decreasing 3-MPA concentrations. While these tests demonstrate amelioration with silver-thiolates, the toxicity of what appears to be the most likely form of silver in aquatic environments (i.e., complexed with $\text{S}(\text{II}^-)$) remains unknown. This represents perhaps the greatest gap in our knowledge of the effects of silver on aquatic organisms in natural environments.

Proof-of-principle experiment: Silver sulfide binding

It is proposed that a series of toxicity tests whose purpose is to examine the binding of Ag(I) by soluble ARS and its effect on toxicity. The idea is that if the molar concentration of ARS exceeds twice the molar concentration of silver:

$$[\text{ARS}] > 2[\text{Ag}] \quad (\text{Equation 1-4}),$$

then no toxicity should occur if the resulting silver sulfide complex ($\text{Ag}_2\text{S}[\text{aq}]$) is not bioreactive. This is the analogous situation to the sediment toxicity prediction that no toxicity occurs if

$$[\text{AVS}] > 2[\text{SEM Ag}] \quad (\text{Equation 1-5}),$$

where AVS is acid-volatile sulfide and SEM (simultaneously extracted metal) Ag is the amount of silver that is extracted simultaneously. The reason in both cases would be the formation of $\text{Ag}_2\text{S}(\text{aq})$ complexes in aqueous solutions and Ag_2S

(acanthite) in sediments. The latter is known to be nontoxic. It is the purpose of the proposed experiments to test the toxicity of $\text{Ag}_2\text{S}(\text{aq})$.

Since this experiment is conceived as a proof-of-principle experiment, short-term (acute) exposures to *Ceriodaphnia* using mortality as the endpoint are suggested. The proposed experimental design is given in Tables 1-7 and 1-8, and consists of both negative (no $\text{Ag}(\text{I})$) and positive (nonsulfide-bound $\text{Ag}(\text{I})$) controls and two treatment regimes of $\text{Ag}_2\text{S}(\text{aq})$ consisting of six concentrations each. The silver-sulfide complex is assumed to have a stoichiometry of Ag_2S , similar to acanthite, and is consistent with available measurements. Details of silver-sulfide preparation are still being developed, but it is presumed that dosing will occur in a 2-step process. First, the sulfide will be stabilized in solution using zinc to form zinc-

Table 1-7 Proposed experimental designs for proof-of-principle experiment

A

Measurement	Concentration					
Ag (nM)	0	1	2	5 ^A	10	20
Ag ($\mu\text{g}/\text{L}$)	0	0.1	0.2	0.5 ^A	1	2
S(-II) (nM)	0	0	0	0	0	0
Zn ²⁺ (nM)	0	0	0	0	0	0

A Estimated LC50 for *Ceriodaphnia*, 48-hour exposure

B

Measurement	Concentration						
Ag (nM)	0	1	2	5	10 ^A	20	100
Ag ($\mu\text{g}/\text{L}$)	0	0.1	0.2	0.5	1 ^A	2	10
Excess Ag (nM)	0	0	0	0	10 ^A	40	90
S(-II) (nM)	5	5	5	5	5	5	5
Zn ²⁺ (nM)	10	10	10	10	10	10	10
Zn ²⁺ ($\mu\text{g}/\text{L}$)	0.6	0.6	0.6	0.6	0.6	0.6	0.6

A Predicted LC50 to 100 for *Ceriodaphnia*

C

Measurement	Concentration						
Ag (nM)	0	10	20	50	100 ^A	200	500
Ag ($\mu\text{g}/\text{L}$)	0	1	2	5	10 ^A	20	50
Excess Ag (nM)	0	0	0	0	50 ^A	150	450
S(-II) (nM)	25	25	25	25	25	25	25
Zn ²⁺ (nM)	50 ^B	50	50	50	50	50	50
Zn ²⁺ ($\mu\text{g}/\text{L}$)	3 ^B	3	3	3	3	3	3

A Predicted LC100 for *Ceriodaphnia*

B Concentration is well below the LC50 for *Ceriodaphnia*

Table 1-8 Measurements to be made in each exposure chamber. All samples filtered (0.45 μm). Sampling and analysis to be done prior to the start, at the water renewal, and after termination.

Measurement	Sampling and analysis considerations
Ag	Clean technique
ARS	See write-up
Zn	Clean technique
Ca, Mg, Na, K, pH, Cl, SO_4^{2-} , HCO_3^- , Alk	Standard methods
DOC	Detection limit (0.1 mgC/L)

DOC = Dissolved organic carbon

sulfide clusters, as discussed above. The clusters are hypothesized to be the primary species for ARS reactivity in natural waters, due to the ubiquitous presence of zinc at levels higher than 10 nM. Second, silver will be added to the stabilized sulfide solution, where it exchanges with zinc in the zinc-sulfide clusters, presumably to form $\text{Ag}_2\text{S}(\text{aq})$. The two $\text{Ag}_2\text{S}(\text{aq})$ treatments are designed to produce varying excess concentrations of nonsulfide-bound Ag(I) that bracket the predicted 50 to 100% mortality concentrations in tests without sulfide. Therefore, if $\text{Ag}_2\text{S}(\text{aq})$ is not reactive, as predicted, then toxicity should correlate with the excess Ag(I) concentrations (nonsulfide-bound silver). Since this procedure involves the preparation of clusters, some preliminary experimentation in the culture media needs to be conducted.

Analytical Methods

Total silver

Just two detection techniques have provided all of the existing reliable data on silver in natural waters: graphite furnace atomic absorption spectroscopy (GFAAS) and inductively coupled plasma-mass spectroscopy (ICP-MS). Application of GFAAS detection requires the use of a preconcentration technique, such as ammonium pyrrolidinedithiocarbamate or diethylammonium diethyldithiocarbamate extraction, as does ICP-MS where ambient silver levels are below 10 to 20 pM. Isotopic discrimination is inherent in ICP-MS methods, therefore ambient level tracer studies with either of the two stable isotopes of silver (^{109}Ag or ^{107}Ag) are possible. Ambient silver levels are not quantifiable with existing electrochemical methods. Few, if any, new detection approaches have emerged in recent years. However, advances in MS instrumentation (e.g., multicollector magnetic sector, ICP-MS, and laser ablation [LA], LA-ICP-MS), coupling chromatography to MS (e.g., HPLC-ICP-MS), and spectrofluorometry offer the possibility of significant improvements in both detection and speciation capabilities for Ag in the near future.

Filtrate silver

A variety of "standard" filtration approaches have been applied to silver in freshwaters, including track-etched membranes (polycarbonate), tortuous-path membranes (cellulose), and high-capacity capsules (polypropylene, polysulfone, etc.). Each approach has its particular advantages and disadvantages in specific environmental conditions. It must be noted that significant differences in filtrate silver levels can result depending upon the specific design and field protocols for use of the filter. These differences are a result of differences in the particle capture mechanisms and capacity of the contrasting filters coupled with the high proportion of silver bound to particles in the 0.1 to 1 μm size range. There can be no blanket recommendations made other than investigators must perform calibration studies with the specific waters they intend to analyze in order to evaluate the size discrimination characteristics of the filters to be used.

Size fractionation

Ultrafiltration is the sole technology that has been used to elucidate the association of silver across the range of submicron particle sizes. Relatively few studies have coupled ultra clean sampling and analysis techniques with strictly controlled UF to produce reliable estimates of the extent of colloid-silver interactions in both estuarine and freshwater systems. The reason for this relative scarcity of data is that determination of the colloid associated silver fraction is by no means routine, particularly in fresh waters, largely due to potential problems with losses due to sorption and ion rejection on the UF media. The magnitude of these artifacts appears to increase as lower size cutoffs are applied. Additionally, quantification is complicated by changes in the size and shape of colloids (especially organic ones) with changes in ionic strength. Therefore, it is critical that complete mass balances be conducted in all UF separations. This is a minimum safeguard that evaluates losses due to sorption, but not other UF artifacts.

Ultrafiltration methods at nominal size cutoffs of 100 kDa, 10 kDa, and 3 kDa have been developed for silver and other trace metals in freshwaters, and as low as 1 kDa in seawater. A wide range of UF media, from cellulose-based to polysulfone derivatives, in several formats (spiral-wound, hollow-fiber, and plates) have been used. While the lack of standardization is unfortunate, the similar results produced by a multitude of approaches suggest that the observed fractionation of silver is true and not a result of artifacts. Clean UF technology has shown that colloid-bound silver is an important, and usually dominant, fraction of filtrate silver. In freshwater and estuarine water, colloid-bound silver often represents more than 50% of filtrate silver and can represent all detectable silver in the filtrate fraction.

Clean techniques appropriate for silver

Clean sampling and analysis techniques are now recognized as essential components of any trace metal study. Though specific details may differ, all depend on the

application of 3 principles: (1) water samples should be permitted to contact only surfaces composed of materials that are intrinsically low in trace metals (e.g., low-density polyethylene, Teflon) and that have been extensively acid-cleaned in a filtered-air environment; (2) samples should be collected and transported with extraordinary care to avoid contamination from field personnel or their gear (Martin M. Shafer, University of Wisconsin, presentation at Argentum III conference); and (3) all other sample handling, preparation, and analysis steps should take place in a filtered-air environment (Class 100 clean room) and using ultrapure reagents (e.g., Benoit et al. 1994). Orders-of-magnitude lower metals levels are now reported for silver levels in many environments as these improved methodologies have been implemented. For example, levels of silver in Lake Michigan, USA, were reported to be in the 1 to 5 nM range as late as the mid-1970s, where it now measures just a few pM (see Table 1-1).

Contamination has both systemic and stochastic components; the former are easier to identify and control. The necessity of any given step of the generally accepted clean handling protocol in reducing or controlling the more random contamination events for a particular metal is harder to quantify. Therefore, the philosophy (particularly in multielement studies) has been to take the conservative approach and apply a broad gamut of field and laboratory contamination control measures. Recent studies addressing contamination source profiles hint that silver may be less prone to contamination in field collection and laboratory processing than metals such as lead and zinc (e.g., Benoit et al. 1994). It seems clear that exhaustive multiacid cleaning procedures for sample bottles and filters may not be required. Relaxing strict clean sampling protocols also may be possible in certain situations. However, any such variation from comprehensive clean procedures must be thoroughly evaluated to minimize contamination biases.

Sample bottle type and preservation

Recent data from the University of Wisconsin Water Chemistry Laboratory (Herrin 1999) indicate that significant losses of silver to Teflon and polyethylene bottles occur in 24 hours or less of unacidified storage and that this silver is not completely recoverable by later acidification and digestion. These losses may represent 30% or more of available silver. These findings are consistent with the work by Wen, Tang et al. (1997) who showed that silver in samples stored unacidified for 2 months could not be completely recovered by subsequent acidification; exposure to UV light was required to recover the silver. In cases where unacidified storage is desirable (e.g., speciation experiments), glass storage containers sorb much less silver than Teflon.

Loss of silver, even in Teflon bottles, can be avoided by acidification with nitric acid at the time of collection.

Size fractionation

Though UF studies have demonstrated that a large majority of silver passing through a 0.45 μm filter is actually associated with colloids in most aquatic environments (including POTW effluents), it is recommended that such fractionation not be performed on a routine basis in characterization of bioassay experiments. The complexity and effort required to avoid potential artifacts in UF fractionations cannot justify, at the current stage of knowledge, the potential benefits. If, however, future work demonstrates that colloiddally associated silver within well-characterized physical or chemical fractions is or is not bioavailable, then this recommendation should be reconsidered. Using analogous reasoning, it is concluded that there is no compelling reason to alter the current 0.45 μm operational cutoff for macrofiltration.

Sulfide determination

It is hypothesized that acute silver toxicity has the potential to be expressed only when levels of silver exceed that of reactive sulfide. Therefore, it is critical to determine the levels of sulfide whenever silver measurements are performed. It is recommended that the methylene blue sulfide protocol be followed (Fischer 1883; Cline 1969). Sulfides present as Fe, Zn, Cd, and H species are fully assessable in this approach. Copper and silver sulfide species are not detected by the methylene blue method, and silver sulfide is not detected electrochemically (see section: *Macroparticles and colloids*). A range of simple thiols, however, is measurable via the methylene blue method when samples are first preserved or preconcentrated via Zn in basic solution. It should be noted that the Cline version of the methylene blue test is recommended, not only because it measures a pool of reduced sulfur compounds that are known to be the most important ones in nature but also because it is relatively simple and easy to use and measures a broad spectrum of reduced sulfur compounds.

Some values of sulfide reported in this work may be underestimated due to long-term (> 2 hours) storage of samples. Sulfide and metal sulfides are prone to adsorb on container walls.

Silver and sulfide in sediments

Silver forms a sulfide mineral (acanthite, Ag_2S), but this mineral has not been documented in any freshwater, estuarine, or marine field study. However, silver should be bound or associated with the common sediment sulfide phases—iron monosulfide (FeS) and pyrite (FeS_2) (Wingert-Runge and Andren 1995). Sediment-bound sulfide that reacts with cold 1 M HCl is termed AVS and releases sulfide from FeS but not from FeS_2 . When the metal that is SEM is less than or equal to the AVS, all metal is assumed to be bound as very insoluble metal sulfides with low porewater metal activity and toxicity. This SEM-AVS approach is now an approved

method (Ankley et al. 1996; USEPA 2000) and has been applied to silver, but there are caveats to interpretation of the data set for silver.

Although sulfide will be released quantitatively by 1 M HCl from the divalent ions of Fe, Zn, Cd, and Pb, sulfide from silver-sulfide solids may not be quantitatively released in short time periods (Rozan and Luther 2001). In addition, Ag(I) reacts with chloride to form complexes and precipitates that reduce the amount of silver that can be measured in solution. Thus, the silver released is underestimated. Silver can be released quantitatively with a nitric acid extraction, but nitric acid oxidizes sulfide and reduces the amount of sulfide that can be determined. Despite these two analytical problems, the amount of silver should always be less than the sulfide in sediments.

However, there are 2 other approaches to the measurement of silver and sulfide that could circumvent these problems. First, 1 M H₂SO₄ could be used instead of 1 M HCl because silver sulfate is soluble. Thus, both sulfide and silver could be measured in a modification of the SEM-AVS method. In addition, a 2-step approach to analysis could be performed to obtain both total sulfide and silver. In this second approach, a subsample can be analyzed by acidic Cr(II) reduction of the sediment (Rozan and Luther 2001), which releases sulfide for measurement from both FeS and FeS₂ and silver associated with these phases. Another subsample can be analyzed by nitric acid digestion, which dissolves both FeS and FeS₂ (Howarth and Merkel 1984) and releases silver from these commonly found metal sulfides. If the silver is less than the total sulfide measured, it can be assumed that the silver is bound in insoluble metal sulfides. Both of these approaches should be investigated in detail before adoption as standard methods.

Conclusions and Recommendations

The authors of this chapter reached consensus on the following conclusions and recommendations:

- Silver can be assumed to be associated mainly with reduced sulfur ligands in almost all natural aquatic systems, both oxic and anoxic.
- The concentrations of free ionic silver, Ag⁺, in aquatic systems are too low to be measured by current techniques and will almost certainly not be a direct factor in toxicity to aquatic organisms.
- Laboratory experiments suggest that the kinetics of ligand exchange are rapid for these highly stable sulfide complexes.
- Silver is associated with particulate and colloidal fractions ranging from less than 1 kDa to greater than 0.45 μm.
- The nature of the colloidal particles has not been characterized yet. Sulfides associated with organic matter and sulfide clusters are likely to be involved as substrates for silver binding.

- Speciation chemistry is critical to an understanding of chronic toxicity and must be considered in all toxicity tests.
- Silver and reduced sulfides need to be measured on the same samples.
- Sampling and analytical techniques need to be used that are appropriate to subnanomolar (< 10 ng/L) silver and submicromolar sulfide concentrations.
- Methods better than the AVS-SEM protocol are needed to measure silver and associated sulfides in sediments.
- Analytical protocols to account for container sorption of sulfides need to be developed for samples stored more than 2 hours.
- Acute and chronic toxicity tests involving silver sulfide complexes in the water phase and in sediments are urgently required.
- Studies of the bioaccumulation of silver sulfides need to be initiated.
- The interaction of NOM and sulfide clusters needs to be elucidated.

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Biological Effects of Silver

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Introduction

Acute toxicity from waterborne silver is probably very rare in the natural world. Laboratory toxicity data derived with highly bioavailable silver (added as silver nitrate) indicate sensitive species fall in the range of 0.85 to 3 $\mu\text{g/L}$ (approximately 8 to 30 nM) in freshwater; in seawater, the sensitivity range is about an order of magnitude higher. Typically, surface water concentrations rarely exceed 0.05 $\mu\text{g/L}$ (0.55 nM) total silver. The U.S. Environmental Protection Agency (USEPA) acute criteria for freshwater and marine dissolved silver concentrations are 3.43 $\mu\text{g/L}$ (34 nM) (at a hardness of 100 ppm) and 1.92 $\mu\text{g/L}$ (19 nM), respectively. Dissolved silver concentrations in U.S. publicly owned treatment work (POTW) effluents are frequently in the 0.1 to 1 $\mu\text{g/L}$ (approximately 1 to 10 nM) range (Gill et al. 1997; Shafer et al. 1998), indicating little risk of acute toxicity in receiving waters except for a few industrial discharges if low dilution occurs. Furthermore, recent studies of metastable sulfide complexes, which appear to be ubiquitous in effluents and receiving waters, suggest that effluent and receiving waters may have the capacity to detoxify on the order of 100 to 300 ng/L (approximately 1 to 3 nM) dissolved silver (refer to Chapter 1). If this hypothesis for ubiquitous metastable sulfide is confirmed, then the acute water-quality criteria (WQC) could be modified to incorporate this protective parameter (much as hardness is applied currently for silver).

Limited laboratory data using silver nitrate for waterborne chronic toxicity indicate effects can occur at concentrations in the 0.2 to 6.3 $\mu\text{g/L}$ (2.1 to 68 nM) range (Hogstrand and Wood 1998). However, the chronic toxicity exposure systems likely were conducted under laboratory conditions that eliminated metastable sulfide, as well as other naturally occurring ligands, from bioassay water. Therefore, it may not represent chronic toxicity in ambient surface receiving waters where metastable sulfide complexes and natural organic matter (NOM) are thought to be ubiquitous. It is imperative, therefore, that in the future silver toxicity is

studied under conditions where silver, metastable sulfide, and NOM are all present at environmentally realistic levels.

Approach to Rates of Silver Equilibrium in Natural Waters and Toxicity Testing

Background

Understanding the rate at which free ionic silver (Ag^+) approaches equilibrium is important for toxicity testing and for assessing silver bioavailability in effluent receiving waters. In toxicity testing within the laboratory, silver is usually added as AgNO_3 , which is not the form of silver found in POTW effluents. Because NO_3^- is a weak ligand and does not appreciably bind the Ag^+ ion, silver is fully dissociated and, therefore, essentially is added in its most toxic form. The added $\text{Ag}(\text{I})$ becomes distributed among the various dissolved, colloidal, and particulate ligands in the system. Bioavailability and toxicity of the added silver ion consequently depend on the degree of association and binding strength of $\text{Ag}(\text{I})$ to these ligands. When conducting toxicity tests, if equilibrium of $\text{Ag}(\text{I})$ with silver-binding ligands is not achieved prior to test organism exposure, then the test may overestimate actual silver toxicity in the system. Therefore, it is essential that sufficient equilibration time be achieved prior to toxicity assessment. It is equally important that a test system contain the important ligands influencing silver speciation in the test waters or effluents under investigation. The concentrations of chemical species in the test environment should be measured by analytical means before performing the test, rather than being assumed from the amounts nominally added.

Rates of reaction (complexation) of $\text{Ag}(\text{I})$ with the various ligands present in aquatic systems may be influenced by ligand type, especially colloidal and particulate ligands, and concentrations of both Ag^+ and ligands in the system. In general, association with dissolved inorganic and organic ligands such as chloride, sulfide, and small organic molecules is rapid, with equilibrium probably achieved within minutes or less (Bell and Kramer 1999). For example, when $\text{Ag}(\text{I})$ is added to zinc sulfide or copper sulfide it binds almost instantaneously, in one minute or less, with release of zinc or copper (Rozan and Luther 2000). However, association with colloidal and particulate ligands, including NOM, may be slower. Although information on Ag^+ reaction kinetics with colloids and particles is limited, factors such as retarded intraparticle diffusion and colloidal pumping may result in slow adsorption kinetics (Schwarzenbach et al. 1993; Gustafsson and Gschwend 1997; Wen et al. 1997; Tang et al. 2000). Time scales of hours or longer may be required to reach a stable distribution of added silver ion among aqueous, colloidal, and particulate phases that may be present in waters where bioavailability is being measured (Piro et al. 1973).

It is important to recognize that the nature and composition of NOM may vary among aquatic systems due to factors such as differences in sources (precursor materials) and age (degree of weathering). Thus, "recent" NOM (such as might be derived from aquatic organisms, i.e., phytoplankton), in general, may contain more lipid and protein-derived organic matter (OM), and thus more sulfhydryl binding sites, than "older" NOM (such as might be derived from older terrestrial systems and lignin-containing plants) (Steinberg and Muenster 1985; Stevenson 1985; Guo and Santschi 1997; Tang et al. 2000). Information on the characteristics of NOM important in reactions with Ag(I) is emerging. For example, recent evidence shows that biogenic thiols as well as inorganic sulfides are present in estuarine waters (Tang et al. 2000; Tang and Santschi 2000). Because information on the influence of NOM character on reaction kinetics with Ag(I) is limited, caution should be used in generalizing from bioavailability experiments involving NOM from only a few sources or sites. Further information is needed on the range in kinetics and strength of Ag(I) binding among different sources and types of NOM and whether these differences are important in measuring and modeling silver bioavailability and toxicity in freshwaters.

Although it is acknowledged that more information is needed on rates of Ag(I) binding to natural colloids, including NOM, it is currently believed that these systems will approach equilibrium within several hours. As a precaution, a 24-hour preequilibration time after addition of AgNO₃ or other Ag salts to bioassay water is recommended for toxicity testing, until additional information is developed. This would require modification of current practices in flow-through tests where mixing times of 5 to 10 minutes prior to exposure are typically used.

In effluents or receiving waters, silver bioavailability may be limited by rates of Ag(I) release from colloidal and particulate ligands in the system. Thus, a Biotic Ligand Model (BLM) equilibrium approach for estimating toxicity could overestimate the actual dose received by an organism exposed to an effluent for a short period of time. Conversely, as an effluent moves downstream, dilution might result in some desorption of bound silver, potentially increasing the fraction of bioavailable silver in the system. However, dilution also would decrease concentration and, as silver-complexing ligands are generally abundant, would likely result in an overall decrease in potential toxicity. Finally, if ligands that bind silver are unstable, loss of these ligands (e.g., oxidation of metastable sulfides, breakdown of organic colloids) could increase silver bioavailability over time. Again, in the case of effluents, concurrent dilution will partly offset these potential increases in bioavailability. Although more information is needed, preliminary research has shown that silver-binding ligands, including metastable sulfides and NOM, are ubiquitous in freshwaters and that acid-volatile sulfides (AVS) are widely distributed in sediments (Ankley et al. 1996; also see section: *Biological effects of silver in sediments*). Thus, decomposition of silver-binding ligands will not likely release Ag(I) into freshwater systems. However, the ligands associated with Ag(I) may vary

temporally and spatially, producing changes in bioavailability (but not necessarily toxicity) of silver.

Research considerations

Research is needed to identify the appropriate equilibration time between silver and all potential ligands, especially NOM, in toxicity tests; to understand differences among NOM types and their ability to complex with silver; and to determine which ligand is effective in reducing aquatic toxicity.

Regulatory consideration

Adequate mixing times (i.e., up to 24 hours, until new information becomes available) should be used in toxicity testing to allow equilibration of silver with all silver complexing ligands, including metastable sulfides, before introducing the test organisms to the system.

Effects of Silver on Aquatic and Terrestrial Species

A major focus of the authors of this chapter is the concern for aquatic species' potential sensitivity to silver. Terrestrial species are relatively insignificant targets for silver toxicity under environmentally realistic conditions. Aquatic species are far more sensitive and far more likely to be exposed.

Aquatic life

Aquatic life sensitivity to silver via aqueous exposure has been widely studied in the laboratory, with acute toxicity studied more intensively than chronic toxicity. In addition to aqueous exposure pathways, evidence exists of viable dietary exposure pathways for silver. Eventually, dietary and dissolved exposure pathways need to be considered in an integrated manner to evaluate the ecotoxic effects of silver. In this section, the focus is on the state of knowledge regarding silver ecotoxicity via aqueous exposure. Dietary exposure is considered further in Chapter 3.

Acute toxicity

The acute effects of waterborne silver on freshwater organisms have been reasonably well quantified with more than 40 organisms evaluated (USEPA 1987; reviewed by Hogstrand and Wood 1998; Wood et al. 1999; see Appendix, Table 2-1). Much less is known about the acute effects of silver on biota in the marine environment although more than 25 organisms have been evaluated (see Appendix, Table 2-3). In general, there is reasonable agreement that, in both freshwater and marine systems, the level and forms of silver that occur are usually far below that which is required to produce acute toxicity in fish. This means that our understanding of the acute toxicity and effects of silver in the aquatic environment have been developed from studies that use environmentally unrealistic levels and forms of silver.

Acute toxicity: Freshwater

The majority of laboratory toxicity studies have been performed using AgNO_3 , with limited data available on other forms of silver. Present USEPA WQC use AgNO_3 data for deriving criteria. Acute silver toxicity to freshwater organisms has been relatively well studied, with species mean acute values (SMAVs) ranging from 0.85 to 1543 $\mu\text{g/L}$ (9.1 to 16,600 nM) across 41 species tested, including species tested since the 1987 USEPA data set (see Appendix, Table 2-1). In freshwater, acute species sensitivity distributions for silver are characteristic of most metals, with invertebrates more sensitive than vertebrates (i.e., fishes) (Figures 2-1 and 2-2). Within the invertebrates, cladocerans and amphipods are more sensitive than aquatic insects, which are more sensitive than other tested invertebrate groups (Figure 2-3). This observed relationship in relative sensitivity among taxonomic groups is generally consistent with that for other metals (e.g., copper and cadmium).

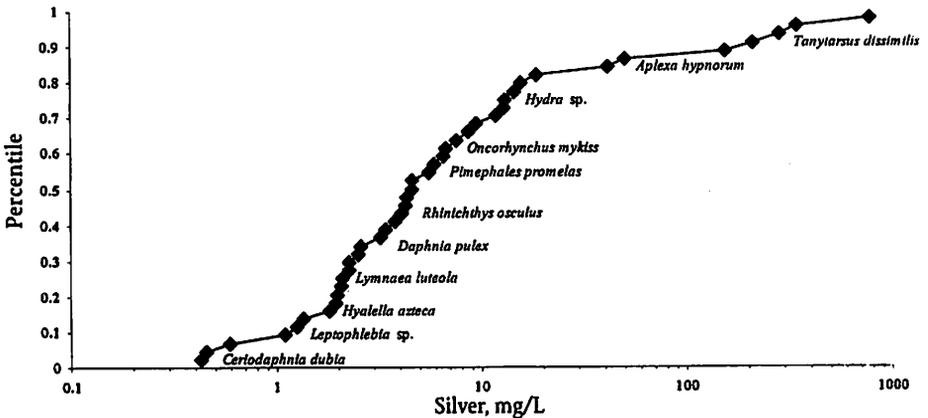


Figure 2-1 Acute sensitivities (SMAV/2) of freshwater organisms to silver

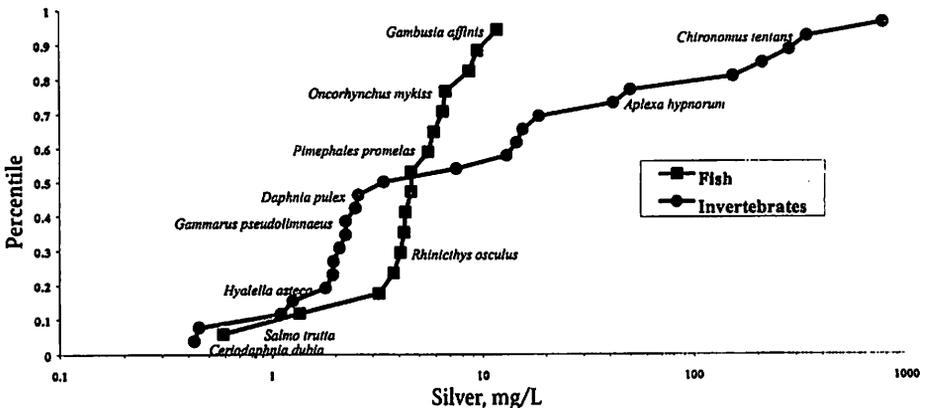


Figure 2-2 Acute sensitivities (SMAV/2) of freshwater fish and invertebrates to silver

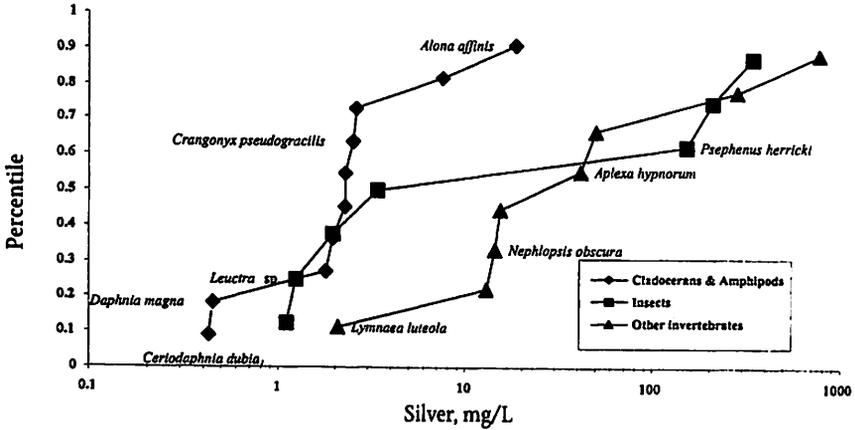


Figure 2-3 Acute sensitivities (SMAV/2) of different freshwater invertebrate groups to silver

Acute toxicity: Marine

Silver acute toxicity to marine organisms has been less intensively studied than has freshwater acute toxicity, but there is a relatively robust data set. SMAVs range from 13.3 to 2700 $\mu\text{g/L}$ (143 to 29,000 nM) across 25 species tested (Figure 2-4; see Appendix, Table 2-3). As with freshwater organisms, certain groups of marine invertebrates (larval bivalves and planktonic crustaceans, such as copepods) are more acutely sensitive than are fish or other invertebrates (Figure 2-5). This type of sensitivity distribution, with larval forms of certain species and copepods more sensitive than other groups, is observed with other metals as well. Some of the more sensitive results may be from endpoints other than mortality, such as growth or other determinations from the more sensitive life stages that are more representative of chronic endpoints.

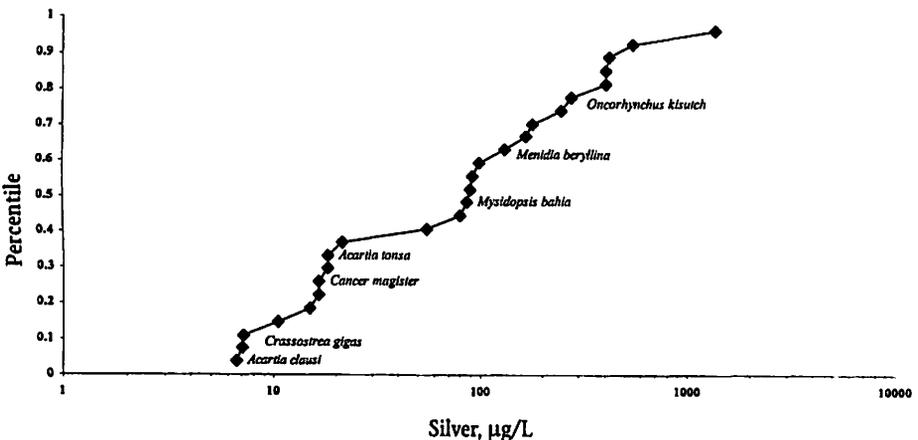


Figure 2-4 Acute sensitivities (SMAV/2) of marine organisms to silver

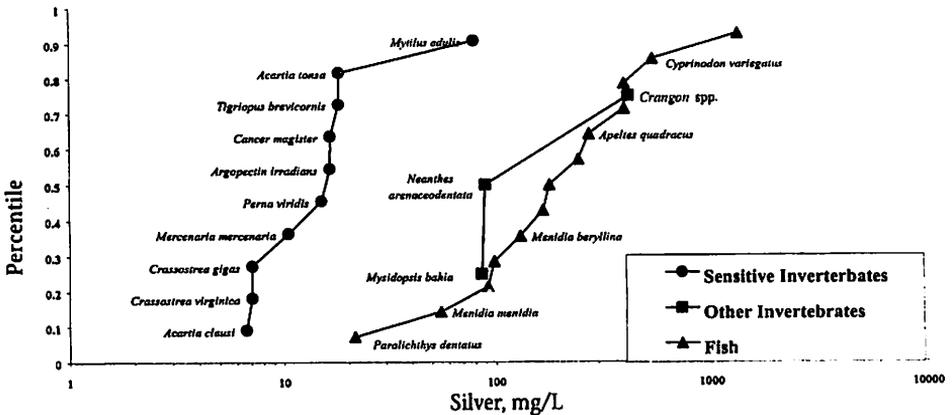


Figure 2-5 Acute sensitivities (SMAV/2) of different marine species groups to silver

Chronic toxicity

Chronic silver toxicity has not been extensively studied (as is the case for most other chemicals). A summary of the limited available data is provided below. As discussed elsewhere, the likely absence of metastable sulfides and other natural ligands in the bioassay water used for chronic laboratory toxicity tests conducted to date has been identified as a potentially significant caveat to the existing chronic database. This leads to the possibility that these studies may overestimate silver bioavailability relative to worst-case, real-world conditions. This caveat should be kept in mind when reviewing chronic toxicity data.

Chronic toxicity: Freshwater

Only 8 freshwater species have been evaluated for chronic silver toxicity (Figure 2-6; Appendix Table 2-2). Species mean chronic values (SMCVs) range from 0.231 to 5.79 $\mu\text{g/L}$ (2.3 to 57.9 nM). Species relative sensitivities observed for these data run counter to the usual pattern for acute toxicity, in that fish are more sensitive than are invertebrates. Cladocerans, in particular, are the least sensitive chronically but most sensitive acutely. In fact, cladoceran chronic sensitivity is less than the acute sensitivity, resulting in acute-to-chronic ratios (ACRs) of less than one. This reversal in sensitivities between relative acute and chronic sensitivities is not consistent with other metals and is very unlikely to be a real effect for silver. Rather, the reversal is likely to be an artifact of experimental design in toxicity testing with daphnids. Specifically, it appears that food addition for daphnids in standard toxicity tests is likely to be binding silver and consequently reducing silver bioavailability in the water column, although silver adsorbed to algae would be consumed.

Although unacceptable for use in calculating a chronic criterion, embryo-larval studies with 10 fish and amphibian species (Birge et al. 2000) provide supporting evidence that the limited chronic toxicity data set for silver does characterize the

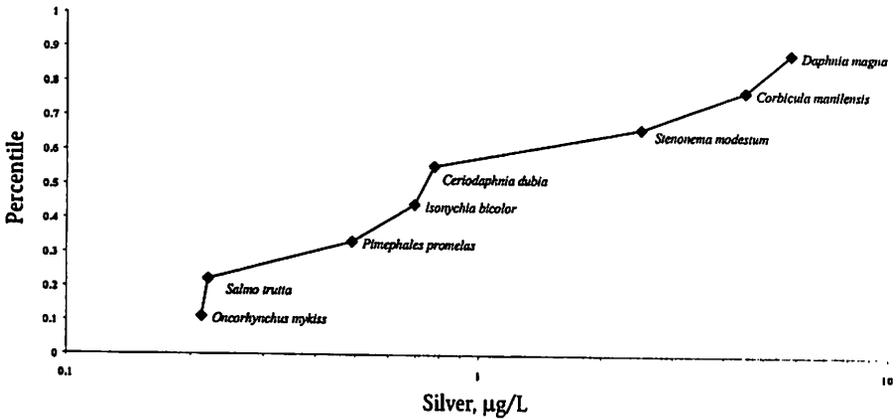


Figure 2-6 Chronic sensitivities of freshwater organisms to silver

range of organism sensitivities. Results from these studies are within the range of results for chronic studies, as defined by the USEPA (Stephan et al. 1985). It also is worth noting that these studies indicate that amphibians are no more sensitive than are fish to silver, in contrast to some other chemicals.

Considering that the most acutely sensitive species have not been adequately evaluated for chronic sensitivity, the chronic toxicity potential of silver clearly has not been fully described. Use of alternative experimental designs to assess daphnid sensitivity is strongly recommended. Alternative designs should consider shorter feeding duration during testing to limit silver binding to food. Alternatively, acute studies could be performed with food added so the chronic response could be more appropriately evaluated. For example, daphnids could be fed once per day for 1 hour prior to test solution renewal or a modified flow-through test could be conducted to reduce the time food is in the exposure chambers.

Chronic toxicity: Marine

Only one invertebrate species, *Mysidopsis bahia*, has been evaluated for chronic sensitivity to silver introduced as silver nitrate (USEPA 1987). Chronic values for *M. bahia* from early life-cycle studies conducted in 5 laboratories ranged from 15 to 87 µg/L (150 to 870 nM) (McKenny 1982). Lussier et al. (1985) conducted a 38-day early life-cycle study using silver nitrate with *M. bahia* and determined chronic values for survival and reproduction of 59 and 19 µg/L (maximum acceptable toxic concentrations [MATCs] of 32 to 108 and 11 to 32 µg/L), respectively (or approximately 590 and 190 nM; 320 to 1080 and 110 to 320 nM, respectively). Similarly, the chronic effects of silver have been evaluated in only two marine fish species, the sheepshead minnow *Cyprinodon variegatus* and the summer flounder *Paralichthys denatus* (Shaw et al. 1997). Mean chronic toxicity value for sheepshead minnow was 449 µg/L (95% confidence limits [CL]: 653 to 174). This was calculated as the geometric mean of no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) for 28-day post hatch mortality and represents the

mean of three tests. This is close to but less than the LC10 that should be a threshold.

The 28-day concentration lethal to 10% of the test subjects (LC10) and 50% of the test subjects (LC50) for the sheepshead minnow, an acutely tolerant species, were 543 (95% [CL]: 410 to 660) and 1095 (95% [CL]: 961 to 1210) $\mu\text{g/L}$, respectively. Using the same formula as that used for the sheepshead minnow above, the summer flounder chronic value would be less than 12 $\mu\text{g/L}$.

An LC50 could not be calculated for the summer flounder, because complete mortality was observed for all silver concentrations tested, the lowest of which was 12 $\mu\text{g/L}$ (i.e., LC100).

These were 28-day post-hatch, embryo-larval studies, conducted under standardized protocols for chronic studies. One hypothesis for the sensitivity to silver observed in this species is their lack of development at hatch. It should be noted that toxicity tests with the summer flounder have not yet been repeated and these results should be viewed as preliminary. Nevertheless, these values are in agreement with results from short-term, embryo-larval exposures of this species (median LC50, 16 $\mu\text{g/L}$; Cardin 1986). Studies evaluating the effects of silver on Na^+/K^+ -adenosine triphosphatase (ATPase) activity in tidepool sculpins and plainsfin midshipmen, after being exposed to AgNO_3 (1.5 to 14.5 $\mu\text{g/L}$ as silver) for 21 days demonstrated physiological mechanism disruptions (Webb et al. 2000).

Recommendations

Considering the identified importance of certain ligands in test dilution waters, dilution waters used in future toxicity studies should be well characterized. Specific to silver, the following should be measured in dilution waters: sulfide (1 nM detection limit), chloride, dissolved organic carbon (0.1 mg/L detection limit), sodium, calcium, alkalinity, hardness, and pH. Silver (total recoverable and dissolved) should be measured in the exposure tanks in all tests and clean sampling techniques employed, especially in chronic studies involving subpart-per-billion concentrations. Annual characterization of dilution waters and food for priority pollutants is also desirable.

There remains a need for more chronic data on organisms that are sensitive to silver, considering a variety of water characteristics.

Freshwater studies: Regulatory considerations

Based on available information, it is recommended that the acute criterion be derived using the existing methodology after the toxicity database has been updated to include studies performed since 1987. It is also recommended that implementation of the acute criterion include use of the BLM, as a site-specific modification (see section: *The BLM approach as a predictive tool for silver toxicity*), to reflect silver bioavailability in the aqueous environment more accurately.

Derivation of a chronic criterion is not recommended until additional studies can be performed. Two problems have been identified with the existing chronic data set:

- 1) potential exclusion of metastable sulfides from test media in most or all chronic toxicity studies, and
- 2) lack of valid daphnid chronic data.

It is recommended against applying an ACR for silver, so far derived largely using insensitive fish species from acute studies, because there is evidence that, for at least some chemicals, a relationship exists between acute and chronic sensitivity (i.e., the ACR increases with decreasing acute sensitivity). Consequently, application of an ACR based on insensitive species (i.e., fish) to estimate chronic sensitivity of sensitive species (i.e., daphnids in acute studies) is not appropriate.

A protocol for chronic cladoceran testing, considering the significance of food presence during the studies, should be developed. Studies evaluating metastable sulfides and chronic daphnid sensitivity to silver should be completed within a reasonable time. A chronic criterion should be implemented after these studies have been completed and interpreted.

Freshwater studies: Research consideration

Testing the chronic sensitivity of additional species is also highly desirable.

Marine studies: Regulatory considerations

The acute marine criterion for silver should be derived using existing methodology after data have been updated to reflect post-1987 acute toxicity test results.

Considering the dearth of chronic toxicity data for marine organisms, development of a marine chronic criterion is not recommended at this time. A chronic criterion should be derived at some point in the future, once sufficient data become available.

Food or trophic transfer studies: Research consideration

Additional research is needed on food and trophic transfer versus waterborne as routes of silver exposure and uptake (see section: *Silver body burden*).

Wastewater treatment plant microbes

Activated sludge microbes have been shown to continuously treat wastewater containing silver at concentrations 100 to 1000 times greater than concentrations known to affect aquatic animals (Pavlostathis and Maeng 1998). Consequently, silver is unlikely to affect activated sludge systems adversely.

Terrestrial plants and animals

When terrestrial plants and animals were exposed to AgNO_3 , the form of silver predominantly used in laboratory aquatic and sediment toxicity studies, they were

far less (100 to 10,000 times) sensitive than aquatic organisms exposed to AgNO_3 (Hirsch 1998c; Ratte 1999). Additionally, the form of silver predominantly found in the terrestrial environment is silver sulfide, which has little or no effect on plants at concentrations 100 to 1000 times greater than found naturally in the environment or biosludges (Ratte 1999). Due to low sensitivity to silver, terrestrial life was not considered a concern to the authors of this chapter, and thus not discussed further.

Mechanisms of Acute Toxicity

The Biotic Ligand Modeling as a predictive tool for silver toxicity

The BLM (Paquin et al. 1999) assesses acute silver exposure on a water chemistry specific basis and predicts mortality in fresh waters (Paquin et al. 1999; McGeer et al. 2000; DiToro et al. 2001). It represents a dramatic advancement in understanding how to model effects of acute silver exposure to freshwater biota accurately. Currently, the key BLM contribution is to function as an improved modifier for predicting acute silver toxicity on a site-specific basis. The BLM has been validated with daphnids, fathead minnows, and rainbow trout. As such, the BLM offers a vast improvement over the "hardness equation" approach (USEPA 1980) in predicting the influence of water chemistry on acute silver effects.

Biotic ligand modelings for silver are based on the original experiments of Janes and Playle (1995), building on ideas expressed by Pagenkopf (1983); these models use derived conditional equilibrium binding constants to model silver binding to rainbow trout gills. Clearly, a strength inherent in these gill loading or tissue residue-based models is the ability to incorporate competitive influences of various cations (e.g., Ca^{2+} , H^+ , and Na^+) as well as the influence of important Ag^+ complexing anions such as NOM, thiosulfate, Cl^- , and possibly metastable sulfide within the modeling framework (Figure 2-7). This mechanistically derived model, originally based on the physiological mechanism of silver loading on rainbow trout gills, estimates toxicity based on predicted Ag^+ loading to theoretical binding sites to produce acute toxicity in aquatic biota.

In freshwater rainbow trout, acute effects of silver exposure result from physiological disruption caused by Ag^+ binding to gills (Morgan et al. 1997; McGeer and Wood 1998; Bury et al. 1999). The primary physiological disturbance, and presumed toxicity mechanism, arising from Ag^+ binding to gills is inhibition of Na^+ and Cl^- uptake at the gill with consequent declines in plasma Na^+ and Cl^- concentrations (Wood et al. 1996; Morgan et al. 1997; Webb and Wood 1998). In turn, a suite of secondary effects, including blood acidosis, generalized stress response, increased plasma total ammonia concentration, fluid volume disturbance, and hemoconcentration, if severe, leads to death from cardiovascular collapse (Wood et al. 1996; reviewed by Hogstrand and Wood 1998; Webb and Wood 1998). This

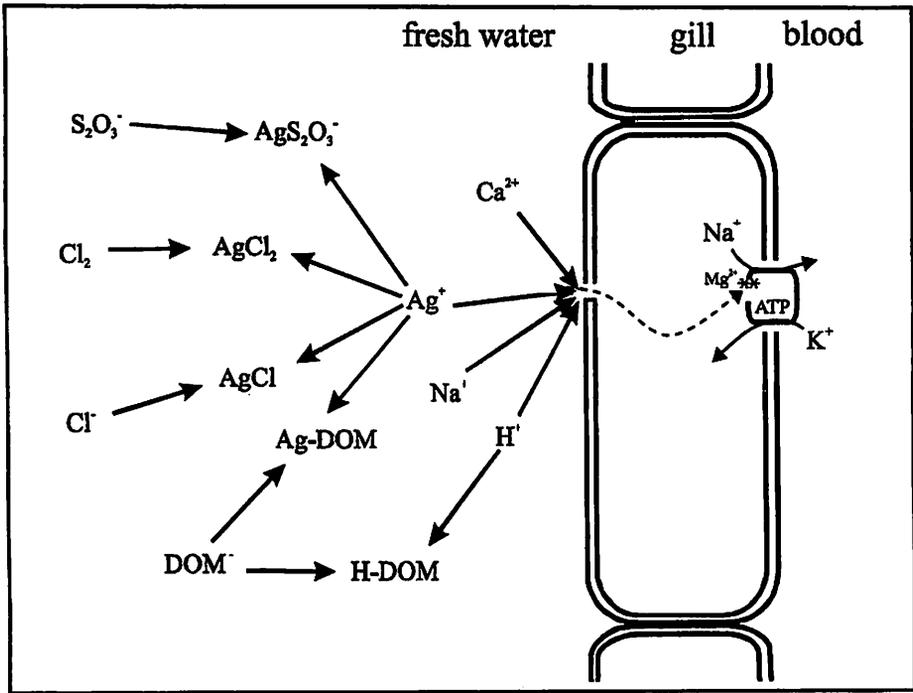


Figure 2-7 Conceptual representation of some of the anionic complexation and cationic competition reactions that influence the binding of Ag^+ to a biotic ligand where it will exert its toxic effect (from McGeer et al. 2000).

disruption of ion uptake in rainbow trout stems primarily from inhibition of gill Na^+/K^+ -ATPase activity. Na^+/K^+ -ATPase is considered to be the key enzyme powering active Na^+ and Cl^- transport in freshwater fish, and it has been established that the inhibitory effect of Ag^+ on Na^+/K^+ -ATPase is due to blocked binding of Mg^{2+} , a cofactor required for adenosine triphosphate (ATP) hydrolysis (Ferguson et al. 1996).

The current BLMs (e.g., Janes and Playle 1995; Paquin et al. 1999; McGeer et al. 2000) should be viewed as an initial platform for predicting silver toxicity and, in principle, show promise for further development and applicability beyond acute toxicity in fresh water. Additionally, validation and improvement of the model for acute silver toxicity in fresh water should be carried out. Validation and model improvement should include testing additional aquatic species and water chemistries to ensure the broad applicability of the modeling framework in fresh water. For example, characterization of silver complexation by various NOM sources is currently limited by little information on availability of measured silver complexation and speciation. Given the ubiquitous nature of NOM and the importance of organic functional groups (e.g., sulfhydryl) to silver speciation, additional experimental work is needed to advance Ag speciation models.

Applicability of the BLM to predict acute silver toxicity in seawater and estuarine waters also should be explored. It is assumed that if the mechanism of acute silver toxicity in saline waters is similar to that of fresh waters, the BLM would apply. However, there is good evidence that the mechanism of silver toxicity in marine fish is not an inhibition of gill Na^+/K^+ -ATPase activity but is related to processes occurring in the gut integument (see Wood et al. 1999). Given the possibility of a different toxicity mechanism, the BLM approach would have to be adapted to include the physical chemistry (conditional equilibrium binding constants) of silver loading onto these different toxic binding sites and exposure characterization in the gut as it relates to measured water column speciation.

Similar to the marine situation, an understanding of the BLM applicability in describing chronic silver toxicity cannot be made without further information on the role of water chemistry and route of uptake on toxicity. Chronic silver exposure includes both waterborne and food route uptake, including trophic transfer, and each has its own complex equilibrium chemistry. Additionally, the possibility of multiple binding sites within the organism, not all of which will produce toxicity, further complicates model development. Experimental work directed at modeling chronic toxicity is complex and would require extensive characterization of water chemistry, silver bioavailability, and endpoint response.

Existing field sites of silver effects in the natural environment (beta sites) or mesocosm studies could provide a unique opportunity to develop the relationships required for modeling but which would require extensive and exhaustive characterization. As knowledge develops, strategies for modeling chronic toxicity in an accurate and scientifically defensible manner may become apparent.

Regulatory considerations

Based on the available information the following should be considered:

- The BLM should be used for site-specific modification of acute criteria in freshwater.
- At the present time, the BLM should not be used for chronic criterion development. The possibility of alternate routes of exposure must be considered.

Research considerations

Research should be considered in the following areas:

- The BLM needs further validation in a wider variety of water qualities and aquatic species.
- There is a need to evaluate the potential applicability of the BLM in estuarine and marine systems.

Mechanisms of Chronic Toxicity

Background

Whereas acute toxicity is defined by short exposures with death typically the endpoint, chronic responses to exposure are not as easily defined. During such exposures, the site of toxicity could be the same as that during acute exposures with only rate of events being slower. Often, however, other more subtle mechanisms lead to chronic toxicity. One problem here is that although water column exposure is the dominating factor for acute toxicity, other uptake routes may be more important in chronic effects. It is not always easy to identify the most sensitive target, and exposures via multiple routes are difficult to conduct under controlled conditions. Unlike acute tests, chronic tests require feeding, and because silver will bind with food, the organisms will be exposed to ingested silver.

The mechanism by which silver causes acute toxicity in freshwater fish has now been identified (reviewed by Hogstrand and Wood 1998; Wood et al. 1999). In brief, exposed fish suffer a progressive net loss of these ions from blood plasma, and eventually die from a suite of internal disturbances, most importantly cardiovascular collapse. Most freshwater invertebrates (e.g., daphnids) have similar pathways of active ion transport, which suggests the mechanism of acute toxicity in these more sensitive organisms is likely to be the same as in freshwater fish, although this has not yet been confirmed. Only free Ag^+ ions, and not complexed forms of silver, seem to cause this ionoregulatory effect, thereby explaining why complexed silver exerts negligible acute toxicity. Understanding this acute toxic mechanism has led to the BLM described above (Janes and Playle 1995; Playle 1998; Paquin et al. 1999; McGeer et al. 2000). These powerful and cost-efficient tools can be used to develop site-specific modifications of acute WQC for silver based on measured receiving water chemistry.

Predicting chronic aquatic toxicity

An extension of this BLM approach to predict chronic toxicity would be very welcome, but would only be valid if the mechanism (i.e., ionoregulatory disturbance) and chemical species (i.e., free Ag^+ ion) are the same as those at chronic exposure levels. Similarly, use of the ACR approach for derivation of chronic WQC implicitly assumes that the mechanism and toxic species are the same as at acute levels. An additional untested assumption of such extrapolations is that chronic silver toxicity, resulting from dietary exposure, is unimportant. Unfortunately, our knowledge of the mechanisms of chronic toxicity is fragmentary, so neither of these approaches can be justified yet. Indeed, there are already some data suggesting these assumptions are invalid and, therefore, that such extrapolations from acute to chronic criteria should not be performed.

A modeling framework for chronic toxicity would have to be specific for chronic endpoints and the factors that regulate these. The scientific background information to build such chronic models is simply not available. Hence, only chronic toxicity tests performed under “real-world” conditions should be used to generate a chronic WQC for silver.

Most chronic toxicity tests have been performed in synthetic waters (i.e., water that has been distilled, deionized, or treated by reverse osmosis) or waters treated by chlorination, dechlorination, or carbon filtration. As a result, it is likely that—unwittingly—these tests have been performed in the absence of metastable sulfide. The ubiquity of sulfide in oxic natural waters, a relatively new finding highlighted at the 1999 Argentum VI workshop (Luther and Tsamakis 1989; Rozan et al. 1999; Tang and Santschi 2000; see Chapter 1), suggests that free Ag^+ ion does not normally exist in natural environments. Total silver levels in fresh surface waters are usually in the low ng/L range. The nanomolar (metastable) sulfide levels naturally present in oxic waters should be sufficient to bind all silver up to the 100 to 300 ng/L (1 to 3 nM) range, the typical NOEC or LOEC levels reported in chronic toxicity studies performed in the absence of sulfides (reviewed by Ratte 1999). If chronic toxicity does occur in the real world, it likely stems from exposure to silver sulfide, not from exposure to free Ag^+ . Thus, results from chronic tests performed in the absence of metastable sulfides and other natural ligands must be interpreted with caution, i.e., the observed effect may be due to very low levels of free Ag^+ ions that are present (an environmentally abnormal situation) and not to silver sulfide complexes (the more normal environmental situation). Indeed, the authors are aware of no information on the toxicity of dissolved silver sulfide complexes. There is clearly an urgent need for such tests, which has led, during this workshop, to the design of exploratory experiments (see Chapter 1).

Route of exposure

Based on studies with marine and freshwater invertebrates, there is evidence that exposure routes other than that from waterborne silver could be critical in defining low-level chronic silver toxicity. Such toxicity seems to occur not by direct contact of organisms with waterborne silver, but rather through organisms ingesting algae or other food particles that have come in contact with waterborne silver at concentrations down to a few ng/L (for extended periods of time, e.g., 72 to 96 hours; Schmittschmitt et al. 1996; Fisher and Hook 1997; Hornberger et al. 1999; Hook and Fisher 2001; also see Chapter 3). In laboratory studies with freshwater cladocerans and marine copepods, silver-loaded algae were used in dietary exposures. The algae concentrated silver by adsorption or absorption, and toxicity was manifested as decreased reproductive potential in the filter feeding crustaceans that ingested the contaminated food.

Evidence of silver effects in the natural environment, although copper or cadmium also was present from POTW wastewater discharges, comes from field studies on

two marine bivalve species in the San Francisco Bay, CA, USA (Lee et al. 2000; also see Chapter 3). In essence, for over two decades there have been dramatic spatial and temporal decreases of silver in water, sediments, and bivalve tissues. Changes in silver tissue levels were inversely correlated to the number of gametes produced. Careful concomitant monitoring of a large number of environmental variables, as well as some physiological indices, suggested that silver was the primary cause of reduced gamete production, although copper and other metals plus other unmeasured substances, also may have been present. Whereas this correlative evidence does not “prove” that silver in the environment impairs reproductive potential, it does raise concern that silver in heavily polluted environments might cause chronic toxicity. This is especially true in light of the observed reduced production of cladoceran and copepod offspring in laboratory studies, in which silver-laden algae, exposed to Ag^+ concentrations (as silver nitrate) as low as 25 ng/L (0.25 nM) for 72 to 96 hours, were fed to the planktonic organisms (Schmittschmitt et al. 1996; Fisher and Hook 1997; Hornberger et al. 1999; Bielmyer et al. 2001; Hook and Fisher 2001; Chapter 3).

The mechanism of reduced gamete and offspring production could involve effects on production and transfer of egg yolk protein (vitellogenin), including accumulation of silver in developing oocytes. Such effects have been observed with cadmium in fish in which cadmium inhibits vitellogenin synthesis (Olsson et al. 1995) and is transported into the oocytes bound to vitellogenin (Ghosh and Thomas 1995). Alternatively, the mechanism could simply be diversion of metabolic resources away from reproduction to metal detoxification (e.g., synthesis of metal binding proteins), but this remains unproven. Nevertheless, chronic toxicity resulting from trophic transfer, filter feeding, or sediment ingestion is yet another reason that may invalidate extrapolation from acute to chronic toxicity. Further research is urgently needed in this area, especially to test conclusively whether silver body burden in bivalves is related to chronic toxicity and whether similar phenomena are seen in fishes and amphibians. It should be noted that, although documented chronic silver toxicity to aquatic vertebrates occurs at levels that are more than one order of magnitude higher than those producing reproductive effects in invertebrates, reproduction has never been studied as an endpoint of silver toxicity in either fishes or amphibians (with the exception of unpublished mesocosm studies, described in Use of field studies and mesocosms in ecological risk assessment of silver).

Although recognizing the preceding caveats, it remains useful to summarize the sparse existing data on mechanisms of chronic toxicity in freshwater fish as revealed by tests performed in the nominal absence of metastable sulfides and other natural ligands. In general, such tests (in the range of 0.1 to 2 $\mu\text{g}/\text{L}$ [1 to 20 nM], added as AgNO_3) indicate that ionoregulatory disturbance occurs in very early life-stages (Galvez et al. 2001; Guadagnolo et al. 2001) and in juvenile rainbow trout (Galvez et al. 1998)—i.e., a similar or identical toxic mechanism to

that seen in acute exposures. In addition, a 5-month exposure of rainbow trout to Ag^+ resulted in small but significant negative effects on smoltification. Specifically, silver-exposed fish were less able to regulate sodium on transfer to seawater (Ferguson and Hogstrand 1997). Exposure of rainbow trout during early life stages to silver exerted a range of effects on growth, hatching time, and ionoregulation at concentrations of $1 \mu\text{g/L}$ (10 nM) or greater (Guadagnolo et al. 2001). These observations on decreased growth are in agreement with the classic work of Davies et al. (1978) on rainbow trout and the recent reports of Davies et al. (1998) on rainbow and brown trout. Nebeker et al. (1983) and Holcombe et al. (1983) reported similar growth inhibition and/or elevated mortality in embryo-larval tests with steelhead (rainbow) trout and fathead minnow, respectively. Given this biphasic response over time, it remains unclear whether ionoregulatory disturbance is the key mechanism, or indeed the only mechanism, that drives chronic responses in freshwater fish. The linkage between ionoregulatory effects and growth or developmental effects remains unproven.

Other factors, such as disturbance of internal homeostasis of other metals (depletion of zinc or copper, perhaps by competition for binding sites on metal-binding proteins) as seen in chronic studies on adult bluegill, largemouth bass (Coleman and Cearley 1974), and rainbow trout (Galvez et al. 1997, 2001) are another possibility. Silver has a known ability to displace zinc and copper from sulfhydryl groups on proteins, including metallothionein (Kagi and Schaffer 1988). Silver is also a powerful inducing agent for synthesis of new metallothionein in fish (Cosson 1994; Ferguson et al. 1996; Hogstrand et al. 1996; Hogstrand and Wood 1998), a process that very likely carries additional metabolic costs. As with observations of invertebrates manifesting decreased fecundity as a result of ingesting Ag -contaminated food, chronic responses in developing fish may simply result from diversion of metabolic resources away from growth processes towards either ionoregulatory processes or metal detoxification.

The critical need to identify targets for chronic toxicity and understand the mechanisms stems from what was learned by understanding acute toxicity, which helped to explain the influence of water chemistry and led to development of the BLM. For chronic responses, a mechanistic understanding of effects will help develop more reliable and cost-effective tools to regulate against potential chronic toxicity.

Research considerations

There is a need to understand the mechanisms of silver toxicity during chronic exposures. Particular emphasis should be placed on the potential toxicity of dissolved silver sulfide complexes and on metabolic, developmental, and reproductive effects. There also is a need to understand the mechanisms of toxicity stemming from the trophic transfer of silver.

Silver Body Burden

There are continuing disagreements concerning the importance of significant body burdens of silver. On the subject of silver body burden in aquatic biota, there was agreement that silver residues in fishes and invertebrates constitute evidence of exposure to bioavailable silver but not necessarily to bioreactive silver, nor does silver body burden necessarily indicate deleterious effects on exposed organisms. The present procedure for developing regulatory criteria should not use silver body burden as a determining factor. Research in progress may or may not support changing the present procedure.

Recent investigations (Birge et al. 2000) have shown that, depending on the biotic species, silver body burden may be useful in distinguishing between bioavailable and non-bioavailable silver in point-source discharges, confirming silver exposure of aquatic biota, and estimating bioavailable Ag concentrations in the water column. Concerning the latter, silver body burden in the stoneroller minnow (*Campostoma anomolum*), determined for organisms from different monitoring stations, correlated strongly with patterns of species richness, abundance, and bioassessment scores for macroinvertebrates. Using a new approach, proportional differences in silver body burden also were used to develop "metal multipliers" for application to total recoverable silver. This permitted calculating the bioavailable Ag, Cd, and Cu in the water column; these values correlated well with macroinvertebrate community metrics. Stoneroller minnows did not appear to be affected by silver or other metals.

At upstream and downstream monitoring stations where no impact was observed, silver body burden measured in stoneroller minnows was in the range of 0.2 to 0.4 $\mu\text{g/g}$ and calculated concentrations of bioavailable silver in suspended sediments ranged from 8 to 16 $\mu\text{g/g}$. The maximum bioavailable silver concentration in the effluent receiving zone was 140 ng/L (1.4 nM). Downstream, bioavailable silver fractions decreased to about 16% of total recoverable silver.

Other studies have focused on the trophic transfer of silver from primary producers to consumers and have established relationships between silver body burden and reproductive failure (Schmittschmitt et al. 1996; Fisher and Hook 1997). In the study by Schmittschmitt et al. (1996), no silver or other heavy metals were added to or detected in the culture water used for the 3-brood test with *Ceriodaphnia dubia*. Neonate production was sharply reduced when silver body burden in *C. dubia* reached a range of 1.8 to 3.8 ng/g after being fed algae containing silver concentrations of 1.1 to 1.3 $\mu\text{g/g}$. However, silver body burden in *C. dubia* decreased to 0.3 to 0.8 ng/g when the algae fed to them contained 3.2 to 6.4 $\mu\text{g/g}$ silver. The authors suggest that this occurred because silver inhibits feeding at these concentrations (Schmittschmitt et al. 1996), a response that has been observed by others (Bitton et al. 1996; Galvez and Wood 1999).

In investigations with marine bivalves, silver tissue residues appeared related to reduced gamete production (Hornberger et al. 1999). However, results were complicated by the presence of copper and other metals. Nevertheless, the link between silver body burden and reproductive performance deserves further attention. Further research on the physiological, toxicological, and ecological significance of silver body burden appears necessary. Particular concerns involve

- 1) determining possible effects of tissue metal residues in reproductive organs, eggs, sperm, and embryos;
- 2) characterizing routes of uptake that contribute to silver body burden;
- 3) quantifying effects of trophic transfer of silver on growth and reproduction; and
- 4) understanding physiological and toxicological mechanisms involved in silver assimilation, metabolism, and depuration.

Regulatory recommendation

At the present time, body burden should not be used as a regulatory endpoint. Body burden can be used as a biomarker of exposure.

Research consideration

More research should be considered relating body burdens of silver to toxicological endpoints.

Biological Effects of Silver in Sediments

Several studies have evaluated the toxicity of silver-amended sediments for benthic organisms. Rodgers et al. (1997) tested the toxicity of 3 silver salts, AgNO_3 , $\text{Ag}(\text{S}_2\text{O}_3)^-$ (a mixture of silver halide and $\text{Na}_2\text{S}_2\text{O}_3$, and AgCl , to *Hyaella azteca* in 4 different freshwater sediments. They found the AgNO_3 salt to be the most toxic of the three. LC50 values for silver after 10 days of exposure were 1.62, 45.4, 60.7, and 379.7 mg/kg for AgNO_3 , whereas for AgCl the 10-day LC50 values were all > 2500 mg/kg, the highest concentration tested. For the silver thiosulfate solution, the 10-day LC50 values were > 569, > 648, > 682, and > 1125 mg/kg (the highest concentration tested). Hirsch (1998b) found that Ag_2S -amended freshwater sediments were not toxic to *H. azteca* over 10 days at the highest tested concentration of 753.3 mg/kg. In a study of silver bioaccumulation from Ag_2S -amended freshwater sediment (Hirsch 1998a), the oligochaete worm *Lumbriculus variegatus* had an estimated silver bioaccumulation factor of 0.18 over a 28-day exposure. Call et al. (1999) obtained 10-day LC50 values of 2750 and 1170 mg/kg of Ag for *Chironomus tentans* tested with AgNO_3 -amended sediments from two lakes. In marine sediments, Berry et al. (1999) tested the amphipod *Ampelisca abdita* using AgNO_3 -amended sediments. In three different sediments, they observed high toxicity (i.e.,

50% to 100% mortality) at sediment exposures starting at 194, 89.5, and 12.9 mg/kg (1.8, 0.83, and 0.12 mmol/kg).

These studies have shown that the sulfide salt of Ag is neither readily bioavailable nor toxic. The thiosulfate and chloride salts also did not exhibit toxicity. The nitrate salt did exhibit toxicity, but at concentrations of Ag in the sediment that were very high in most cases. Differences in the basic characteristics of the sediments and their resultant capacities for binding the silver resulted in sediment-specific toxicity.

Application of the simultaneously extracted metal or acid-volatile sulfide U.S. Environmental Protection Agency method

This method involves measuring the sediment-bound sulfide that reacts with cold 1 M HCl AVS while determining the simultaneously extracted metal (SEM). When $[\text{SEM} - \text{AVS}] \leq 0$, all metal is assumed to be bound as very insoluble metal sulfides, yielding negligible porewater metal activity. This activity correlates to acute toxicity for several metals. Extensive laboratory studies have verified this method for the metals Cd, Cu, Zn, Ni, and Pb. Several laboratory and field validation studies have confirmed the method for Cd and Zn. Recent studies with silver by Berry et al. (1999), Call et al. (1999), Hirsch (1998a), Crecelius et al. 1997, and Rodgers et al. (1997) have shown that the method can be extended to both marine and freshwater sediments. In applying the method to silver, however, certain adjustments must be made (see Chapter 1). Ambient sediment quality criteria might be developed based upon concentrations of AVS plus organic carbon for anoxic waters and organic carbon plus amorphous Fe(II) oxides for oxygenated waters (Call et al. 1999).

Certain cautionary notes to the application of simultaneously extracted metal or acid-volatile sulfide

Recently, certain studies suggested the possibility that some metals (Cd, Ni, and Zn) bioaccumulate in sediment-dwelling organisms and bivalves when excess AVS is present. In these studies, however, as well as in field validation studies, no-adverse-effects were observed. Research on benthic invertebrates in San Francisco Bay, CA, USA, suggests that ingested silver and copper, in the presence of AVS, adversely affected reproduction, although there may have been water column exposure or other stressors present (Lee et al. 2000). On the other hand, Hirsch (1998a) reported no adverse growth or reproductive effects from exposure to silver, as silver sulfide, following bioaccumulation in oligochaetes.

There is no doubt that silver should be included among metals whose acute sediment toxicity is proposed to be controlled by AVS. However, misgivings about the SEM-AVS guideline, raised by two workshop participants, were that the method relies on anoxia to protect oxygen-requiring organisms, it only predicts nontoxicity, and it categorizes sediments much more on the basis of AVS than on SEM. Pore

waters in sediments with AVS are anoxic, so benthic organisms would not allow themselves exposure to the environment proposed to be controlling their exposure to metals. Although sediments are, by far, commonly nontoxic, the fact that the guideline is usually correct does not verify its underlying validity. Lastly, when sediments are categorized into those that AVS exceeds SEM and those that it does not, SEM concentrations in both groups are similar whereas AVS levels are very different. In effect, the guideline regulates sediments on the basis of AVS and not metal concentrations.

In response, it should be noted that metal behavior in the oxic layer is affected by the anoxic layer chemistry and by oxidation kinetics of metal sulfides. At the present time, neither of these seems to discredit the SEM-AVS approach. Additionally, SEM concentrations vary greatly, and it is in those sites that have relatively high metal (or very low AVS) concentrations that the SEM-AVS method is particularly valuable. Finally, it would not seem inappropriate to regulate one substance on the basis of another, if it is indeed the property of the latter that regulates the former.

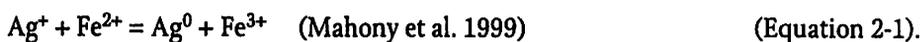
Additional sinks for silver in sediments

Sediment organic carbon

Previous work (Mahony et al. 1996; Mahony et al. 1997) has shown that adsorption of some heavy metals (Cd, Cu, Pb) to sediments can be correlated to sediment OM. This provides an additional correlative mechanism that regulates porewater activity of the metal. If carbon-normalized binding constants are known, then regulation of the metal relative to sediment toxicity can be further established. Thus, at a given organic carbon percentage and porewater concentration equal to the water-quality criterion, the sorbed metal concentration, in addition to the sulfide bound component, can be calculated and incorporated into the equilibrium sediment guideline (ESG). For silver, organic carbon with organic reduced sulfur groups is known to be a strong complexing agent. Some preliminary binding constants have been determined (refer to Chapter 1, Table 1-4).

Sediment iron

Preliminary studies with Ag^+ seem to argue for multiple binding sites. For example, Call et al. (1999) observed that freshwater sediments appeared to bind Ag^+ to an extent that was greater than what could be explained by AVS and organic carbon combined and suggested that amorphous iron oxides were involved. Others have determined that iron hydroxide and oxyhydroxide (goethite) have an adsorptive affinity for silver and other metals (Wingert-Runge and Andren 1993, 1995). Recently it was shown that Fe(II) may be a somewhat unique sink for silver in sediments according to the reaction



Further investigations of this process in sediments are under way and, if its validity is fully established, then the recommendation is that iron also be incorporated into the determination of ESG for silver. It should be noted that this would be especially applicable in sediments with little or no AVS or organic carbon (Mahony et al. 1996; Call et al. 1999). Preliminary studies argue for multiple binding sites (Call et al. 1999).

Sediment metal sulfides

Finally, it is of minor significance that Ag_2S is the most stable of the environmentally important metal sulfides. Thus, sediments containing metal sulfides other than FeS would have these as sinks for silver insofar as silver would replace the metal in them. Because silver concentrations are usually low, it is predicted that consequent release of other metals would not be sufficient to produce toxicity.

Regulatory recommendations

The following regulatory considerations for sediments are recommended:

- The SEM-AVS method, suitably adjusted, should be applied to silver in assessing the potential for its acute toxicity in sediments.
- Organic matter should be incorporated into determining the ESG for silver, when a more complete set of binding constants is available.
- Additional binding capacities of particulate OM and iron for silver should be applied when calculating any site-specific ESGs.

Use of Field Studies and Mesocosms in Ecological Risk Assessment of Silver

Rationale for use

Evidence that exposure of aquatic and terrestrial organisms to environmentally relevant silver species and concentrations has or has not caused population-level effects would be useful to better understand or apply laboratory findings to the environment. Hopefully, such studies would lead to sound regulations for silver. There are several uncertainties in conducting a risk assessment for silver. Whereas acute aquatic and sediment toxicity are relatively well understood for silver, chronic toxicity mechanisms and dietary routes of exposure require more evaluation. Field studies, conducted under natural conditions where the aquatic community is exposed to silver, or studies in experimental ecosystems ("mesocosms") are types of test system in which population-level and community-level effects of silver exposure (solely or in combination with other metals) could be studied. Such studies would encompass chronic, multiple route exposures and include trophic-level transfer.

The panel was aware of only two mesocosm studies (Gorsuch and Ewell 1977; Ewell et al. 1993; Ratte 1999) using silver thiosulfate and two published field studies (Birge et al. 2000; Lee et al. 2000) that included silver. Yet several mesocosm studies have been conducted on other chemicals, including pesticides (Crossland and La Point 1992; Graney et al. 1994), metals (Giesy et al. 1981; Niederlehner et al. 1985), and surfactants (Belanger 1994). In Europe, a number of workshops have been held and a working document has been prepared outlining the use of mesocosms to study the environmental hazard of chemicals. The conclusion of scientific experts at these workshops has been that lentic, freshwater mesocosms are particularly useful to study chemical fate, trophic transfer, and the identification of population-level and community-level responses to chemical exposure (refer to La Point and Persoone 1999). Such field studies would be conducive to understanding the effects of silver exposure at environmentally realistic concentrations.

Whereas natural field studies are useful in identifying bioavailable silver and in viewing population dynamics as a correlate to silver in the environment, there are some interpretive difficulties because other variables (stressors, e.g., physical, biological, chemical) may be responsible for changes in populations. Mesocosm studies can help reduce, or at least account for, such variables. Using experimental ecosystems allows a focus on silver fate, alone, or a focus on community responses including trophic transfer through the epibenthos, planktonic cladocera and into fish. Ideally, the dosing regime and chemical or physical milieu will be selected only after careful discussions among toxicologists, ecologists, and chemists.

Do laboratory experiments support and agree with field studies? In general, the answer to this question would be "no." Water-effect ratio (WER) studies that include complexing agents such as NOM, suspended particulates, and other ligands generally show that toxicity to fishes and daphnids is greatly reduced, by as much as 60 times over that in test solutions with AgNO_3 (Erickson et al. 1998). However, there is a range of WERs; for example, Diamond et al. (1990) measured a WER of less than 1.0 for silver in the New River, VA, USA. Some whole effluent toxicity (WET) studies of POTW effluents have shown no effects when silver has been present (most likely as particulate silver) at concentrations that would have been lethal if silver nitrate had been used. For example, as part of National Pollutant Discharge Elimination System (NPDES) requirements, short-term chronic experiments were conducted with 8 sets (2 sets per year) of 7-day fathead minnow and 7-day ceriodaphnid studies in CO, USA, using 100% effluent and 4 serial dilutions, containing up to 33 $\mu\text{g/L}$ (average 8 $\mu\text{g/L}$) of silver. There were no observed effects on growth or development of the minnows or reproductive impairment of ceriodaphnids below 25% in all studies and up to 100% effluent in some studies (Kodak Colorado Division 1995 to 1998). Unpublished results (data included in Ratte 1999) of a second Kodak field study, conducted for one year in flow-through ponds using silver thiosulfate added at 1 mg/L (10,000 nM), demonstrated that

fathead minnow reproduction took place and that these fish grew normally, as did American toad tadpoles observed in the first study (Gorsuch and Ewell 1977). The green algae, *Chlamydomonas*, *Chroococcus*, and *Chlorella* were abundant, as were zooplankton, chironomids, and corixids. These results are consistent with early life stage laboratory findings using silver thiosulfate and fathead minnows (LeBlanc et al. 1984).

Research consideration

Field tests and mesocosm studies should be considered in the risk assessment of silver and are highly recommended to understand trophic transfer, silver fate, and population responses.

APPENDIX TO CHAPTER 2

Table 2-1 Acute silver toxicity data for freshwater organisms ($\mu\text{g/L}$)

Species	LC50	SMAV	Reference
<i>Alona affinis</i> (cladoceran)	37	37	Ghosh et al. 1990
<i>Aplexa hypnorum</i> (snail)	83	83	Holcombe et al. 1987
<i>Barbus sophore</i> (two spot barb)	7.55	7.55	Khangarot and Ray 1988a
<i>Bufo melanostictus</i> (common Indian toad)	4.1	4.1	Khangarot and Ray 1987
<i>Caenorhabditis elegans</i> (nematode)	100	100	Williams and Dusenbery 1990
<i>Ceriodaphnia dubia</i> (cladoceran)	0.79	0.85	Diamond et al. 1997
<i>Ceriodaphnia dubia</i> (cladoceran)	0.92		Rodgers et al. 1997
<i>Ceriodaphnia reticulata</i> (cladoceran)	1.4	3.9	Elnabarawy et al. 1986
<i>Ceriodaphnia reticulata</i> (cladoceran)	11		Mount and Norberg 1984
<i>Channa punctatus</i> (snake-head catfish)	18.89	18.89	Khangarot and Ray 1988a
<i>Chironomus tentans</i> (midge)	676	676	Rodgers et al. 1997
<i>Cottus baridi</i> (mottled sculpin)	5.3	8.5	Goettl and Davies 1978
<i>Cottus baridi</i> (mottled sculpin)	13.6		Goettl and Davies 1978
<i>Crangonyx pseudogracilis</i> (amphipod)	5	5	Martin and Holdich 1986
<i>Cyprinus carpio</i> (common carp)	2.7	2.7	Rao et al. 1975
<i>Daphnia magna</i> (cladoceran)	0.9	0.9	Holcombe et al. 1987
<i>Daphnia pulex</i> (cladoceran)	1.9	5.2	Elnabarawy et al. 1986
<i>Daphnia pulex</i> (cladoceran)	14		Mount and Norberg 1984
<i>Gambusia affinis</i> (mosquitofish)	23.5	23.5	Diamond et al. 1990
<i>Gammarus pseudolimnaeus</i> (scud)	4.5	4.5	Lima et al. 1982; Call et al. 1983
<i>Hyalella azteca</i> (scud)	1.9	3.6	Diamond et al. 1990
<i>Hyalella azteca</i> (scud)	6.8		Rodgers et al. 1997
<i>Hydra</i> sp. (hydra)	26	26	Brooke et al. 1986
<i>Ictalurus punctatus</i> (channel catfish)	17.3	17.3	Holcombe et al. 1987
<i>Isonychia bicolor</i> (mayfly)	6.8	6.8	Diamond et al. 1990
<i>Jordanella floridae</i> (flagfish)	9.2	9.2	Lima et al. 1982; Call et al. 1983
<i>Lepomis macrochirus</i> (bluegill)	13	13	Holcombe et al. 1987
<i>Leptophlebia</i> sp. (mayfly)	2.2	2.2	Brooke et al. 1986
<i>Leuctra</i> sp. (stonefly)	2.5	2.5	Diamond et al. 1990
<i>Lymnaea luteola</i> (pond snail)	4.2	4.2	Khangarot and Ray 1988b
<i>Moina dubia</i> (cladoceran)	4.5	4.5	Ghosh et al. 1990
<i>Nephropsis obscura</i> (leech)	29	29	Holcombe et al. 1987
<i>Oncorhynchus kisutch</i> (coho salmon)	11.1	11.8	Nishiuchi 1979
<i>Oncorhynchus kisutch</i> (coho salmon)	12.5		Nishiuchi 1979
<i>Oncorhynchus mykiss</i> (rainbow trout)	5.3	13.4	Davies et al. 1978; Goettl and Davies 1978
<i>Oncorhynchus mykiss</i> (rainbow trout)	6		Holcombe et al. 1987
<i>Oncorhynchus mykiss</i> (rainbow trout)	6.2		Davies et al. 1978; Goettl and Davies 1978
<i>Oncorhynchus mykiss</i> (rainbow trout)	6.9		Lemke 1981
<i>Oncorhynchus mykiss</i> (rainbow trout)	8.1		Davies et al. 1978; Goettl and Davies 1978
<i>Oncorhynchus mykiss</i> (rainbow trout)	8.4		Lemke 1981
<i>Oncorhynchus mykiss</i> (rainbow trout)	8.6		Nebeker et al. 1983
<i>Oncorhynchus mykiss</i> (rainbow trout)	9.2		Nebeker et al. 1983
<i>Oncorhynchus mykiss</i> (rainbow trout)	9.7		Lemke 1981
<i>Oncorhynchus mykiss</i> (rainbow trout)	9.7		Nebeker et al. 1983
<i>Oncorhynchus mykiss</i> (rainbow trout)	11.5		Lemke 1981

-continued-

Table 2-1 continued

Species	LC50	SMAV	Reference
<i>Oncorhynchus mykiss</i> (rainbow trout)	13		Davies et al. 1978; Goettl and Davies 1978
<i>Oncorhynchus mykiss</i> (rainbow trout)	14		Lemke 1981
<i>Oncorhynchus mykiss</i> (rainbow trout)	17.87		Lemke 1981
<i>Oncorhynchus mykiss</i> (rainbow trout)	240		Lemke 1981
<i>Oncorhynchus mykiss</i> (rainbow trout)	170		Lemke 1981
<i>Oncorhynchus mykiss</i> (steelhead)	9.2	9.2	Nebeker et al. 1983
<i>Orconectes immunis</i> (crayfish)	560	560	Holcombe et al. 1987
<i>Philodina acuticornis</i> (rotifer)	1400	1543	Buikema et al. 1974
<i>Philodina acuticornis</i> (rotifer)	1700		Buikema et al. 1974
<i>Pimephales promelas</i> (fathead minnow)	7.8	11.1	Erickson et al. 1998
<i>Pimephales promelas</i> (fathead minnow)	3.9		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	5		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	5.3		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	5.6		Lemke 1981; Nebeker et al. 1983
<i>Pimephales promelas</i> (fathead minnow)	6.3		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	6.7		Holcombe et al. 1983
<i>Pimephales promelas</i> (fathead minnow)	7.4		Lemke 1981; Nebeker et al. 1983
<i>Pimephales promelas</i> (fathead minnow)	9		Holcombe et al. 1987
<i>Pimephales promelas</i> (fathead minnow)	10.7		Lima et al. 1982; Call et al. 1983
<i>Pimephales promelas</i> (fathead minnow)	10.98		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	11.1		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	11.75		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	16		EG&G Bionomics 1979; LeBlanc et al. 1984
<i>Pimephales promelas</i> (fathead minnow)	110		Lemke 1981
<i>Pimephales promelas</i> (fathead minnow)	150		Lemke 1981
<i>Poecilia reticulata</i> (guppy)	6.44	6.44	Khengarot and Ray 1988a
<i>Psephenus herricki</i> (water penny beetle)	> 306	> 306	Diamond et al. 1990
<i>Rana hexadactyla</i> (frog)	25.7	25.7	Khengarot et al. 1985
<i>Rhinichthys osculus</i> (speckled dace)	4.9	8.2	Goettl and Davies 1978
<i>Rhinichthys osculus</i> (speckled dace)	13.6		Goettl and Davies 1978
<i>Salmo trutta</i> (brown trout)	1.17	1.17	Davies et al. 1998
<i>Simocephalus vetulus</i> (cladoceran)	15	15	Mount and Norberg 1984
<i>Stenonema modestum</i> (mayfly)	3.9	3.9	Diamond et al. 1990
<i>Tanytarsus dissimilis</i> (midge)	420	420	Holcombe et al. 1987
<i>Thymallus arcticus</i> (Arctic grayling)	6.7	8.6	Nishiuchi 1979
<i>Thymallus arcticus</i> (Arctic grayling)	11.1		Nishiuchi 1979
<i>Tubifex tubifex</i> (tubificid worm)	31	31	Khengarot 1991

SMAV = Species mean acute value

Table 2-2 Chronic silver toxicity data for freshwater organisms ($\mu\text{g/L}$)

Species	NOEC	LOEC	Chronic Value	SMCV	Reference
<i>Ceriodaphnia dubia</i> (cladoceran)	0.53	1.14	0.78	0.78	Rodgers et al. 1997
<i>Corbicula manilensis</i> (Asiatic clam)	2.6	7.8	4.5	4.5	Diamond et al. 1990
<i>Daphnia magna</i> (cladoceran)	20	41	29	5.9	Nebeker 1982
<i>Daphnia magna</i> (cladoceran)	10.5	21.2	14.9		Nebeker 1982
<i>Daphnia magna</i> (cladoceran)	8.8	19.4	13.1		Nebeker 1982; Nebeker et al. 1983
<i>Daphnia magna</i> (cladoceran)	3.4	8	5.2		Nebeker 1982; Nebeker et al. 1983
<i>Daphnia magna</i> (cladoceran)	2.7	3.9	3.2		Nebeker 1982
<i>Daphnia magna</i> (cladoceran)	1.6	4.1	2.6		Nebeker 1982; Nebeker et al. 1983
<i>Daphnia magna</i> (cladoceran)	0.8	1.22	0.99		Rodgers et al. 1997
<i>Isonychia bicolor</i> (mayfly)	0.31	1.56	0.70	0.70	Diamond et al. 1990
<i>Oncorhynchus mykiss</i> (rainbow trout)	0.36	0.51	0.43	0.21	Nebeker et al. 1983
<i>Oncorhynchus mykiss</i> (rainbow trout)	0.09	0.17	0.12		Davies et al. 1978
<i>Oncorhynchus mykiss</i> (rainbow trout)	0.15	0.22	0.18		Davies et al. 1998
<i>Pimephales promelas</i> (fathead minnow)	0.37	0.65	0.49	0.49	Holcombe et al. 1983
<i>Salmo trutta</i> (brown trout)	0.20	0.25	0.22	0.22	Davies et al. 1998
<i>Stenonema modestum</i> (mayfly)	1.84	3.4	2.5	2.50	Diamond et al. 1992

NOEC = No-observed-effect concentration

LOEC = Lowest-observed-effect concentration

SMCV = Species mean chronic value

Table 2-3 Acute silver toxicity data for saltwater organisms ($\mu\text{g/L}$)

Species	LC50	SMAV	Reference
<i>Acartia clausi</i> (copepod)	13.3	13.3	Lussier and Cardin 1985
<i>Acartia tonsa</i> (copepod)	37.8	36.46	Lussier and Cardin 1985
<i>Acartia tonsa</i> (copepod)	30.9		Schimmel 1981
<i>Acartia tonsa</i> (copepod)	66		Schimmel 1981
<i>Acartia tonsa</i> (copepod)	35.8		Schimmel 1981
<i>Acartia tonsa</i> (copepod)	23.5		Schimmel 1981
<i>Acartia tonsa</i> (copepod)	36.4		Schimmel 1981
<i>Acartia tonsa</i> (copepod)	36.3		Lussier and Cardin 1985
<i>Apeltes quadracus</i> (fourspine stickleback)	546.6	546.6	Cardin 1986
<i>Argopectin irradians</i> (bay scallop)	33	33	Nelson et al. 1976
<i>Atherinops affinis</i> (topsmelt)	183	183	Shaw et al. 1998
<i>Cancer magister</i> (crab)	33.1	33.1	Dinnel et al. 1983
<i>Crangon</i> spp. (sand shrimp)	> 838	> 838	Dinnel et al. 1983
<i>Crassostrea gigas</i> (Pacific oyster)	11.91	14.21	Coglianese and Martin 1981
<i>Crassostrea gigas</i> (Pacific oyster)	15.1		Coglianese and Martin 1981
<i>Crassostrea gigas</i> (Pacific oyster)	11.94		Coglianese 1982
<i>Crassostrea gigas</i> (Pacific oyster)	19		Dinnel et al. 1983
<i>Crassostrea virginica</i> (Eastern oyster)	5.8	14.15	Calabrese et al. 1973
<i>Crassostrea virginica</i> (Eastern oyster)	24.2		MacInnes and Calabrese 1978
<i>Crassostrea virginica</i> (Eastern oyster)	35.3		MacInnes and Calabrese 1978
<i>Crassostrea virginica</i> (Eastern oyster)	32.2		MacInnes and Calabrese 1978
<i>Crassostrea virginica</i> (Eastern oyster)	13		Zarogian 1981
<i>Crassostrea virginica</i> (Eastern oyster)	7		Zarogian 1981
<i>Crassostrea virginica</i> (Eastern oyster)	3		Zarogian 1981
<i>Crassostrea virginica</i> (Eastern oyster)	37		Zarogian 1981
<i>Cymatogaster aggregata</i> (shiner perch)	355.6	355.6	Dinnel et al. 1983
<i>Cyprinodon variegatus</i> (sheepshead minnow)	441	1084	Schimmel 1981
<i>Cyprinodon variegatus</i> (sheepshead minnow)	898		Schimmel 1981
<i>Cyprinodon variegatus</i> (sheepshead minnow)	1356		Schimmel 1981
<i>Cyprinodon variegatus</i> (sheepshead minnow)	1510		Schimmel 1981
<i>Cyprinodon variegatus</i> (sheepshead minnow)	1876		Schimmel 1981
<i>Cyprinodon variegatus</i> (sheepshead minnow)	1065		Shaw et al. 1998
<i>Fundulus heteroclitus</i> (mummichog)	2700	2700	Dorfmann 1977
<i>Menidia beryllina</i> (inland silverside)	260	260	Shaw et al. 1998
<i>Menidia menidia</i> (silverside)	110.1	110.1	Cardin 1986
<i>Mercenaria mercenaria</i> (clam)	21	21	Calabrese and Nelson 1974
<i>Mysidopsis bahia</i> (mysid)	249	171.8	Lussier et al. 1985
<i>Mysidopsis bahia</i> (mysid)	256		Schimmel 1981
<i>Mysidopsis bahia</i> (mysid)	300		Schimmel 1981
<i>Mysidopsis bahia</i> (mysid)	86		Schimmel 1981
<i>Mysidopsis bahia</i> (mysid)	313		Schimmel 1981
<i>Mysidopsis bahia</i> (mysid)	65		Schimmel 1981
<i>Mysidopsis bahia</i> (mysid)	132		Schimmel 1981
<i>Mytilus edulis</i> (blue mussel)	159	159	Nelson et al. 1988

-continued-

Table 2-3 continued

Species	LC50	SMAV	Reference
<i>Neanthes arenaceodentata</i> (polychaete)	151	178.6	Pesch and Hoffman 1983
<i>Neanthes arenaceodentata</i> (polychaete)	145		Pesch and Hoffman 1983
<i>Neanthes arenaceodentata</i> (polychaete)	260		Pesch and Hoffman 1983
<i>Oligocottus maculosus</i> (tidepool sculpin)	331	331	Shaw et al. 1998
<i>Oncorhynchus kisutch</i> (coho salmon)	487.5	487.5	Dinnel et al. 1983
<i>Paralichthys dentatus</i> (summer flounder)	47.7	42.76	Cardin 1986
<i>Paralichthys dentatus</i> (summer flounder)	8		Cardin 1986
<i>Paralichthys dentatus</i> (summer flounder)	15.5		Cardin 1986
<i>Paralichthys dentatus</i> (summer flounder)	565		Shaw et al. 1998
<i>Parophrys vetulus</i> (English sole)	800	800	Dinnel et al. 1983
<i>Perna viridis</i> (green mussel)	30	30	Mathew and Menon 1983
<i>Pseudopleuronectes americanus</i> (winter flounder)	196.3	196.3	Cardin 1986
<i>Scorpoenichthys marmoratus</i> (cabezon)	> 800	> 800	Dinnel et al. 1983
<i>Tigriopus brevicornis</i> (copepod)	36.37	36.37	Menasria and Pavillon 1994

SMAV = Species mean acute value

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Biological Processes

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Introduction

When linking environmental chemistry of Ag(I) (oxidized form of silver) to its potential hazards, it is necessary to consider how Ag(I) interacts with organisms. This includes uptake, distribution, and elimination of Ag(I) in relation to environmental forms of Ag(I) (see Chapter 1), as well as observed biological effects (see Chapter 2). The species of Ag(I) absorbed, the route of uptake, and the rate of absorption seem to be factors that determine whether or not accumulated Ag(I) has effects on organisms. Thus, there is a distinction between bioavailable Ag(I) and bioreactive Ag(I), the former being Ag(I) that is available for uptake by organisms and the latter referring to the fraction of accumulated Ag(I) that has the potential to react with specific targets, causing effects.

The free Ag^+ ion, or loosely bound Ag(I), has been shown to be toxic to many aquatic animals at low concentrations. The reason for this potency is the very strong affinity of Ag(I) for sulfhydryl groups; Ag(I), indeed, seems to exert acute toxicity by binding to such a group on a specific enzyme. Interestingly, this strong affinity for S(II-) (reduced sulfur) may affect both bioavailability and toxicity of Ag(I) because in natural environments S(II-) is almost always in molar excess of Ag(I). Although sediment short-term toxicity tests indicate that Ag(I)S(II-) is benign on an acute time scale, it has become apparent that some organisms can take up and accumulate Ag(I) bound to both organic or inorganic S(II-) complexes (Griscom et al. 2000; Lee, Griscom et al. 2000; see Chapter 2). Moreover, recent evidence suggests that such Ag(I) complexes might be associated with chronic effects, some of which may occur at environmentally realistic concentrations of silver.

This chapter has focused on six areas of importance in considering the links between silver in the environment, its uptake by organisms, and its subsequent effects. Even though several of these topics are discussed in other chapters, it is felt that they are important to consider in the context of pharmacokinetics and other biological and chemical processes.

How Does Silver Cross Membranes?

Background

In aquatic organisms, the three major epithelia that Ag(I) could initially enter are those of dermis, gill, and gut. Each of these epithelia consist of several cell types, which have different functions in the physiology of the organism. The presence of different receptors for molecules, different modes of internalization of materials, and different ways of handling molecules may be anticipated. For cells in general, the major modes of entry of a chemical species are passive diffusion through the cell membrane, endocytosis, diffusion through specific ion channels, facilitated diffusion through specific compound transport systems, and active transport through energy-driven carriers. For species that are deliberately absorbed by a cell, there usually will be designated pathways for entry through transport proteins or surface receptors. However, nonessential compounds may inadvertently be taken up through transport pathways designed for essential molecules, or they may pass by simple diffusion through the cell membrane if they are sufficiently lipophilic. Transporting cells in the integument, including the gills, typically have transporters for hydro-minerals. Intestinal cells, because of their importance in food uptake, also have many selective molecule transporters, e.g., for amino acids, carbohydrates, etc., and often have systems for the incorporation of relatively large molecules. Some animal species, such as bivalves, are even able to ingest colloids and larger particulates by endocytosis.

In exploring the processes for Ag(I) transport through a membrane into a cell, it is noted that few controlled experiments have actually been designed to address mechanisms of Ag(I) uptake. Nevertheless, some studies are available that show Ag(I) uptake and provide indirect evidence of how the transport may have occurred. The attempt here is to develop a framework in which these data may be rationalized in chemical terms. Such a framework also must endeavor to address the problem that the chemical form, in which Ag(I) is presented to a cell, can lead to Ag(I) uptake (bioavailability) that may or may not result in effects (bioreactivity). Because of the fragmentary information on Ag(I) transport mechanisms, the following paragraphs contain speculative suggestions.

Low molecular weight Ag(I) compounds

Low molecular weight Ag(I) compounds are defined as "dissolved" (using the current U.S. Environmental Protection Agency [USEPA] operational definition of filtration through a 0.45 μm filter) and are subdivided into two classes. One class is weakly bound Ag(I), designated $\text{AgL}_{(w)}$, where ligands might be, for example, carboxylic acids (formation constant: $\log K_f$ 0.7), amines ($\log K_f$ 3.1), or chloride ($\log K_f$ 3.1). The other class is strongly bound ligands, designated $\text{AgL}_{(s)}$, where ligands might be thiosulfate ($\log K_f$ 8.8), cysteine ($\log K_f$ 11.9), or glutathione (\log

K_f 12.3). All these ligands contain functional groups that can exist in natural environments, but are not necessarily major ligands for Ag(I) in the environment.

For Ag(I) to enter a cell through a selective ion channel, it must be very weakly ligated or at higher concentrations potentially present as the aquated ion Ag^+ because ion channels will have hydrophilic amino acid residues (i.e., aspartates, threonines, serines, and glutamates) at the receptor and channel sites. Ag(I) would only undergo ligand exchange with such functional groups if it is an $AgL_{(w)}$ type. In this case, the receptor could effectively ligate Ag(I) and transport it into the cell. A recent paper by Bury and Wood (1999) presented evidence that Ag^+ , added as $AgNO_3$ ($\log K_f$ 0.3) in a relatively "clean" environment, might be transported into the gill cells of a rainbow trout by a proton-coupled Na^+ channel. Transport by Ca^{2+} or K^+ channels was ruled out by competitive experiments with both ions. Uptake of Ag^+ was reduced by increased Na^+ and, most significantly, by the specific Na^+ channel blocker phenamil. Furthermore, bafilomycin A1, an inhibitor of V-type H^+ ATPases (adenosine triphosphatases), also blocked Ag^+ influx suggesting that Ag^+ influx (like Na^+ influx) may be coupled to H^+ efflux. Once the Ag(I) entered the cell, it remained highly bioreactive and interfered with the Na^+/K^+ -ATPase on the basolateral side of the cells, probably by binding to a cysteine at the Mg^{2+} binding site of the protein (Ferguson et al. 1996; Morgan et al. 1997). It is conceivable that the intracellular Ag(I) is effectively compartmentalized and moved relatively quickly to the basolateral side of the cell. Even if there were a ligand exchange of Ag(I) with a glutathione-SH, the resultant Ag-thiolate could exchange with the likely critical cysteine of the ATPase and inhibit the enzyme, but it must remain available for associative ligand transfer and not be buried in the interior of another large protein.

Experiments on rainbow trout in a clean medium, with $AgCl^0(aq)$ as the Ag(I) source, showed appreciable uptake of Ag(I) into the cell but with markedly lower bioreactivity than for Ag(I) derived from $AgNO_3$ (Hogstrand et al. 1996; McGeer and Wood 1999). The binding strength of $AgCl^0(aq)$ ($\log K_f$ 3.1) is strong enough to reduce ligand exchange at the sodium channel site, resulting in low transport rate into the cell. Presumably there would need to be an alternative mode of $AgCl^0$ transport to account for the observed bioaccumulation. It can only be speculated about the nature of this uptake mechanism. Passive diffusion either as a small neutral aggregate or as an NH_3AgCl^+ complex would be chemically conceivable although not necessarily rapid. However, such diffusion could take place across the entire gill surface as opposed to uptake through Na^+ channels, which would occur only at the specific cells (i.e., chloride cells) where the channels are present. Ligand exchange onto a lysine, methionine, or cysteine side chain of a gill surface protein followed by transport into the cell by an as yet unknown protein is conceivable, in which case Ag(I) would be "going along for the ride."

In similar clean-medium experiments with rainbow trout, Ag thiosulfate showed high Ag(I) uptake but low bioreactivity (Wood et al. 1996). Because the $\log K_f$ of

$\text{Ag}_2\text{S}_2\text{O}_3$ is relatively high at 8.8, silver thiosulfate might enter a cell as a complex by an endocytotic route, by ligand transfer of Ag(I) to a protein cysteine-SH, with subsequent transport by an unknown transporter, or through an anion transporter. Again the cellular uptake site would leave the Ag(I) in a relatively nonbioreactive state, perhaps because of the high binding affinity to thiosulfate or its putative exchange ligand. It is conceivable that gill cells in the rainbow trout have developed a sulfhydryl-rich receptor or transporter for the purpose of tightly binding metals, wanted or unwanted, at the cell surface. Such a system also would strongly bind $\text{AgL}_{(w)}$ species but might not have the capacity to cope with high concentrations of $\text{AgL}_{(w)}$ (low K_m and low v_{max}). These speculations remain to be explored by further experiment.

Uptake of Ag(I) by the fish gill is followed by elimination to other parts of the body. This elimination process is surprisingly rapid and almost all of the gill Ag(I) has redistributed to other tissues within hours after being taken up by the gill from the water (Mayer et al. 1996). One reason for this rapid elimination of Ag(I) from the gill cells may be the presence of effective Ag(I) transport system on the basolateral membrane (facing the blood) of the gill epithelium. Very recently, Bury, Grosell et al. (1999) showed that there is a P-type ATPase in the basolateral membrane of rainbow trout gills that transports Ag(I). It is tempting to suggest, although presently not known, that this silver-ATPase activity is responsible for the rapid elimination of Ag(I) from gill tissue.

Studies of *Ceriodaphnia dubia* with the Ag thiolates, Ag-cysteine and Ag-glutathione, in clean media showed acute toxicity at concentrations slightly higher than those observed during administration of AgNO_3 as the Ag(I) source (Bielmyer et al. 2001). It seems likely that these small silver complexes enter the animal via amino acid and peptide transporters, respectively. Alternatively the uptake mechanism could involve ligand exchange onto a protein cysteine-SH and subsequent transport through the membrane. No experimental information was available to demonstrate transport or mechanisms of toxicity.

Large molecules, aggregates, or colloids containing Ag(I)

Little appears to be known about uptake of very large molecules, aggregates, or colloids that contain Ag(I). This group would contain molecular weights from 1 to 100 kDa. Thus, all these compounds will pass through a 0.45 μm filter and be considered "dissolved" according to current regulations. Lower molecular weight species, discussed above, can conceivably carry out transfer of Ag(I) at a cell surface by ligand transfer, but this would be more difficult for higher molecular weight species and it would seem that endocytotic pathways would be the only available ones. Some comment should be provided on natural organic matter (NOM), a common aggregate to which Ag(I) binds, and one relevant study should be noted. Native NOM, encompassing both particulate and "dissolved" (e.g.,

passage through a 0.45 μm filter) sizes, normally contains sufficient S(II-) to have ambient Ag(I) bound as either an Ag-thiolate or as an $\text{Ag-metal sulfide complex}$ associated with the NOM (Manolopoulos et al. 1999). Entry of Ag-NOM into cells can only be by endocytosis, as noted above, or ligand exchange to a protein cysteine-SH followed by transport into the cell. Because of the size of NOM aggregates, most of these probably contain binding sites for Ag(I) both at the surface and internally. One would anticipate ligand transfer of Ag(I) from peripheral or "surface" bound Ag(I) to be fast as it would be more easily accessible for ligand exchange. In contrast, Ag(I) bound to cryptic S(II-) groups in the interior of the NOM would be expected to be very slow to exchange with membrane thiols.

In a recent paper, Carvalho et al. (1999) reported uptake of Ag(I) from colloidal macromolecular organic matter (OM) containing radiolabelled Ag(I) by a brown shrimp (*Penaeus aztecus*). During a 14-day uptake study, the hepatopancreas was the site of highest accumulation. Contrary to expectation, evidence was presented that the gill rather than the gut was the major epithelium where cellular entry took place. Ag(I) presented as colloidal macromolecular OM showed different pharmacokinetics than weakly associated Ag(I) . In clean media, weakly bound forms of Ag(I) were found to accumulate primarily in the abdomen instead of the hepatopancreas.

Particulate matter

Suspended and sediment particles are both placed in this category. There appears to be a consensus that uptake of metals, such as Ag(I) , is stimulated in some animal species when the water contains inorganic particles (e.g., iron oxides, clays, silica, etc.) that are coated with organic materials (Decho and Luoma 1994; Gagnon and Fisher 1997). Bioaccumulation of Ag(I) by bivalves has been extensively examined (Hornberger et al. 1999; Roditi et al. 1999, 2000a, 2000b; Griscom et al. 2000; Lee, Griscom et al. 2000). Bivalves are able to ingest sediment particles and, in the gut, internalize Ag(I) into the cells (Wang et al. 1996; Griscom et al. 2000). Possible processes could be endocytosis with enzymatic stripping, transport of Ag(I) with peptides or amino acids and/or processing of carbon materials inside the cells. Alternatively, one could envision bacterial conversion of Ag(I)S(II-) to less strongly ligated Ag(I) , which in turn could be assimilated by the bivalves. In most estuarine sediments it is likely that molar concentrations of acid-volatile sulfide (AVS) would exceed molar concentrations of silver. Thus, Ag(I) must often be bound to S(II-) , perhaps as Ag_2S (acanthite) or more likely as a mixed-metal sulfide complex with, for example, copper or zinc. Nevertheless, bioaccumulation by aquatic benthos is common in those circumstances. Speciation of the Ag(I) within the organism is currently not known, but reduced gamete production in environmentally Ag(I) exposed bivalves might be connected to Ag(I) burden (Hornberger et al. 1999). Clearly, the speciation of Ag(I) in these organisms is an important area for further study.

In summary, several forms of Ag(I) are transported across respiratory and/or body surfaces and into tissues. Weakly complexed Ag(I) (or aquated Ag^+ at higher concentrations typical of toxicity studies but not natural environments), AgCl, $\text{Ag}[\text{S}_2\text{O}_3]_n$, Ag-cysteine, and Ag-glutathione have all been shown to be bioavailable. The route of uptake seems to depend upon the strength of the silver-ligand complex presented to the organism as well as the species of organism. The uptake pathway, in turn, influences whether or not the accumulated Ag(I) becomes bioreactive. In addition to Ag(I) species of exposure, bioreactivity of Ag(I) is influenced by method of exposure (i.e., water or diet) and the physiology of the organism involved. Additional studies are needed to clarify these relationships. Exposure to colloidal Ag(I) results in silver within tissue of some species. Uptake of silver from Ag(I)S(II-) complexes, or from Ag_2S , apparently takes place, but the absorbed Ag(I) species have not been clearly identified. Not all these forms necessarily exist in the environment. Proposed transport mechanisms are related to ligand exchange, but should be better understood. Uptake by algal cells is important because this is the initial introduction into a potentially sensitive food web.

Dietary Exposure

Many studies have suggested that dietary Ag(I) associated with food particles is available for bioaccumulation in several different organisms. Assimilation efficiency measures physiological absorption of ingested metals within soft tissue of an organism following evacuation of unabsorbed material and is used to determine bioavailability (Wang and Fisher 1999). Note that assimilation is not the same as bioaccumulation, which refers to the accumulated body burden (i.e., assimilation minus elimination). Assimilation efficiency of Ag(I) associated with various ingested particles (e.g., algae, natural suspended particles, oxic and anoxic sediments) determined for several organisms, such as zooplanktons, polychaetes, and bivalves, clearly demonstrates that uptake of Ag(I) does occur through the diet (Luoma and Fisher 1997; Wang and Fisher 1999). These assimilation values range from 4% to 50%, depending on animal species and food type (Table 3-1). Lee, Griscom et al. (2000) found that a marine bivalve *Macoma balthica* can assimilate Ag(I) from ingestion of Ag_2S precipitated on glass beads. The assimilation efficiency determined for Ag_2S (14% to 28%) was comparable to those determined for Ag(I) on oxic particles. The assimilation efficiency determined for a mussel *Mytilus edulis* with the two food types above was only approximately 3%. Clearly, there is a large variability in reported assimilation efficiencies for Ag(I) (Table 3-1). Part of this variability may arise from differences in doses and other dissimilarities between experimental conditions, but there seem to be considerable disparities between biological species.

Galvez and Wood (1999) reported that juvenile rainbow trout fed a diet containing Ag_2S (3000 mg Ag kg^{-1} or 28 mmol kg^{-1} diet) accumulated Ag(I) in liver 4-fold

Table 3-1 Assimilation efficiency percent of Ag(I) reported for aquatic invertebrates

Animal	Food	Assimilation efficiency %	Reference
Ciliate: <i>Fabrea salina</i>	prymnesiophyte	22	A
Copepod: <i>Acartia tonsa</i>	diatom	17	B
Copepod: <i>Temora longicornis</i>	2 diatoms	8 - 19	C
	natural seston	15	
Polychaete: <i>Nereis succinea</i>	oxic sediments	16 - 30	D
Mussel: <i>Mytilus edulis</i>	2 diatoms,	4 - 34	E
	2 chlorophytes,		
	2 dinoflagellates,		
	1 prasinophyte		
Mussel: <i>Mytilus edulis</i>	oxic sediments	4 - 15	F, G
Oyster: <i>Crassostrea virginica</i>	prymnesiophyte	44	H
Clam: <i>Macoma balthica</i>	1 diatom,	38 - 49	H
	1 prymnesiophyte		
Clam: <i>Mercenaria mercenaria</i>	1 diatom,	22 - 35	H
	1 prymnesiophyte		
Zebra mussel: <i>Dreissena polymorpha</i>	2 diatoms,	4 - 16	I
	1 chlorophyte,		
	1 cyanophyte,		
	natural seston		
Seastar: <i>Marthasterias glacialis</i>	Mussels	69	J
Clam: <i>Potamocorbula amurensis</i>	Algae sediment	18 - 27	K
Clam: <i>Macoma balthica</i>	Ag ₂ S on glass beads	14 - 28	L
Clam: <i>Mytilus edulis</i>	Fe-oxyhydroxides	~ 3	L

A Fisher et al. 1995

E Wang et al. 1996

I Roditi and Fisher 1999

B Reinfelder and Fisher 1991

F Griscom et al. 2000

J Fowler and Teyssi 1997

C Wang and Fisher 1998

G Gagnon and Fisher 1997

K Griscom et al. 2000

D Wang et al. 1999

H Reinfelder et al. 1997

L Lee et al. 2000

higher than control trout after 56-day feeding. However, juvenile rainbow trout (*Oncorhynchus mykiss*) provided a diet containing biologically incorporated Ag(I) (approximately 3 mg Ag kg⁻¹ or 0.03 mmol/kg diet) displayed liver concentrations 12-fold higher than those of controls after 3 months (Galvez et al. 1996), suggesting that biologically incorporated Ag(I) is more bioavailable to trout than Ag₂S mixed with the food. Even with the biologically incorporated Ag(I), the concentrations of dietary Ag(I) used was much higher than those in possible natural diets of fish. Thus, the highest concentration of Ag(I) recorded in animal whole body soft tissues was 320 µg g⁻¹ (3.0 µmol g⁻¹) dry weight (dw) found in mud snail, *Nassarius obsoletus*, from San Francisco Bay, CA, USA, (Luoma and Phillips 1988). Ag(I) associated with the cytosolic fraction of algal cells was more available for assimilation by herbivorous zooplankton than Ag(I) adsorbed on the cell surface (Bielmyer et al. 2001) as shown for other trace metals (Reinfelder and Fisher 1991). This relationship could have a significant implication for trophic transfer of Ag(I) due to

the position of algal cells in the food chain. Thus, available information suggest that bioavailability of Ag(I) from ingested food is strongly influenced by the nature of Ag-food association and differs considerably among animal species.

Suspended particles are processed by benthic filter-feeders and pelagic secondary consumers (e.g., zooplankton) and sediment particles by deposit feeders. Once within the gut of an animal, ingested particles would be assimilated with aid of digestive fluids containing high concentrations of organic ligands such as amino acids and proteins (Chen and Mayer 1998). It has been hypothesized that during digestion, Ag(I) bound to organic molecules could be released or leached from food particles and the Ag(I) could then react with amino acid or protein.

Subsequently, Ag(I) bound to these organic molecules could be transported into individual cells via specific transporters for macromolecules in the gut epithelium. Additionally, some invertebrates (e.g., *M. balthica*) allocate ingested food particles to the digestive gland where intensive intracellular digestion occurs, which could enhance uptake of Ag(I) (Decho and Luoma 1996). Based on this information, it appears that the chemistry and physiology of the gut in different animals (e.g., redox state, particle retention time) would likely affect assimilation of particle-associated Ag(I).

The relative contribution of Ag(I) bioaccumulation from dietary uptake has been estimated by employing a biokinetic model (Wang et al. 1999) and microcosm studies (Lee, Lee et al. 2000). These results suggest that dietary uptake plays a significant role in Ag(I) bioaccumulation in some invertebrate species. For example, Wang et al. (1999) estimated that by using a biokinetic model, 65 to 95% of accumulated Ag(I) was derived from dietary route in a marine deposit-feeding polychaete, *Nereis succinea*. Lee, Lee et al. (2000) found in a laboratory microcosm study that the deposit feeding worm, *Neanthes arenaceodentata*, obtained Ag(I) predominately from ingestion of contaminated sediments. Some marine animals also could absorb Ag(I) from imbibed water via the gut epithelium, but the magnitude of Ag(I) bioaccumulation via drinking is thought to be negligible compared to uptake from diet.

There is no known evidence that Ag(I) accumulated via dietary uptake causes acute toxicity. However, some recent studies indicate that Ag(I) transported through an algal-zooplankton food chain may cause decreased fecundity in zooplankton (Schmittschmitt et al. 1996; Fisher and Hook 1997; Bielmyer et al. 2001). Schmittschmitt et al. (1996) demonstrated that fecundity of *C. dubia* decreased significantly after ingestion of the algae *Selenastrum capricornutum*, which had been previously exposed to Ag(I) in the dissolved phase ($20 \mu\text{g L}^{-1}$ or 180 nM), yielding a total Ag(I) concentration of $1.1 \mu\text{g g}^{-1}$ (10 nmol g^{-1}) algae (dw). The effects of Ag(I) on reproduction in the cladoceran were not observed when the organisms were exposed directly to low levels of Ag(I) in the dissolved phase, but appeared when algal cells were first exposed to the same dissolved concentrations and then fed to the cladoceran (Bielmyer et al. 2001).

Thus, experimental evidence shows that dietary uptake occurs for Ag(I) and, at least in some animals, it may even be the dominant uptake route during chronic exposures. Research also strongly suggests that there may be effects leading to reduced reproductive capability in some animals that accumulate Ag(I) in this way. Assimilation efficiencies are highly variable among food-types and between different species and should be studied with the goal of establishing generalizations. The roles of dietary exposure in Ag(I) accumulation and toxicity should be quantified in a variety of species to reflect biological diversity in terms of taxa and feeding strategy. At least in molluscs, Ag(I) from anoxic sediments or Ag₂S on glass beads are assimilated after ingestion. Because of this, the influence on AVS-normalization procedures in setting regulatory limits for Ag(I) needs to be reviewed. Bioreactivity and biospeciation of Ag(I) accumulated via the diet should be further investigated.

Waterborne Exposure

Pharmacokinetics

Published bioconcentration factors (BCFs) resulting from waterborne Ag(I) exposures are relatively low in teleost fish compared to other aquatic organisms. The BCF value following a 2-day radioactive pulse of ^{110m}Ag at 11.9 µg L⁻¹ (110 nM) Ag(I) (added as AgNO₃) was only 2.4 for rainbow trout (Galvez et al. 2001), which is comparable to a BCF of 2.4 for brown trout after 57 days of exposure to dissolved AgNO₃ under similar conditions (Garnier et al. 1990). The similarity in whole body BCF values between studies, despite the large difference in exposure time, suggests that Ag(I) levels in the whole body equilibrate quickly in fish. In comparison, fathead minnows (*Pimephales promelas*) exposed to Ag(I) thiosulfate resulted in BCF of 1.8 (Terhaar et al. 1977), suggesting that the bioavailability of these forms of Ag(I) to these fish are within the same order of magnitude. Regardless of route of uptake, bioaccumulated Ag(I) appears to distribute predominantly to the liver. At steady state, Ag(I) in the liver represents approximately 60 to 70% of the whole body Ag(I) burden (Mayer et al. 1997; Hogstrand and Wood 1998; Wood et al. 1999). Kinetics of uptake and distribution of Ag(I) have been studied during exposure to waterborne Ag(I) (Mayer et al. 1997; Galvez et al. 2001; Wood et al. 2002). Exposure of European eel (*Anguilla anguilla*) or rainbow trout to Ag(I) as Ag⁺ or AgCl⁰ resulted in a very rapid uptake of Ag(I) by the gill epithelium and an equally rapid elimination from the gills. From the gills, Ag(I) was more or less directly redistributed to the liver although temporary retention in the tissues surrounding the intestinal tract has been observed during some conditions (Mayer et al. 1997; Galvez et al. 2001). Accumulated Ag(I) was very slowly eliminated from the body.

Effects of aquatic speciation

Like many toxic metals, Ag(I) has been shown to exert an acute toxic response at the gill epithelium of teleost fish. The physiological mechanism of acute Ag(I) toxicity has been identified as a severe reduction of Na⁺ and Cl⁻ uptake at the gills resulting from branchial Na⁺/K⁺-ATPase inhibition. Recent studies have demonstrated that acute Ag(I) toxicity in fish is strongly influenced by the geochemical speciation of the metal. For example, weakly ligated Ag(I) (added as AgNO₃) is at least 10³ times more toxic than the strongly ligated Ag(I) thiosulfate (LeBlanc et al. 1984; Hogstrand et al. 1996). At the time Hogstrand et al. (1996) suggested, although it was not formally tested, that acute Ag(I) toxicity to fish adhered to the free ion activity model, which states that only the free Ag⁺ ion results in toxicity. To test this hypothesis, a series of toxicity and physiology tests were conducted on juvenile rainbow trout. Toxicity tests were performed in low ionic-strength and S(II-)-depleted water. Silver was added as AgNO₃ and reconstituted with either Cl⁻ or NOM to manipulate predicted Ag⁺ levels. Elevation of the Cl⁻ concentration from 50 to 1500 μM increased the 96-hour LC50 by a factor of 4.3, whereas elevating the NOM concentration from 0.3 to 5.8 mg L⁻¹ carbon, resulted in a 4.1-fold protection against Ag(I) toxicity. Using geochemical speciation analysis, the acute Ag(I) toxicity in juvenile rainbow trout was correlated to the Ag⁺ species alone (Hogstrand and Wood 1998; Bury et al. 1999; McGeer and Wood 1999).

The strong relationship between Ag⁺ and toxicity was also observed during physiological studies by Bury, McGeer et al. (1999) and McGeer and Wood (1999). In these studies, reductions in plasma Na⁺ level and inhibition of gill Na⁺/K⁺-ATPase activity in rainbow trout were found to correlate with waterborne Ag⁺. Interestingly, no discernible relationship existed between gill silver burdens and water Ag⁺ concentrations. Similar effects were noted following exposure with silver-thiosulfate (Hogstrand et al. 1996). Exposure to Ag(I) thiosulfate (at very high concentrations) resulted in similar gill Ag(I) burdens as fish exposed to silver added as AgNO₃, although only the latter was acutely toxic. In addition, exposure to very high concentrations of silver-thiosulfate resulted in liver Ag(I) levels 300-fold above control levels; this is compared to Ag(I) levels in livers of fish exposed to weakly ligated Ag(I), which were approximately 3-fold above basal concentrations. There were no discernible effects, but the tests were of short duration, and it is not known if there would be chronic effects from such a tremendous accumulation. What can be said is that the Ag(I) accumulated in the liver during these conditions was at least initially reactive enough to trigger a biological response, since it elicited a very strong induction of metallothionein synthesis (Hogstrand et al. 1996). Reproductive effects, which seem to be sensitive to Ag(I) in some invertebrate organisms, have not been investigated in fish. The key point here, however, is that when Ag(I) bound to a strong ligand (thiosulfate) was presented to the fish, there was little toxic effect at the gills, which is the site for acute toxicity.

Bioavailability and Bioreactivity of Silver

The bioreactivity concept

It is clear that metal speciation not only governs metal binding and bioavailability but also the intracellular bioreactivity of Ag(I). The mechanisms behind this distinction remain to be demonstrated and the following should be regarded as a working model to explain bioreactive versus nonbioreactive Ag(I). According to this model, in order for Ag(I) to be bioreactive and cause toxic effects it must not only enter any cell, but also be present in the appropriate type of cell in a reactive form. This may seem confusing, because once Ag(I) has entered the organism its previous speciation in the water column should become irrelevant, unless Ag(I) has entered as a complex with a high stability constant. In the freshwater fish gill, Ag^+ , or loosely associated Ag(I), is thought to be taken up from the water and accumulated in ionocytes (i.e., chloride cells), which contain very high numbers of the intracellular target for Ag(I) toxicity, the Na^+/K^+ -ATPase. Yet these ionocytes comprise only 5% to 10% of the gill surface. Currently the mechanism of the apical uptake is unsettled, but there is pharmacological evidence that Ag(I) enters as Ag^+ through the apical protein channel that transports Na^+ (Bury and Wood 1999). This uptake process, which is driven by internal and external factors, might concentrate Ag(I) at a high effective dose at or near the target of toxicity (Figure 3-1A; Hogstrand and Wood 1998; Wood et al. 1999). In contrast, waterborne Ag(I), originating as AgCl^0 , $\text{Ag}(\text{S}_2\text{O}_3)_n$ (silver thiosulfate), or Ag-dissolved organic matter (DOM), may not have this directional pathway, and would therefore enter the gill in a less specific manner occurring across the other cell types that make up the majority of the epithelium (Hogstrand and Wood 1998; Wood et al. 1999). As the target of toxicity is much less abundant in these other cell types, Ag(I) can accumulate without causing acute toxicity (Figure 3-1C and Figure 3-1D). If Ag(I) enters the gill as a stable complex, it may be present in the target cells (i.e., ionocytes) in a nonbioreactive form. This case could apply, for example, during exposure to silver-thiosulfate: it is possible that most of the absorbed Ag(I) passes across the epithelium associated with thiosulfate (Figure 3-1D). The Ag(I) that leaves thiosulfate during the passage across the epithelium is likely exchanged only with high-affinity ligands, such as glutathione and metallothionein, as evidenced by metallothionein induction in combination with nominal toxicity (Figure 3-1C; Hogstrand et al. 1996).

In some species, such as the fathead minnow (*Pimephales promelas*), water Cl^- offers little protection against Ag(I) toxicity and, thus, uptake of Ag(I) from AgCl^0 is as toxic as that when Ag(I) is present as Ag^+ (Bury, Galvez et al. 1999). This could be explained by the suggested model if the affinity of the sensitive uptake sites is higher in fathead minnow than in rainbow trout so that a significant directional uptake of Ag^+ occurs even if Ag(I) is present as AgCl^0 in the water. A higher affinity for silver at these sites would increase their competitiveness for silver and there-

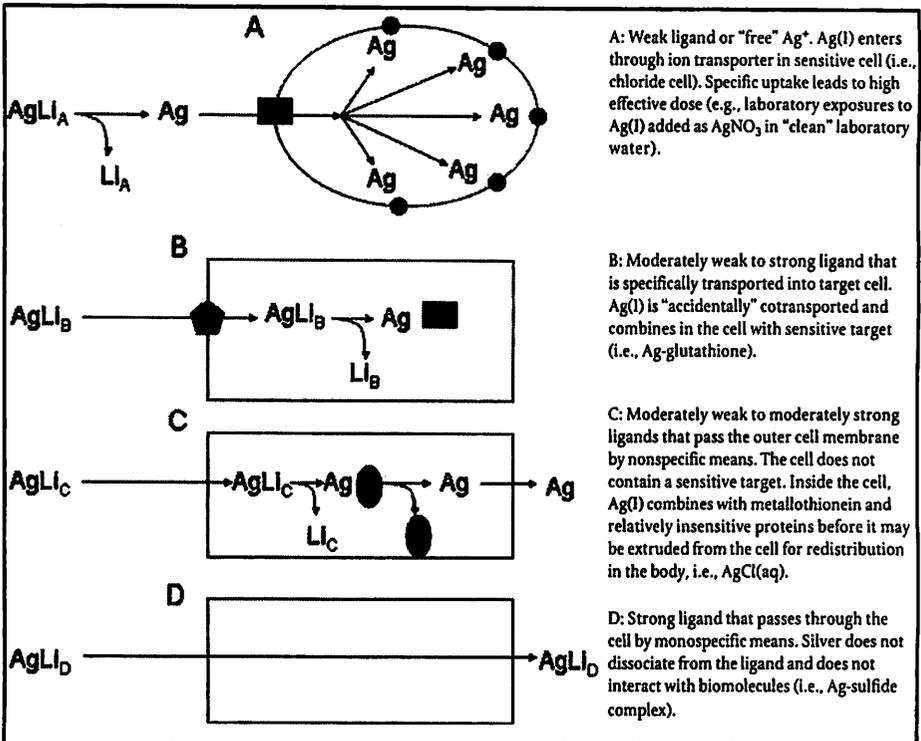


Figure 3-1 Ag(I) bioavailability. Ag(I) is bioavailable in all cases (A through D) but only bioreactive in cases A and B.

fore reduce the protective effect offered by chloride. This effect can be simulated, using a Biotic Ligand Model (BLM) approach where the affinity of the site for toxicity is increased for Ag(I) and other cations.

Effects of exposure route

Further evidence that bioavailable Ag(I) should be distinguished from bioreactive Ag(I) comes from comparisons of responses to hepatic Ag(I) that originated from waterborne and dietary Ag(I) exposures, respectively. During exposure to waterborne Ag(I) (added as AgNO_3) there was a 4-fold increase in hepatic Ag(I) burden over 7 days, leading to a significant induction of metallothionein synthesis (Hogstrand et al. 1996). Exposure to Ag(I) via the diet, on the other hand, resulted in a similar increase in hepatic Ag(I) concentration during a 16-day period, but without concomitant metallothionein induction (Galvez et al. 1996). The difference in response could either reflect the differences in uptake route or accumulation rate (4-fold increase during 7 versus 16 days). An important observation here is that all accumulated Ag(I) was not equally bioreactive and presently it is not fully understood how to separate the two.

Is silver in the environment bioreactive?

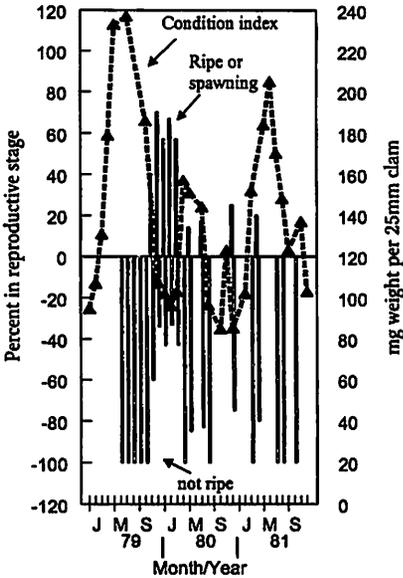
Clearly, across geochemical conditions and exposure regimes there is not a linear relationship between Ag(I) tissue burdens in gill and response measures such as Na^+ and Cl^- depletion, Na^+/K^+ -ATPase inhibition, or metallothionein induction. A relationship between tissue Ag(I) burden and toxicity can be seen during exposures in the laboratory to the free Ag^+ ion, but it appears that Ag^+ is present at extremely low concentrations in nature and that virtually all Ag(I) in the environment is bound in thermodynamically stable S(II-) complexes and to a lesser extent to organothiols (see Chapter 1). Field studies are appropriate to determine if Ag(I) is accumulated by organisms exposed in their natural aquatic environments and if any such accumulated Ag(I) is bioreactive.

Studies on marine mollusks from San Francisco Bay and contaminated sites in Great Britain have demonstrated that Ag(I) in natural environments indeed can be bioavailable (Truchet et al. 1990; Hornberger et al. 1999; Griscom et al. 2000). Furthermore, work conducted in San Francisco Bay indicates that accumulated silver might be bioreactive. These studies have been described in some detail because they are the only field studies available suggesting that silver potentially could be associated with chronic effects in natural aquatic environments. One of these is a time series study of Ag(I) and Cu bioaccumulation in the bivalve *M. balthica* at a mudflat in South San Francisco Bay (Hornberger et al. 1999). Archived specimens were sectioned and reproductive potential (i.e., presence of fully developed sperm or eggs) was examined (Figure 3-2). Animals were analyzed for silver, copper, and zinc at near monthly intervals throughout the study period (1974 to 1976; 1979 to 1981; 1982 to 1984; 1987 to 1989). A variety of metals, grain size, and total organic carbon (TOC) in sediments were studied over the entire time series; these conditions were variable seasonally and year-to-year, but no unidirectional trends were observed. Many other environmental conditions were carefully monitored and, again, no unidirectional trends were observed. Unidirectional trends were observed for Ag(I) and Cu(I/II), but not for Zn(II).

Silver levels in sediments declined from approximately $1.6 \mu\text{g g}^{-1}$ (15 nmol g^{-1}), dw, in the late 1970s to $0.2 \mu\text{g g}^{-1}$ (1.8 nmol g^{-1}) in the late 1990s. Concurrently, sediment copper levels dropped moderately from approximately $85 \mu\text{g g}^{-1}$ ($1.3 \mu\text{mol g}^{-1}$) to approximately $50 \mu\text{g g}^{-1}$ ($0.78 \mu\text{mol g}^{-1}$). Silver, as well as copper, tissue concentrations declined from extremely high levels (silver: approximately $100 \mu\text{g g}^{-1}$; copper: approximately $300 \mu\text{g g}^{-1}$) in 1975 to 1982 to levels about 5-fold background in the late 1990s (silver: approximately $10 \mu\text{g g}^{-1}$; copper: approximately $50 \mu\text{g g}^{-1}$). These data suggest that exposure to bioavailable silver and copper changed substantially over the study. During the period of greatest Ag(I) and Cu(I/II) exposure, few animals from Palo Alto mudflat ever contained mature gametes (Figure 3-2; Hornberger et al. 1999). In one year during this period, 1982 to 1983, a reproductive cycle typical of other mudflats in San Francisco Bay (Nichols and Thompson 1985) was observed, which might be explained with a very

high concurrent freshwater discharge. Reproductive inhibition was again observed in 1985 and 1987. In 1989, the year of lowest metal exposure, nearly all animals contained mature gametes in nearly all months (Figure 3-2A and 3-2B).

A Reproductive status, *M. balthica*
1979 to 1981
Means: Cu = 266; Ag = 90 $\mu\text{g/g}$



B Reproductive status, *M. balthica*
1988 to 1989
1988: Cu = 53; Ag = 20 $\mu\text{g/g}$
1989: Cu = 35, Ag = 11 $\mu\text{g/g}$

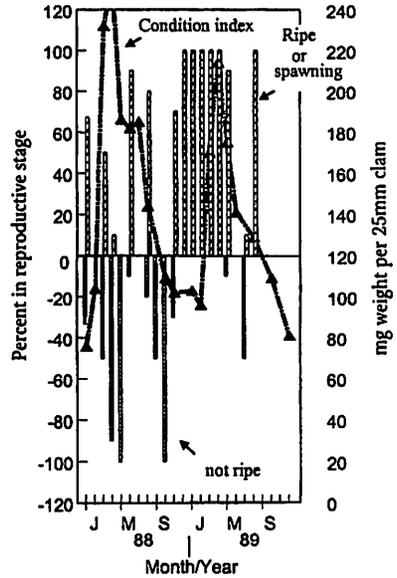


Figure 3-2 Condition and reproductive status in the clam *M. balthica* at a site in South San Francisco Bay in (A) 20 near monthly samples in 1979 to 1981 and (B) 18 near monthly samples in 1988 to 1989. Reproductive data from 1974 to 1976 and 1983 to 1985 are not shown, but are consistent with trends shown here. Vertical bars on positive axis represent the percent of clams (approximately 10 individuals were sectioned for each monthly sample) with mature sperm or eggs in their gonads. Bars on the negative axis represent the percent of animals that did not contain mature sperm and eggs in their gonads. The line represents the condition index, which is the weight of tissue in an animal with a shell length of 25 mm. The condition index varies as clams add and lose lipid seasonally. No other environmental variable changed unidirectionally other than pollutant concentrations over this sampling period or among the years when reproduction was studied.

The significance of the reproductive depression was substantiated by development of increased tolerance to both Ag(I) and Cu(I/II) during the period of increased metal exposure (Luoma 1977). Furthermore, during the period of greatest exposure to metals (1979 to 1980), spillover of Ag(I) and Cu(I/II) from metallothionein-like protein associations to low molecular weight compounds was reported (Johansson et al. 1986). Shift of intracellular Ag(I) away from metallothionein is a sign of metal movement toward sensitive target sites and has been related to physiological

stress in some studies (Sanders et al. 1983). Overall, this evidence does not prove that the population of bivalves at Palo Alto was threatened, but it does indicate that Ag(I) and/or Cu(I/II) stress might have been present. Thus, at Palo Alto, environmental Ag(I) and Cu(I/II) exposures were positively correlated with reproductive impairment. Many other potential covariates were examined and ruled out as possible causes of the effects. It remains possible that organic toxicants, which were not measured, played a role in the observed pattern.

The second field study was conducted in North San Francisco Bay with the bivalve, *Potamocorbula amurensis* (Samuel N. Luoma, U.S. Geological Survey, unpublished observations). The approach was much the same (see Brown and Luoma 1995). Samples were collected at near-monthly intervals between 1991 and 1999. Tissue concentrations were employed to evaluate metal exposures. Hydrography and hydrology were carefully documented throughout the study (*P. amurensis* is a filter feeder so water column hydrography is probably more important than sediment chemistry). Five sites were studied over the period, from the head (landward reach) toward the mouth (seaward reach) of the estuary, along the salinity gradient. Salinity is more variable at these stations and over time than at Palo Alto in South San Francisco Bay. Food availability probably follows the salinity gradient, from the head (high availability) to the mouth (low availability) of the estuary; allochthonous food from the freshwater delta may be a very important source of nutrition for these animals. A Cd(II) contamination gradient also consistently occurs from the head toward the mouth (Brown and Luoma 1995). Other metal exposures are more complicated, but understood. Ag(I) contamination occurred at the midestuarine sites near a military base between 1991 and 1994 in this study. No additional metal contamination occurred, nor was the gradient in Ag(I) contamination associated with any other estuarine parameter (such as annual mean salinity), food, sediment, or hydrographic gradient. The potential of exposure to organic toxicants does exist and studies continue to evaluate that possibility. The biological response was the same as at Palo Alto. The frequency of occurrence of mature gametes was reduced at the higher Ag(I) exposures, and the normal cycle of occurrence (as defined by comparison to less contaminated sites) was altered. As the Ag(I) contamination dissipated, the normal cycle of gamete production returned. The Ag(I) contamination episode was therefore correlated with reduced reproductive capabilities of the clams.

The approach used in these field studies eliminated many confounding environmental variables because of the long-time series and multifaceted data collection. Although copper body burden covaried with the effect in Palo Alto, this was not the case in North San Francisco Bay. Elevated levels of cadmium were present in North San Francisco Bay but did not correlate with reduced gamete production. It should be noted that organic contamination was not studied and it is possible that non-metal toxicants could have caused or contributed to the effects observed. This possibility is currently being examined. However, evidence at hand suggest that

Ag(I) could have been involved in chemical interference with the production of mature gametes in these two episodes. Following on the finding by several investigators that Ag(I) in diet causes interference with reproduction in copepods and cladocerans (Schmittschmitt et al. 1996; Fisher and Hook 1997; Bielmyer et al. 2001), this effect of Ag(I) and its causes deserve further detailed investigation.

Within these two field studies, the observation of reproductive impairment was correlated with Ag(I) body burden. However, the tissue concentration at which effects were elicited was not the same in the two bivalves used in the two studies. The concentration of Ag(I) in tissues coincident with impaired reproduction in *M. balthica* was between 16 to 80 $\mu\text{g g}^{-1}$ (15 to 74 nmol g^{-1}), dw. Even though this is an extensive data set, it is limited for such a statistical analysis, so the uncertainty is high with regard to a threshold level. The data set for *P. amurensis* is more extensive because data are available from multiple sites. The threshold for the effects was 5 to 10 $\mu\text{g g}^{-1}$ (46 to 90 nmol g^{-1}), dw.

The field results are consistent, to some degree, with laboratory results because no simple relationship was observed between Ag(I) exposure and the associated effect. A universal relationship of this sort may not be possible with total tissue burdens. This does not mean, however, that no such relationship exists; the field studies outlined above indicate that within a biological species and a general geographical area total silver body burden might relate to chronic effects. It also should be recognized that the observation of substantial Ag(I) bioaccumulation may be a flag that Ag(I) was a pollutant of concern in both studies, even though a universal threshold tissue burden could not be established. Bioaccumulation results can be indicative of situations where organisms may be exposed to Ag(I). Further study is therefore necessary to delineate the toxicological significance of environmentally accumulated Ag(I). Bioaccumulation also may be a useful first-tier indicator of susceptible species in natural systems. For example, extremely high Ag(I) concentrations, approximately 100 $\mu\text{g g}^{-1}$ (930 nmol g^{-1}), wet weight, have been reported in the liver of beluga whale (*Delphinapterus leucas*) (Becker et al. 1995; Mackey et al. 1996), although Ag(I) contamination in beluga whale has not been linked to health effects. There also is evidence from work with invertebrates that the tendency to accumulate metals, including Ag(I), in a particular species is inversely correlated to the metals tolerance of that species as well as to its abundance in metal polluted areas (Birge et al. 2000). Further, metal accumulation in a moderately metal sensitive species, the central stoneroller minnow (*Camptostoma anomolum*), was found to be strongly correlated with poor macroinvertebrate diversity (Birge et al. 2000). This suggests that investigation of the role of metals, including silver, in relation to the health and bioadaptability of these organisms might be of value.

Intracellular speciation of Ag(I)

To some extent, what determines if absorbed Ag(I) is toxic or not depends on the molecular makeup of the cell in which Ag(I) is accumulated. Because of the presence of sulfhydryl-containing amino acids, and amino acid residues in cells, all intracellular Ag(I) is bound to biomolecules. Of special importance are metallothionein (Mayer et al. 1997) and probably glutathione, which are suggested to have log K values for Ag of greater than 17 (Hamer 1986; Kägi and Schäfer 1988) and 12.3 (Adams and Kramer 1999), respectively. Typical intracellular concentrations of these particular biomolecules are 15 to 60 μM for metallothionein (Hogstrand et al. 1996) and 5 to 10 mM for glutathione (Mason and Jenkins 1995). Thus, speciation likely occurs by molecular interactions between biomolecules. The functional significance of this is that intracellular distribution and movement of Ag(I) apparently are not random, but rather linked to biological pathways.

As indicated previously, cell-specific distribution of Na^+/K^+ -ATPase and Ag(I) in fish gills may explain why different forms of bioavailable Ag(I) shows tremendous differences in bioreactivity. The capacity to detoxify Ag(I) is another factor that seems to be of importance in determining Ag(I) toxicity. In a cell culture study using 2 different salmonid cell-lines, one of which had a compromised metallothionein expression, it was found that the cells lacking metallothionein were about 5 times more sensitive to Ag(I) than the ones that displayed a normal expression of the protein (Mayer et al. 1996). Other studies have shown that metallothionein protects against Ag(I) inhibition of isolated Na^+/K^+ -ATPase (Hussain et al. 1994, 1995; Ferguson et al. 1996). These in vitro results can be related back to the whole animal. Following a 2-day exposure to radioactively labeled Ag(I) ($11.9 \mu\text{g L}^{-1}$ or 110 nM added as $^{110\text{m}}\text{AgNO}_3$ to S(II-)-depleted water), Ag(I) levels in the cytosolic fraction of the liver from rainbow trout increased from 35% to 72% of the total cellular burden between days 8 and 19, respectively (Galvez et al. 2001). Using size-exclusion chromatography, it was determined that most (approximately 70%) of the $^{110\text{m}}\text{Ag}$ content in the liver cytosol eluted at a molecular weight characteristic of metallothionein. In comparison, the cytosolic distribution of $^{110\text{m}}\text{Ag}$ in gills was less specific, with binding of the metal to several fractions, including metallothionein. Only a small portion (approximately 15%) of the gill cytosolic Ag(I) load was bound to metallothionein. The considerably lower capacity of the gills compared the liver to bind Ag(I) to metallothionein renders the former more vulnerable to Ag(I) toxicity, which is reflected in the fact that the gill is the primary target for acute Ag(I) toxicity. Similarly, a recent study indicates that the metal-sensitive mayfly (*Stenonema* sp.) has a reduced ability to produce metallothionein (Cain et al. 2001). Another approach used by many invertebrate species to detoxify metals is to immobilize them in intracellular inclusion bodies, made up by membrane-surrounded concretions of inorganic metallosulfur complexes (Truchet et al. 1990). The formation of nontoxic granules can lead to accumulation of body burdens of recalcitrant, inactive Ag(I) in tissues, and thereby

uncouple immediate toxicity from a response. Long-term fate of these granules has not been studied.

An earlier report by Truchet et al. (1990) on the common periwinkle *Littorina littorea* and the peppery furrow shell *Scrobicularia plana*, collected from the Looe estuary (Great Britain), did examine Ag(I) speciation and showed, histologically and chemically, that Ag(I) accumulated mainly extracellularly in basement membranes. Ag(I) was present as clusters of Ag_2S granules along with copper sulfide. In a related laboratory study with 4 bivalve mollusks (Berthet et al. 1992), likewise, it was found that Ag(I) was present as granules of Ag_2S in a variety of organs and cell type, depending on species. Some of the accumulated Ag(I) (10% to 20%) also was found in a soluble protein fraction, presumably bound to protein SH-groups.

Whereas not all bioaccumulated Ag(I) is bioreactive, bioreactive Ag(I) can be bioaccumulated in realistic situations. Bioreactivity of Ag(I) is governed by factors such as Ag(I) species assimilated, route and rate of uptake, and capacity of Ag(I) accumulating cell types to detoxify Ag(I). Free Ag^+ ion exposure results in bioaccumulation of highly bioreactive Ag(I) at the gill, but Ag^+ concentrations in natural environments are vanishingly low. Exposure to chloro-complexed and thiosulfate-complexed Ag(I) result in bioaccumulation of Ag(I) of low apparent bioreactivity in several fish species. Dietary uptake apparently results in accumulation of bioreactive Ag(I) in zooplankton. The effect appears to be linked to uptake from a soluble intracellular fraction in algal cells. Available evidence suggests that dietary Ag(I) is not bioreactive in fish, but so far possible effects on gamete formation have been ignored. Associative evidence suggests bioreactive Ag(I) occurred in bivalves at silver-contaminated sites in nature. At present, it is not understood how to separate bioaccumulated Ag(I) from bioreactive Ag(I), and mechanisms that facilitate or inhibit formation of bioreactive Ag(I) in tissues must be better characterized. Studies to this end should include Ag(I) reactions on target cells and speciation of Ag(I) within organisms.

Identification and Characterization of the Chronic Toxicity of Silver

Significant advances have been made in the characterization of the acute toxicity of Ag(I) to freshwater fish (see Chapter 2). In contrast, very little is known of the chronic toxicity of Ag(I). The establishment of chronic water-quality criteria (WQC) requires the derivation and application of chronic values; i.e., greater than no-observed-adverse-effect level (NOAEL) less than lowest-observed-adverse-effect level (LOAEL). Ideally, chronic values would be derived from laboratory toxicity tests that involve exposure of the test organisms through the life cycle (chronic exposure), allowing measurement of toxicity endpoints distinct from those observed during acute exposure. Preferentially, these endpoints involve characteris-

tics such as fecundity and scope for survival in natural environments where predators and competitors are present.

However, since full life-cycle laboratory studies with fish are complex and expensive, subchronic toxicity studies often are used as surrogates. Such experiments have been performed with rainbow trout, during which effects of Ag(I) (added as AgNO₃) on embryo development and larval growth and survival were evaluated (Davies et al. 1978). While established subchronic tests are informative about the effects of Ag(I) on the specific endpoints measured, they provide no information on other relevant indicators of chronic toxicity, such as reduced gamete numbers and quality, nor do they provide insight into the potential for toxicity to the embryo resulting from maternal transfer of Ag(I) to the eggs. Currently, the establishment of chronic WQC relies heavily upon the use of acute-to-chronic ratios (ACRs) to estimate chronic values for species that the acute toxicity of the chemical has been characterized but the chronic toxicity has not been evaluated. These ratios are derived from toxicity values for species in which both acute and chronic toxicity values have been measured.

The validity of the ACR approach is contingent upon the general assumptions that ACRs will be constant over the range of natural water chemistries and animal species. ACR also assumes that waterborne Ag(I) is the prevalent cause of chronic toxicity. There are several reasons why these assumptions may not be valid for silver. Acute toxicity to Ag(I) is caused by ionoregulatory dysfunction at the body surface. This is a highly speciation-dependent phenomenon and not all accumulated silver species contribute towards the toxic effect. In contrast, available evidence indicates that chronic toxicity may operate through a different mechanism (i.e., gamete formation) and that there may be a direct correlation between accumulated Ag(I) and observed effect. Furthermore, chronic silver toxicity may be dominated by dietary silver whereas ACR is based on waterborne exposure, which is the critical administration route for acute toxicity. Chronic values that are greater than the corresponding acute values have been reported for Ag(I), resulting in ACR values less than 1 and clearly raising concerns about the validity of the approach. Chronic values that are greater than acute values may reflect differences in speciation and bioavailability of silver between acute and chronic exposures. For example, the requirement for feeding during chronic exposures may increase the NOM content of the exposure media, reduce the bioavailability of the silver, and reduce toxicity associated with the total silver concentration in the media.

Results from recent laboratory studies and correlative field evidence suggest that Ag(I) may target reproduction in some invertebrate species, resulting in reduced fecundity (see Chapter 2). Epidemiological studies in humans and reproductive studies in rodents have failed to identify any significant reproductive toxicity associated with silver exposure (summarized in U.S. Department of Health and Human Services 1990). Notably, studies designed to evaluate the chronic, reproductive toxicity of silver to fish and other oviparous vertebrates are lacking.

Confirmation of possible reproductive toxicity of silver to invertebrates is required, as well as the extension of such studies to oviparous vertebrates. Identification of the mechanism of chronic toxicity of Ag(I) will facilitate the establishment of WQC by identifying groups of organisms that may be susceptible to this mechanism of toxicity, as well as those that would not be likely to be susceptible due to differences in reproductive physiology, and would allow for the appropriate use of subchronic toxicity assessments that include the sensitive target as an endpoint of toxicity. Knowledge of the mechanisms of chronic toxicity could possibly allow the development and use of ACRs among species that share common physiological targets of chronic Ag(I) toxicity.

Ag(I) biogeochemistry is sufficiently unique to warrant particular considerations in designing full or partial life-cycle toxicity assessment with this metal. These considerations include the following:

- exposures that incorporate as much of the organism's life cycle as is technically and practically possible;
- incorporation of both aqueous and dietary routes of Ag(I) exposure into the study design;
- evaluation of a variety of species representing various trophic levels;
- use of environmentally relevant and well characterized silver speciation for exposures;
- effects related to measured, not nominal, Ag(I) concentrations; and
- measurement of appropriate endpoints of chronic toxicity, particularly as related to fecundity and population impact.

In summary, insufficient information is available to confidently define chronic values of silver and, at this point, ACR is not an appropriate approach for Ag(I). Fecundity and other abilities to be competitive within an ecosystem are important and relevant to populations. Chronic toxicity tests must be more than simply extended acute tests and must include exposure to dietary forms of bioavailable silver. Speciation of Ag(I) and the chemistry of test water must be controlled and appropriately monitored and should be consistent with natural waters. Likewise, bioaccumulation and bioreactivity should be monitored. Full life-cycle toxicity studies with Ag(I) on carefully chosen organisms are needed to define the chronic toxicity of this metal, to identify putative endpoints of chronic toxicity, and to provide insight into mechanisms of chronic toxicity. A mechanistic understanding of the chronic toxicity of Ag(I), in turn, will allow the design and conduct of partial life-cycle studies that incorporate relevant endpoints. Available information suggest that effects on critical life-cycle processes, such as maternal transfer and early life stages, deserve special consideration. A mechanistic understanding of chronic toxicity also could allow for the use of ACRs among species that share common targets of chronic Ag(I) toxicity.

Silver Sensitivity

Background

It is important to determine the most sensitive receptors of silver toxicity. This may be in terms of specific organisms, certainly, but also may be in terms of a particularly sensitive biological process, organ, or environment. Such knowledge would allow a better focus of efforts from a scientific standpoint, as well as from the regulatory aspect. For the biologist-chemist, the advantage is the ability to concentrate limited resources on specific and delineated tasks. The regulator, similarly, is provided with an indicator, or at least an area of concern, that can be closely monitored against environmental damage. Currently, there is not a great deal of data relating to organisms, sites, or locations that may be the most sensitive to silver toxicity.

Acute toxicity

A large body of information is available in the scientific literature reporting the acute toxicity of Ag(I) to freshwater organisms of various species. Laboratory data are typically provided for aquatic model species, such as rainbow trout, fathead minnow, *Daphnia* sp., or *C. dubia*. Several reports have shown that the site of relevance for acute toxicity of freshwater fish is the gill, causing interruption of the ionoregulatory system. Freshwater invertebrates are generally more sensitive than freshwater fish, but it has not been rigorously shown that Ag⁺ is the only form that causes toxicity, or that the gill is the site of concern in invertebrates (confer with Chapter 2; Wood et al. 1999). However, given its low abundance and geochemistry, Ag(I) is not likely to be an acute toxicity problem in freshwater environments (confer with Chapter 4). Although there are less data available for saltwater systems, acute effects of Ag(I) seem even less likely to be problematic because of the high concentrations of chlorides, which would bind any Ag(I) not complexed by S(II-). This is borne out by studies that show that Ag(I) is much less acutely toxic in brackish water and seawater because of the elimination of free Ag⁺ and loosely bound Ag(I) (Shaw et al. 1997, 1998; Ferguson and Hogstrand 1998; Hogstrand and Wood 1998; Wood et al. 1999). In marine fish, uptake of waterborne Ag(I) may be both through the gills and via absorption at the gut (reviewed in Wood et al. 1999). This does not alter the fact that in both freshwater fish and marine fish species, the primary cause of acute Ag(I) toxicity is disruption of the ion transport system and volume regulation (confer with Chapter 2; Hogstrand and Wood 1998; Wood et al. 1999). There is, however, an important difference between the 2 groups in that the site of acute Ag(I) toxicity in marine fish is believed to be the gut, and not the gills as in freshwater species. It is recommended that work on acute toxicity be focused on updating ambient WQC and validation of the BLM so it can be used in adjusting the criteria. The BLM needs to be tuned and validated with natural freshwaters, but the majority of the work will need to be on brackish and marine systems in which it is still unknown whether or not BLM can be used to model toxicity.

Chronic toxicity

The most sensitive and environmentally realistic endpoint is probably a chronic one. Work by Schmittschmitt et al. (1996), Fisher and Hook (1997), and Bielmyer et al. (2001) show that fecundity of some species of zooplankton is reduced if they are fed phytoplankton that contain silver. While biomagnification does not occur, Ag(I) may be concentrated by the phytoplankton by adsorption onto the frustule and incorporation into cellular material, increasing the dose to the zooplankton above ambient concentrations. An exposure through the food chain such as this also is significant because the form that Ag(I) is presented and the route of exposure both affect the manifestation of toxicological effects, when compared to water-only exposure. As previously discussed (see section: *Dietary exposure*), there are data indicating that gamete formation in bivalves may prove to be sensitive to Ag(I), and particularly to Ag(I) associated with the sediment. Reproductive endpoints may prove to be important, but work is just beginning on investigation of this phenomenon.

Added research is needed to elucidate the primary effects of chronic exposure, which appear to be focused on the reproductive system, in the most sensitive species. It is important to point out that, while reproductive disturbances have only been noted in a few invertebrate species, such endpoints of Ag(I) effects have not been investigated for more than a handful of species, belonging to two phylogenetic groups (*Crustacea* and *Bivalvia*). In the investigation, other areas of potential sensitivity that are not known at this time may become important. As an example, two recent articles reported concentrations of Ag(I) in beluga whale liver of about $100 \mu\text{g g}^{-1}$ (930 nmol g^{-1}), wet weight (Becker et al. 1995; Mackey et al. 1996), by far the highest values that have been noted in the literature. There are no known effects from this silver accumulation in beluga whales, but it is recommended that when concentrations such as these are noted, they should be investigated to determine if there are any consequences.

In conclusion, sensitive species and sensitive processes must be identified to understand or predict potential ecosystem effects. Reproductive effects appear to be a sensitive chronic endpoint of Ag(I) stress. In laboratory studies, it has been demonstrated that feeding of Ag-exposed phytoplankton to cladocerans and copepods results in reduced fecundity. This may be true even when the phytoplankton are exposed to environmentally realistic concentrations of Ag(I). Because phytoplankton efficiently take up Ag(I) complexes, understanding Ag(I) biotransfer and bioreactivity in the phytoplankton-based food web is important. The only reported possible effect of Ag(I) on natural populations was a reduction in gamete production in bivalves from the San Francisco Bay area. It is possible that bivalve sensitivity to Ag(I) may be enhanced because of dual digestive routes and long gut residence times in these animals. These issues are just emerging, and it is recommended that research define and clarify the potential for effects in the natural environment.

Sediment Processes

Environmental Ag(I) concentrations are very low ($< 0.5 \mu\text{g L}^{-1}$; 5 nM [unfiltered]; confer with Chapter 1). In addition, Ag(I) has a high affinity for adsorption on particle surfaces and aqueous Ag(I) concentrations are predominately associated with particulate and colloidal forms. The tendency for Ag(I) to exist in colloidal and particulate forms can result in accumulation in sediments. For example, long-term monitoring has shown that patterns of Ag(I) accumulation in sediments are related to Ag(I) discharges from upstream publicly owned treatment works (POTWs) (Shafer et al. 1998; Hornberger et al. 1999). The combination of low water-column concentrations and a tendency for accumulation in sediments makes Ag(I)-contaminated sediment a potentially significant route of exposure for benthic organisms, and thus a possible pathway for Ag(I) to enter the food web.

Bioavailability of Ag(I) in sediments appears to be limited by the chemical nature of sediment Ag(I) deposits. Accumulation of S(II⁻) in sediments has been documented to determine the fate and bioavailability of metals by formation of insoluble and stable metal sulfides (Ankley et al. 1996). Ag(I) forms some of the most stable metal sulfide complexes. Furthermore, the high stability of silver sulfides, relative to other metal sulfides, means that Ag(I) can successfully displace other metals from available sulfides. Thus, whenever there is molar excess of S(II⁻) in sediments, Ag(I) should exist bound to reduced sulfur. Given the low environmental concentrations of Ag(I) it is anticipated that there will always be sediment sulfide concentrations in excess of sediment Ag(I).

Exposures of benthic invertebrates to sediment Ag(I) in the form of Ag₂S have been made to evaluate bioavailability and toxicity of silver sulfides. In 10-day exposures to *Hyallela azteca*, Ag₂S had no effect on mortality and no effect on body weight despite very high concentrations of added Ag(I) (up to 750 mg kg⁻¹ or 6.9 mmol kg⁻¹ dw; Hirsch 1998b). In a similar experiment, 28-day exposures of *Lumbriculus variegatus* to sediment with added Ag₂S, had no observed effects on mortality, reproduction, or body dw. There were, however, higher Ag(I) concentrations measured as total body burden (80 $\mu\text{g Ag g}^{-1}$ or 0.74 $\mu\text{mol g}^{-1}$ dw) in animals exposed to sediments containing 440 mg kg⁻¹ (4.1 mmol kg⁻¹) Ag(I). This low level of accumulation was used to estimate a sediment bioaccumulation factor of 0.18, which supports the idea that silver sulfide in sediments has generally low bioavailability or bioreactivity (Hirsch 1998a). Metal spiking experiments of ionic Ag⁺ (added as AgNO₃) can be used to produce silver sulfides within anoxic sediments. Acute exposure of benthic invertebrates to such silver-spiked sediments did not result in toxicity when silver levels were lower than measured AVS (Berry et al. 1999). A long equilibration time (> 100 days) was used in these exposures to convert the added Ag(I) to Ag₂S. Since the kinetics of Ag(I) complexation with sulfides are believed to be very rapid (minutes; see Chapter 1), it is not clear why these long equilibration times were necessary. One explanation could be that the

Ag(I) concentrations added were much higher than what would be typical of environmental exposures. It also is not clear whether similar equilibration times would be necessary at the very low concentrations of Ag(I) normally found in the environment to be incorporated within sediment sulfides.

These short-term laboratory studies suggest that silver sulfides in sediments is neither bioavailable nor bioreactive (Hirsch 1998a, 1998b). However, the long-term field studies discussed earlier (see section: *Dietary exposure*) suggest that exposure of the bivalves *M. balthica* and *P. amurensis* to natural silver-contaminated sediments can lead to bioaccumulation of silver that might have been bioreactive (Hornberger et al. 1999). Measurements of Ag(I) accumulation in these organisms over a 20-year period correlated with acid-extractable Ag(I) concentrations in sediments. In these sediments, AVS by far exceeded extractable Ag(I) (Lee, Griscom et al. 2000). Similar correlation was found between measurements of Ag(I) accumulation in *M. balthica* and acid-extractable Ag(I) in estuaries around the UK (Luoma et al. 1995). These results raise some important questions about the bioavailability and bioreactivity of Ag(I) in natural sediments and possible species differences in uptake of Ag(I). It remains to be shown if these environmentally exposed organisms are accumulating Ag(I) bound to sediments, which would presumably be incorporated in sediment sulfides, or if other routes of exposure are significant sources (such as Ag(I) bound to organothiols or water-column particulates).

To summarize, in most natural sediments AVS likely exceeds the concentration of Ag(I), indicating that Ag(I) is likely bound to S(II-) compounds. Therefore, it is of importance to investigate and characterize any bioavailability and bioreactivity of Ag(I) bound up in metal sulfides and organic thiolates. Some information on this topic is available. *Lumbriculus* and amphipod bioassays show no toxicity when AVS exceeds Ag(I) concentrations on molar basis. However, bivalves may assimilate Ag(I) bound to metal sulfides (i.e., FeS, FeS₂) in anoxic sediment, thereby rendering silver bioavailable. In fact, 0.3 to 1.0 M HCl-extractable Ag(I) correlates with Ag(I) bioaccumulation in environmentally exposed bivalves across broad geochemical gradients (Luoma and Bryan 1982; Luoma et al. 1995). Moreover, Ag(I) is bioaccumulated and correlated with negative effects on reproduction when both water column and sedimentary inorganic S(II-) levels are in excess of Ag(I) (Hornberger et al. 1999). Thus, S(II-)-bound Ag(I) complexes may be both bioavailable and bioreactive to some organisms and there is a conceivable risk that Ag(I) might be mobilized to the food web through this route. The significance of S(II-) and AVS measurements on Ag(I) speciation and resultant bioavailability needs to be better understood.

General Conclusions and Recommendations

Regulatory recommendations

There seems to be strong agreement among chemists that Ag(I) in aquatic environments exists almost entirely bound to inorganic S(II-) and organic-reduced sulfur ligands, which are chemically very stable (confer with Chapter 1). It is, however, important to note that whatever the speciation, environmental Ag(I) is de facto bioaccumulated. At this time, bioreactivity of intracellular Ag(I) accumulated from such sources is not well known. Therefore, geochemical normalizations to AVS or "methylene blue measurable sulfide" may oversimplify the bioavailability concept. Regulatory implementation of bioavailability concepts will require consideration of biological factors, such as conceivable microbial remobilization of Ag(I), ability of some species to assimilate Ag(I) from silver sulfides, and possibility of effects via food-chain transfer. Thus, regulatory criteria must reflect biological realities, especially to protect from chronic effects on populations and communities and/or avoid oversimplifications about effects.

At the present time, the following are considered particularly important regulatory recommendations:

- **Body burden:** Bioavailable Ag(I) should not be regarded uniformly equal to bioreactive Ag(I). There is no universal relationship between accumulated Ag(I) and toxicity, although trends may be observed between accumulation and effects in key species in similar environments.
- **BLM:** Consider using the BLM approach for site-specific adjustments of acute toxicity, but not before validation and fine-tuning is completed and any substantial limitations have been identified.
- **ACRs:** Do not use ACRs to derive chronic WQC. Chronic limits have to be determined using endpoints relevant to chronic exposures and be modified by environmental factors that influence chronic toxicity.
- **Extractable sulfides (e.g., AVS):** Be cautious about using acid extractable sulfides as modifiers of silver criteria. Evidence exists that Ag(I)sulfides are both bioavailable and perhaps bioreactive to some organisms.

Research recommendations

Ultimately, it would be very advantageous to establish biologically universal links among some measure of tissue residues of Ag(I) (i.e., bioreactive Ag(I), non-metallothionein-bound Ag(I), cell-specific Ag(I) accumulation, total tissue Ag(I)) and effects detrimental to organisms. There seem to be possibilities for links, but they lie in understanding distributions, speciation, or processes within organisms, tissues or cells. Future studies should therefore consider uptake mechanism, distribution between cell types and tissues, intracellular speciation of Ag, chronic toxicity studies with "chronic endpoints."

Uptake mechanisms

Depending on geochemical speciation and uptake route, some forms of Ag(I) may go directly to sites of toxic action (e.g., Ag^+ to Na^+/K^+ -ATPase); other forms may diffuse more nonspecifically through the tissues or cell and not reach the sites of concern. Dietary exposure to Ag(I) may involve transport of potentially toxic forms of Ag(I); this is a hypothetical explanation for the apparent toxicity of dietary Ag(I) uptake in zooplankton. At least in mollusks, Ag(I) may be assimilated from anoxic, S(II)-rich sediments or Ag_2S after ingestion. Clearly, identification of uptake mechanisms for Ag(I) in different organisms is the key to understanding which forms of Ag(I) can be bioavailable and lead to bioreactivity.

Distribution between cell types and tissues

Different types of cells may accumulate Ag(I) differently. Forms of Ag(I) that are transported into susceptible cells may cause effects more readily than those transported into less susceptible cells. Alternatively, species or developmental stages that are rich in susceptible cells may be more vulnerable to Ag(I) effects (e.g., inhibition of Na^+/K^+ -ATPase seems especially vulnerable, and cells differ vastly in their content of this enzyme; Perry 1997). Therefore, it is of importance to identify the distribution of Ag(I) to sensitive cell types as function of exposure to environmentally realistic forms of Ag(I).

Intracellular speciation of Ag

Just as geochemical speciation of Ag(I) is important in understanding transport and fate of Ag(I) in the environment, studies on the intracellular speciation of Ag(I) in an organism helps us to understand its bioreactivity. Subcellular or tissue-specific fractionation of Ag(I) (in geochemical terms, intracellular speciation) differ depending upon the form of the Ag(I) that is introduced to the species, the rate of accumulation, and the exposure route. This could be one explanation of why bioaccumulated Ag(I) is bioreactive in some instances but not others. Furthermore, organisms may detoxify Ag(I) differently and this also may interfere with a simple relationship between total body Ag(I) and adverse effects. Thus, studies are recommended that can help to identify the intracellular localization of Ag(I) in relation to exposure regime.

Chronic toxicity studies with "chronic endpoints"

It cannot be assumed that endpoints for acute toxicity are the most sensitive chronic endpoints. Similarly, the forms of Ag(I) that are acutely the most toxic may not necessarily be the forms that are most potent or relevant in producing chronic effects. Because of observed indications that chronic toxicity (e.g., reproductive disturbance) may occur at environmentally realistic concentrations and forms of Ag(I) (whereas acute toxicity does not), it is of outmost importance that the sensitive species and endpoints are identified, the mechanisms of chronic toxicity

become unraveled, and the chronic toxicity testing is designed so that appropriate uptake pathways and effects are considered.

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Risk Assessment

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Implications for Regulation

The results of the preceding sections have significant implications with respect to assessing the risk associated with ambient levels of silver in the environment and to the regulatory measures that are implemented to manage this risk. The implications of these results are discussed in this section in the context of the standard framework for risk assessment (USEPA 1992, 1996b). This framework includes the following steps: problem formulation, exposure assessment, effects assessment, and risk characterization. Although this approach is adopted for organizational purposes, it is premature to expect that the results discussed will constitute a definitive risk assessment for silver. Rather, the results are intended to provide an overview of the current state of the science as it relates to completion of a risk assessment for silver. Although, in some respects, the results to be presented serve as a screening-level assessment for silver, significant data gaps and uncertainty exist. Areas where further research is warranted to reduce uncertainty to acceptable levels, should a detailed risk assessment for silver be performed, will be highlighted.

Problem Formulation

In risk analysis, the problem formulation step defines the problem to be addressed, develops assessment endpoints (i.e., what are we trying to protect), and develops a plan for analyzing and characterizing the risk. In this section, preliminary hypotheses are put forward, conceptual models are developed, and assessment endpoints and measures of effects are identified.

This workshop focused on releases of silver to aquatic systems. Aquatic systems receive inputs of silver primarily from industrial effluents, sewage treatment plant outfalls, runoff from sludge-amended soils, and sometimes from unregulated point-source discharges. Elevated concentrations of silver are usually associated with anthropogenic activities such as mining and photographic processing. Silver recovery from photo processing effluents has increased in efficiency over recent

years in response to both environmental concerns and economic demand for reclaimed silver (Eisler 1996; Purcell and Peters 1998). Nearly all photo processing final effluents are diluted with other wastewaters when entering a wastewater treatment plant (WWTP), then silver is mostly removed (> 94%) as it passes through the WWTP (Shafer et al. 1998). Final WWTP discharges of silver into aquatic systems are usually at low concentrations, and most of this silver is bound to particles, organic colloids, thiosulfate, sulfide, dissolved organic matter (DOM), and chloride. The amount of total silver existing as uncomplexed, ionic Ag^+ in the environment, as a result of WWTP discharges, is therefore very low (Lytle 1984; Purcell and Peters 1998).

This section evaluates sensitive aquatic species and vulnerable aquatic systems to determine if they are at risk from silver loading. The conceptual model will present pathways of silver to aquatic systems and routes of exposure to aquatic organisms. Because of the lack of information, not all pathways will be addressed. Current ambient concentrations of silver in aquatic systems and aquatic chemistry issues of silver will be presented in the *Exposure assessment* section. Uncertainties and data needs will be presented. This information will be used with chronic toxicity data for aquatic biota that are presented in the *Effects assessment* section. Additional testing requirements and study limitations will be discussed. The exposure and effects sections will be used to characterize the risk from silver to these aquatic organisms. Lines of evidence and associated uncertainties will be discussed.

The effects of silver on wildlife, other terrestrial biota, and humans will not be addressed in detail in this document. The following sections present a brief discussion of the impacts of silver on these endpoints and explain why they have not been considered in further detail.

Terrestrial biota

In water treatment facilities, silver is removed by adsorption to sludge and by precipitation of silver sulfide and metallic silver. Terrestrial animals could be exposed to silver via contact with biosolids from water treatment plants. However, current understanding of the fate and transport properties of silver sulfide and metallic silver suggests that silver will not mobilize in these soil applications. Plant uptake experiments by Hirsch (1998a) showed little in the way of significant effects, with only slight uptake by some members of the lettuce family. In addition, there were no effects on survival or growth in studies on the terrestrial earthworm (Beglinger and Ruffing 1997; Hirsch 1998b).

Human health

Within the assessment of potential receptors of environmental silver, it is important to consider humans and to evaluate potential health risks. Risk to humans, as with risk to any biological receptor, is dependent on the inherent hazard associated with silver and the relative exposure to humans. From the standpoint of inherent

toxicity potential to humans, silver is different from other more common environmental metals because it is not associated with adverse health effects or chronic toxicity in humans, and there is no evidence for carcinogenicity, neurotoxicity, or reproductive or developmental toxicity from exposure to silver. In 1991, the U.S. Environmental Protection Agency (USEPA) reevaluated the available toxicity data for silver and similarly concluded that silver is not associated with chronic adverse effects in humans.

Subsequent to this review, and in an effort to reflect this knowledge of silver, the maximum contaminant level (MCL) for silver was deleted and replaced with a higher, nonenforceable secondary maximum level goal for drinking water based on the endpoint of argyria (USEPA 1991). Argyria, a cosmetic pigmentation of the skin, mucous membranes, and eyes, results from chronic overexposure to silver and, importantly, is not considered an adverse health effect. This distinction was critical in the USEPA's decision to delete the MCL. It also is important to note that the USEPA reference dose (RfD) for silver, a daily exposure level deemed to be acceptable for humans, also is based on the endpoint of argyria. Argyria has not been reported, and is not expected to occur, as a result of environmental exposures. Thus, based on the knowledge of the toxicological properties of silver, there is little basis for concern over the hazard that silver presents, a critical factor in the evaluation of health risk (see Appendix 1 to Chapter 4 for additional discussion of exposure or risk assessment for humans).

Wildlife

A screening-level, ecological risk assessment has been completed for wildlife, with the following used as indicator species: red tailed hawk, mallard duck, red fox, and mink (see Appendix 2 to Chapter 4 for a summary). The average and maximum reported levels of silver in selected biota that might serve as food sources for these indicator wildlife species were based on results included in a comprehensive review by Eisler (1996). Intake of silver from water also was considered. Based on this screening-level analysis, the primary route of exposure was a result of silver in food, with water intake accounting for 5% or less of the average daily intake. Even assuming that all dietary silver associated with food and water was bioavailable to the indicator species, the hazard quotient (HQ) for each of the indicator species was low. With average food concentrations assigned, the HQ ranged from about 0.001 for the hawk to about 0.03 for the mallard, or about 0.1% to 3% of the RfD. When the maximum reported silver concentrations in food were assigned as a sensitivity analysis of the results, the HQ ranged from about 0.01 to 0.1.

The results obtained at the average concentration of silver in food are viewed as being most relevant with regard to assessing the potential for chronic effects due to dietary exposure to silver. A value of HQ less than or equal to 0.03, corresponding to an average dietary intake that is less than 3% of the RfD, is considered low enough to indicate that adverse effects are not likely to occur. This conclusion is

justified in light of the conservative assumptions incorporated in this analysis. These assumptions include the use of a no-observed-effect concentration (NOEC) for the RfD for mammals, silver in food is 100% bioavailable, area and dietary use factors = 1, consideration of both average and maximum reported silver concentrations in food sources, and 100% of the mallard duck's diet is aquatic macro invertebrates. However, it also must be recognized that, although the review by Eisler (1996) upon which the exposure levels are based was extensive in scope, it was not clear whether or not the data reflect silver levels in biota at highly impacted sites, such as an acid mine drainage site. To the degree the data do not reflect such conditions, the results do not necessarily represent a worst-case scenario and must be qualified in this respect.

Aquatic life

In the remaining discussion of the implications of recent scientific findings, direct exposure via the water column of aquatic ecosystems will be focused on. The rationale for this is that silver enters aquatic systems from aqueous discharges and/or bound to solids from waste treatment plants and via runoff from agricultural systems or from runoff from land application of silver-containing sludge (Research Triangle Institute 1998). Exposure via the dietary route may be important, but insufficient data are presently available to assess the dietary pathway of exposure (both in the water column and in sediments), and, thus, the risk assessment cannot address this issue. Due to the fact that the benthic sediment is the ultimate repository of metals such as silver in aquatic systems, this pathway will be considered as well.

Assessment endpoint and measures of effect

The generic assessment endpoint for silver in aquatic systems is to ensure survival, growth, and reproduction of sensitive aquatic species. Chronic endpoints considered in this review are fish growth and invertebrate reproduction. These endpoints are ecologically relevant and are sensitive measures of effects. Due to a lack of information, effects on fish from the dietary pathway will not be addressed.

Conceptual model

The conceptual model for this risk analysis is based upon silver discharge to fresh water, where chemical speciation reequilibration occurs, along with adsorption to particles and algae, and sedimentation (see Figure 4-1). Planktonic aquatic invertebrates may be exposed to silver through the water column, or by feeding on algae or sediment particles. Benthic organisms are exposed to silver via sediment ingestion, by contact with the sediment interstitial water, and by interaction with the overlying water column (e.g., circulation and/or filtering of overlying water through sediment burrows). Fish exposure results from the water column or by feeding in chronic exposures. Trophic level effects also are considered.

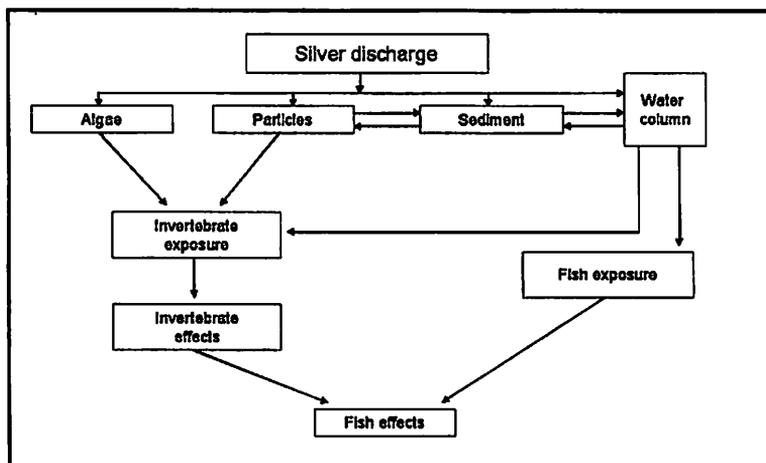


Figure 4-1 Conceptual model for silver risk analysis

Exposure Assessment

Silver speciation in natural waters

Background

As a type B, “soft” metal, silver(I) binds preferentially with donor atoms in the order $S > I > Br > Cl$ approximately $N > O > F$. As a result of its low (charge or radius) ratio, the water exchange kinetics for the silver ion are very fast, and, thus, it will tend to equilibrate rapidly with competing ligands in solution.

Predominant forms of silver in natural waters include silver complexes with simple inorganic ligands (e.g., chloride, thiosulfate, sulfide, and polysulfide), polynuclear inorganic ligands (polysulfides), and organic ligands (simple organic ligands, and natural organic matter); free Ag^+ will be a very minor species (see chapter 1). Given the very low concentrations of dissolved silver usually found in natural waters, the concentrations of these ligands will normally greatly exceed those of silver, implying that silver speciation will be relatively independent of the concentrations of other co-occurring metals.

For oxic waters, if reduced sulfur species are absent, equilibrium calculations indicate that silver speciation will be sensitive to the chloride concentration, e.g., in simple $Ag-Cl$ systems the mono-chloro complex ($AgCl^0$) becomes important (> 50%) at ambient chloride concentrations above approximately 0.5 mM (about 54 mg/L). However, in the presence of reduced sulfur species (e.g., sulfide, organic thiols), $Ag(I)$ will bind preferentially to these ligands, and $AgCl_n$ species will no longer be important in fresh waters. Even in sea water, silver sulfide complexes should predominate over the mono-chloro and di-chloro complexes.

Natural waters

Recent measurements have demonstrated that metastable reduced sulfur species persist in oxic natural waters despite the presence of oxygen (Luther and Tsamakis 1989; Kuwabara and Luther 1993). Reported concentrations of "reactive sulfide," methylene blue measurable sulfide (MBMS), are often greater than 10 nM (Manolopoulos and Kramer 2001); values in sewage effluents tend to be much higher (up to 100 nM) (Shafer et al. 1998; Adams and Kramer 1999a, 1999b). The nature of these reactive sulfide forms is presently unknown, but possible candidate species include dissolved sulfide (stabilized by complexation to Zn or Cu) and polysulfide clusters (possibly coated with, or buried within, natural organic matter [NOM]). Laboratory experiments have shown that reactions of dissolved Ag with such reduced sulfur species occur rapidly. Organic thiols such as cysteine or glutathione would not be determined as reactive sulfide, but if present, they too would bind silver strongly (e.g., $\log K_{Ag\text{-}cys^-} = 11.9$).

Even at the lower end of the reported concentrations for reactive sulfide (10 nM), these S(II) species will normally greatly exceed ambient silver concentrations. Under such conditions, given the very high binding affinity of reduced sulfur species for Ag, the free silver ion concentration would be extremely low. Equilibrium calculations suggest that values for the free Ag^+ ion will normally be less than 10^{-14} M at circumneutral pH (Bell and Kramer 1999), i.e., well below the detection limits of even the most sensitive analytical techniques.

Using the current Silver Gill Ligand Model (Janes and Playle 1995), and fixing the free Ag^+ ion concentration at a maximum value of 10^{-14} M, the amount of silver binding to rainbow trout gills would not be biologically significant (< 0.01% of the gill binding sites occupied by Ag). Even at a free Ag^+ concentration of 10^{-12} M, only about 1% of the gill sites would be occupied by silver. Note, however, that in its current form the Silver Gill Ligand Model does not take into account the possible formation of ternary (gill-S)-Ag-(S-R) complexes, where "gill-S" would be a sulfur-containing gill ligand, and "S-R" represents the generic reduced S(II) species that is thought to dominate Ag(I) speciation in the exposure medium.

Silver interactions with aquatic organisms (biological membranes)

The interaction of a metal with an aquatic organism involves the following 4 steps: (1) advection or diffusion of the metal from the bulk solution to the biological surface, (2) diffusion of the metal through an outer protective polysaccharide or glycoprotein layer, (3) sorption or surface complexation of the metal at passive binding sites within the protective layer or at sites on the outer surface of the plasma membrane, and (4) uptake or "internalization" of the metal (transport across the plasma membrane). Once within the cell, the metal may interact with a great variety of intracellular sites with obvious metabolic consequences.

To elicit a biological response from a target organism and/or to accumulate within this organism, a metal must interact with or traverse a cell membrane. The impor-

tant features of the plasma membrane barrier are its overall hydrophobic, phospholipidic character, the presence of proteins—some of which may traverse the lipid bilayer—and the existence of transport proteins and/or ion channels that facilitate the movement of ions across the membrane (Simkiss and Taylor 1995). Possible transport mechanisms for silver are shown in Figure 4-2 (see also Chapter 3, Figure 3-1).

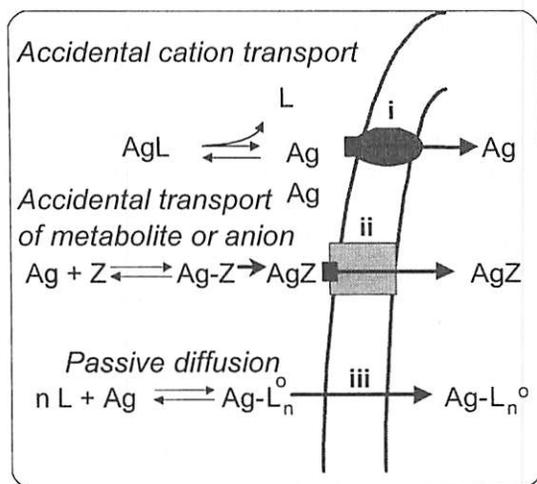


Figure 4-2 Mechanisms by which silver may cross biological membranes. For simplicity, charges are not shown for mechanisms (i) and (ii).

Cationic metals and their hydrophilic complexes cannot enter biological cells by simple diffusion but must instead cross the plasma membrane via ion channels or by mediated diffusion. The normal uptake route will be as a cation, via route *i*, since silver is an unessential metal, it must enter via a transport system normally used for transport of other essential cations (e.g., Na, K, or Cu(I)). Note that since the concentration of free Ag^+ is normally very low in natural waters (see section: *Silver speciation in natural waters*), the actual species reacting at the membrane surface is likely to be a $Ag(I)$ complex, i.e., a ligand-exchange reaction between the complex in solution and the transport site on the membrane.

Other experimental evidence suggests that silver also may enter cells via route *ii* (e.g., as a negatively charged chloro-complex or thiosulfato-complex) or route *iii* (as the neutral $AgCl^0$ complex) (Engel et al. 1981; Hogstrand et al. 1996; Wood et al. 1996; Fortin and Campbell 1998; Reinfelder and Chang 1999). Transport by such routes occurs in parallel to route *i* and thus can lead to greater than anticipated silver accumulation. However, given the conclusions of section *Silver speciation in natural waters* regarding the speciation of silver in natural waters, it appears unlikely that transport of chloro-complexes or thiosulfato-complexes will be biologically significant in natural systems.

Acute toxicity of silver to freshwater fish involves ionoregulatory disruption through inhibition of basolateral Na^+/K^+ -ATPase (adenosine triphosphatase) in the gill (reviewed by Wood et al. 1999; Chapter 2). Proven mitigating factors against this toxic effect of silver are NOM, water Cl, and to a lesser degree cations such as Ca^{2+} , Mg^{2+} , and Na^+ (e.g., Erickson et al. 1998). Particulate matter also probably reduces the effect of silver by binding Ag^+ . NOM and particulate matter themselves are not toxic to fish (Lake and Hinch 1999; Richards et al. 1999). Due to the characteristically lower concentrations that typically occur, NOM and particulate matter are both less important complexing factors in brackish water and seawater compared to fresh water (Turner 1995).

In marine fish, the intestine is the probable site for silver uptake and acute toxicity because marine fish drink seawater and excrete excess ions at their gills instead of taking up ions at the gills. The acute toxicity to silver in marine systems is much lower than in fresh water (Chapter 2). Overall, the physiological effects of silver on marine fish are poorly understood and there are differences in sensitivity to silver among fish species (e.g., elasmobranchs; Wood et al. 1999). Speciation modeling of silver interactions at the gills and gut of marine fish (e.g., the Biotic Ligand Model [BLM] approach) demonstrated conceptually the minimal binding of silver at the gills and relatively greater binding of silver at the gut (Wood et al. 1999).

The general conceptual approach of the original Silver-Gill Accumulation Model of Janes and Playle (1995) has recently been adapted for use in predicting the acute toxicity of silver to aquatic organisms. The BLM of acute toxicity (Paquin, Santore et al. 1999; as described in USEPA 1999; DiToro et al. 2001) relates a critical level of silver accumulation at the biological site of action, the biotic ligand, to the LC50 of the test organism. Although, in the case of fish, the biotic ligand corresponds to the physiologically active sites where silver binds to the gill membrane, this is not necessarily the case for other types of organisms. As more is learned about the physiological basis of these interactions, a more mechanistically-based model should be possible. In the interim, it has been shown that this approach is of practical utility in predicting metal toxicity to fishes and invertebrates, even in the absence of a more fundamental mechanistic understanding of these phenomena (MacRae et al. 1999; Meyer 1999).

Silver exposure pathways

The principal exposure pathways for silver in aquatic systems are via the water column and sediment. Exposure levels to be discussed herein will be evaluated using field measurements. It is noted, however, that it is often necessary in the context of a regulatory setting to use mathematical models, such as fate and transport and bioaccumulation models, to estimate exposure levels in the absence of field data. Such models also are used to predict future exposure levels in the water column, sediment, and biota under alternative risk management scenarios. As an example, models are needed to set effluent discharge permit limits that meet water-quality standards in the context of a total maximum daily loading (TMDL)

analysis. Appendix 3 to Chapter 4 includes a further discussion of the use of models for exposure assessment purposes for metals and discusses areas where further development is needed for these models to be more useful in conducting exposure assessments for metals.

Water column

The conceptual model (Figure 4-1) depicts the primary exposure pathway for silver as direct uptake from water by fishes and invertebrates in both freshwater and marine ecosystems.

Both porewater and surfacewater assessments are based upon the available laboratory acute and chronic water exposure toxicity studies. The problem formulation step identified the water column in aquatic ecosystems as the primary pathway for further assessment in this risk assessment. The rationale for this is that silver enters aquatic systems from some industrial effluents and publicly owned treatment works (POTWs) and/or bound to solids from POTWs or from runoff from land application of silver-containing sludge (Research Triangle Institute 1998). Further, there was a consensus of opinion within the risk assessment review group that insufficient data are available at present to assess the importance of the dietary pathway of exposure (both in the water column and in sediments). There are data that indicate that silver uptake via the diet can occur and that it might be significant, especially as it relates to bioaccumulation (Luoma et al. 1995; Hornberger et al. 2000; Lee et al. 2000). This pathway deserves further investigation, particularly as it relates to assessing the potential for chronic toxicity. The sediment pathway assessment also uses water (pore water) as the assessment medium. These data are summarized in the effects characterization portion of this chapter (section *Risk characterization*).

Aquatic sediment

Aquatic sediments generally are viewed as serving as a long-term repository (sink) for silver in aquatic environments. Metals such as silver accumulate in benthic sediments in association with particulate material that settles from the water column in the relatively quiescent, depositional regions of a water body. This deposited material, over a time scale of several months to several years, can be subsequently transported downward with respect to the sediment-water interface via bioturbation and burial (DiToro 2000). Within the deeper anoxic bedded sediment layers, the reactive silver tends to be incorporated into iron sulfide (FeS, FeS₂). Seasonal variation in the depth of the oxic layer may lead to the subsequent oxidation of metal sulfides within the surficial sediment layer. This process can result in elevated levels of dissolved silver in pore water, and a trace amount of silver may then diffuse back into the overlying water column. However, Ag(I) is particle reactive and diffusion occurs relatively slowly in undisturbed systems, such that silver will tend to accumulate in net depositional areas, where on balance the effect is a loss of silver from the water column and burial in the sediment.

Generally, it is accepted that dissolved silver in sediment pore water represents an important exposure route of silver to aquatic life (Berry 1999). The processes that control transfer of this dissolved silver across biological membranes are likely to be similar to those described above for the water column. The dissolved concentration is to a large extent controlled by the acid-volatile sulfide (AVS) level in the sediment. Silver is somewhat different than other metals that were initially included in the metals mixtures equilibrium sediment guidelines (ESG) because it is monovalent. It is for this reason that twice the molar concentration of silver reacts with an equimolar concentration of AVS. Also, silver sulfide forms a very insoluble sulfide, and it is not solubilized in the standard AVS extraction.

Berry et al. (1999) demonstrated the utility of using interstitial water measurements of silver and AVS concentration to assess biological effects of silver. Figure 4-3 presents results of a reanalysis of previously published data (Rodgers et al. 1997). As shown, mortality in freshwater sediments is not sediment specific when silver concentration is expressed in terms of $([Ag]/2) - AVS$ (4-3A), whereas it is when expressed on a dry weight (dw) basis (Figure 4-3B). For saltwater sediments, it was similarly shown that silver toxicity to *Ampelisca abdita* is sediment specific over a range of sediment types when silver concentration is expressed on a dw basis (Figure 4-4A). However, when the silver exposure level is expressed in terms of $([Ag]/2) - AVS$ (Figure 4-4B) or in terms of interstitial water toxic units (IWTU = LC50 based on pore water concentration or LC50 in a water only exposure; Figure 4-4C), it is not sediment-specific. That is, if simultaneously extracted metal (SEM) silver exceeded AVS, AVS was not measurable, IWTU greater than 0.5, and the sediment was generally toxic. Conversely, if AVS was at measurable levels, the silver was not toxic. Collectively, these results support the use of AVS and silver IWTUs in predicting the acute toxicity of silver in sediments. Any sediment with measurable AVS should have low concentrations of silver in the interstitial water and any silver there would probably be bound to sulfides. Thus, acute toxicity of silver via the pore water route of exposure should not be observed. However, the cautionary notes in Chapter 1 should be recognized.

Ingestion of particulate silver is another possible route of exposure. Recent studies have shown that some metals in sediments, including Cd(II), Ni(II), and Zn(II), may bioaccumulate in sediment-dwelling organisms and bivalves, even when excess AVS is present (Hare et al. 1994; Lee et al. 2000). However, these studies did not directly link the accumulation of these metals to toxic effects. The analogous studies have not yet been conducted for silver. As discussed in Chapter 2, accumulation of ingested silver has been implicated as a cause of effects in some benthic invertebrates (*Macoma balthica* and *Potamocorbula amurens*) in San Francisco Bay, California, USA (Hornberger et al. 1999; Lee et al. 2000). However, the possibility of other stressors (e.g., other metals or pesticides) being the cause of these observed effects cannot be completely ruled out.

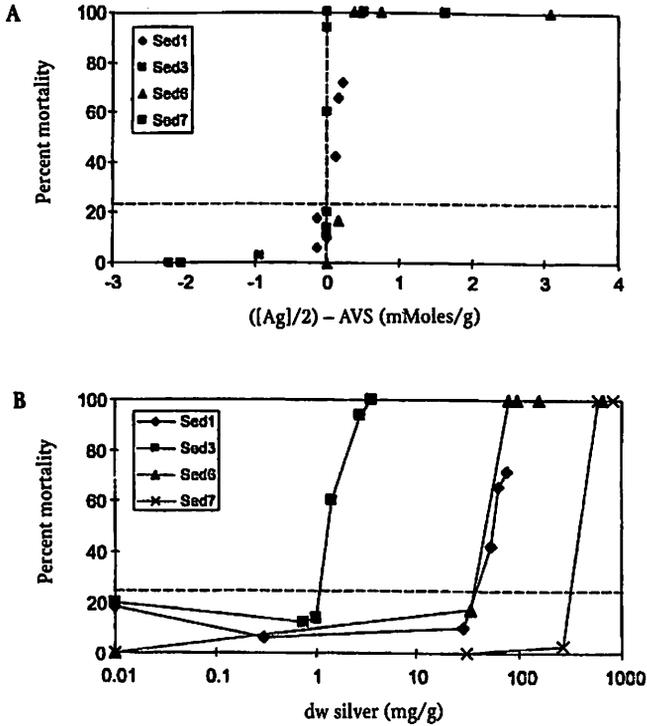


Figure 4-3 Percent mortality of the amphipod *Hyallela azteca* in 4 freshwater sediments spiked with silver (data from Rodgers et al. 1997). Sediments below the dashed line at 24% mortality are not considered toxic. A vertical dashed line at $SEM-AMS = 0$ (A) indicates the predicted break point in toxicity (Berry 1999).

Characterization of ambient Ag levels

As noted previously, this overview will rely on measured levels of silver to characterize the exposure levels of silver in aquatic systems. Measurements of silver in the water column and in bedded sediments will be considered, including the pore water in sediments.

Water column

Caution must be exercised in use of data to characterize the levels of silver in natural waters. This is because it has frequently been recognized that older historical data are often unreliable due to the failure to use clean sampling and analytical techniques (Prothro 1993; Hunt 1995; Hunt and Lewis 1995; HydroQual 1995; Kinnerson 1995). To avoid this difficulty, the data summarized herein are from relatively recent studies where great care was taken to ensure that clean metals sampling and analytical techniques were used.

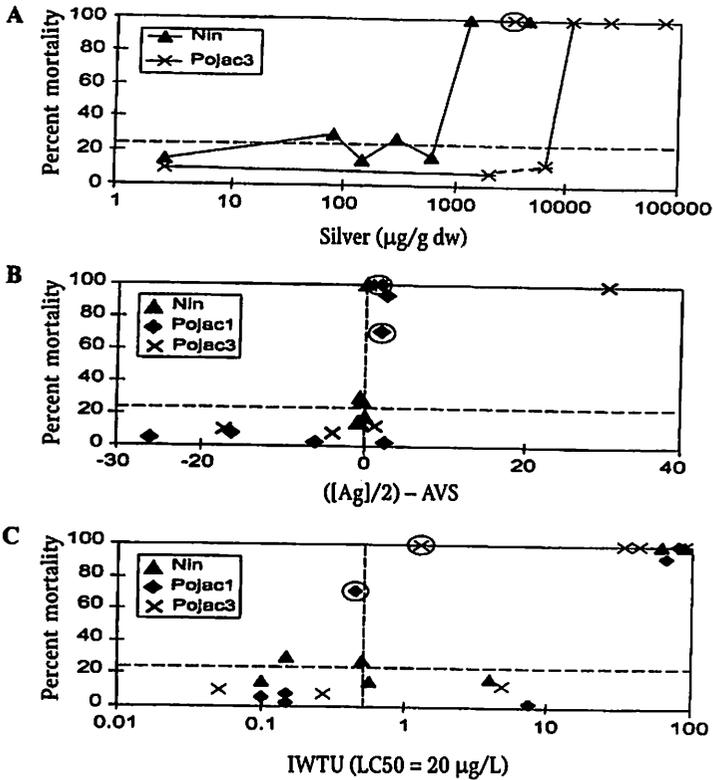


Figure 4-4 Percentage mortality of the amphipod *Ampelisca abdita* as a function of (A) dry weight silver concentration, (B) $([\text{Ag}]/2) - \text{AVS}$, and (C) interstitial water toxic units (IWTU). Sediments below the dashed line are not considered toxic. Vertical lines indicate predicted break points in toxicity. Data points believed to be the result of interstitial water ammonia are circled and not connected by lines in (A) (Berry 1999).

Nin = Ninigret pond sediment
 Pojac = Pojac point sediment

Freshwater results based on different sample filtration techniques are summarized on the left panel of Figure 4-5 (Benoit 1994; Wolfe and Fitzpatrick 1996; Gill et al. 1997; Wen et al. 1997; Shafer et al. 1998). These data were generally collected from sites downstream from POTWs that were known to discharge relatively high concentrations of silver. Median and 95th-percentile total silver concentrations of about 0.06 and 2 $\mu\text{g/L}$ (0.56 and 18 nM) characterize the upper distribution, respectively. The middle probability distribution represents operationally defined dissolved silver that was measured after a 0.45 μm filtration. The median concentration is about 0.015 $\mu\text{g/L}$ (0.14 nM) and the 95th-percentile is about 0.35 $\mu\text{g/L}$ (3.2 nM). This operationally defined measure of dissolved silver is expected to include not only free Ag^+ , but other inorganic and NOM-complexed forms of Ag as

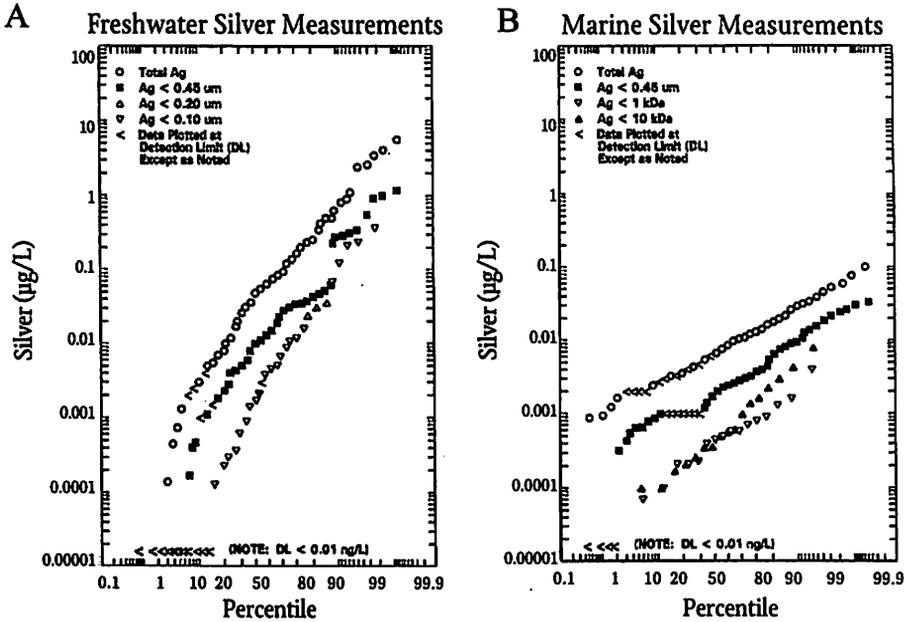


Figure 4-5 Probability distributions of ambient silver concentrations in freshwater settings (left panel: data from Benoit 1994; Wolfe and Fitzpatrick 1996; Gill et al. 1997; Wen et al. 1997; Shafer et al. 1998) and Marine Settings (right panel: data from Sañudo-Wilhelmy and Flegal 1992; Smith and Flegal 1993; Benoit 1994; Benoit et al. 1994; Sañudo-Wilhelmy et al. 1996; Wen et al. 1997)

well. Concentrations in the lower distribution, including measurements made using 0.1 or 0.2 μm size separations, are about an order of magnitude lower than the total silver measurements, with median and 95th-percentile concentrations of about 0.004 and 0.25 $\mu\text{g/L}$ (0.037 and 2.32 nM), respectively. It is expected that ionic silver levels in these samples are less than the concentrations of silver represented by this lowest distribution. This lower probability distribution (pooled data for $< 0.2 \mu\text{m}$ and $< 0.1 \mu\text{m}$) is considered to provide an upper bound estimate of the maximum bioavailable silver in these water samples.

Similar results for silver in marine waters are shown on the right panel of Figure 4-5. As shown, estuarine and coastal waters have silver concentrations that are typically lower than in freshwater systems (Sañudo-Wilhelmy and Flegal 1992; Smith and Flegal 1993; Benoit 1994; Benoit et al. 1994; Sañudo-Wilhelmy et al. 1996; Wen et al. 1997). Median and 95th-percentile concentrations of total silver are approximately 0.008 and 0.04 $\mu\text{g/L}$ (0.079 and 0.37 nM), respectively. The operationally defined 0.45 μm total dissolved concentrations, median and 95th-percentile, are about 0.002 and 0.015 $\mu\text{g/L}$ (0.019 and 0.14 nM), respectively. The probability distribution of data that represent the $< 1 \text{ kDa}$ fraction of silver is about a factor of 5 lower than this.

It should be recognized that the preceding freshwater and marine data are not necessarily representative of silver concentrations at locations where the receiving water is impacted by acid mine drainage or by discharge of an industrial point source effluent. To the degree this is the case, additional data may be warranted on a site-specific basis when such situations are encountered.

Aquatic sediment

Silver concentrations in benthic sediments vary considerably from site to site. Maximum concentrations of total silver measured in sediments of water bodies in the U.S. over the last 25 years have ranged from 0.07 to 20 μg /g dw of sediment (μg /g dw, or 0.65 to 185 nM/g dw) (Eisler 1996). Maximum levels in marine sediments near Pacific coast cities ranged from 1.5 to 3.5 μg /g dw (13.9 to 32.5 nM/g dw; Freeman 1979, as reported by Eisler 1996). Puget Sound sediments (0 to 20 cm) were reported to contain 0.67 μg /g dw (6.21 nM/g dw) and the silver concentration decreased with further increases in sediment depth up to a total depth of 265 cm (as reported by Eisler 1996; Bloom and Crecelius 1987). Pre-1986 Southern California coastal basins were reported to have much higher levels of 14 to 20 μg /g dw (130 to 185 nM/g dw; more recent data not available). These concentrations are consistent with maximum concentrations of greater than 10 μg /g dw (greater than 93 nM/g dw) that were reported for San Francisco Bay sediments (Bryan and Langston 1992; as reported in Eisler 1996).

It is important to recognize that the preceding measurements represent total sediment silver, and as such, they probably represent a significant overestimate of extractable, bioavailable silver in sediments. Even so, if it is assumed that all of this silver was SEM silver, the maximum reported value of 20 μg /g dw is equivalent to 185 nM/g dw. Most sediments typically contain high enough levels of AVS (i.e., greater than 10,000 nM/g dw) to react with this amount of silver to form the insoluble metal sulfides that incorporate silver. Thus, porewater concentrations of free Ag are expected to be very low in most settings for which data have been reported.

For the period 1992 to 1997, Hornberger et al. (1999) reported surficial sediment silver concentrations in San Francisco Bay of 0.38 ± 0.17 μg /g dw (3.52 ± 1.57 nM/g dw), with maximum levels less than 1 μg /g dw (9.3 nM/g dw). These concentrations were measured using a 2-hour extraction with weak acid, a procedure that is similar to the USEPA AVS-SEM method. Pursuant to the previous discussion, measurement of this amount of silver as SEM suggests that AVS levels have been exceeded by the sediment silver (recall that Ag_2S is not considered to be extractable using this method). It then follows that porewater silver levels may be elevated, and the potential for toxicity via this route of exposure (i.e., Ag in pore water) exists. However, measured porewater levels in San Francisco Bay sediments are in fact low (total dissolved silver < 0.05 μg /L [0.46 nM]; Rivera-Duarte and Flegal 1997). This apparent inconsistency may reflect the presence of silver in other metal sulfide phases.

Thus, as noted previously, there is somewhat of an inconsistency with regard to our current understanding of the overall process. However, the important result is that porewater levels of silver are still low, even when extractable silver is present at measurable levels. Thus, based on this limited data and the above discussion, the potential for adverse effects via this route of exposure (i.e., silver in pore water) is considered small.

Assessment Effects

The sensitivity of aquatic life to silver via aqueous exposure has been widely studied in the laboratory, with acute toxicity being studied much more intensively than chronic toxicity. The data are derived from laboratory studies using standard procedures with low DOM and low reactive sulfide concentrations in the test water. These conditions maximize the potential for toxicity to be expressed. These data were used for this risk assessment because the collective wisdom of the scientific community over the past several years has been that the primary route of exposure to aquatic organisms is via the water column and, in the case of fish, via the gill. Data are beginning to accumulate from several lines of evidence that dietary uptake contributes to silver body burden residues, but it currently is not known if dietary uptake in natural systems results in toxicity. The exposure pathway identified for assessment was direct transfer of silver from water to algae, invertebrates, and fishes.

Acute toxicity

Freshwater species

As discussed previously (Chapter 2), the acute toxicity of silver to freshwater organisms has been relatively well studied with species mean acute values (SMAVs) ranging from 0.85 to 1543 $\mu\text{g/L}$ (7.88 to 14,300 nM) across 43 species tested (including species tested since 1987). In synthetic fresh water, acute species sensitivity distributions for silver are similar to those of most metals, with invertebrates on average being more sensitive than vertebrates (i.e., fishes) (Chapter 2, Figure 2-1). Within the invertebrate group, cladocerans and amphipods are more sensitive than aquatic insects, which are more sensitive than other invertebrate groups that have been tested. This relationship in relative sensitivity between groups is generally consistent with that observed for several other metals (e.g., copper, cadmium).

Marine species

The acute toxicity of silver to marine organisms has not been studied as extensively as it has for freshwater organisms, but as reviewed in Chapter 2, a relatively robust data set is still available. SMAVs range from 13.3 to 2700 $\mu\text{g/L}$ (123 to 25,000 nM) across 26 species tested (Chapter 2, Figure 2-4). Similar to the situation for

freshwater species, a specific group of invertebrates (larval bivalves and planktonic crustaceans, such as copepods) is more sensitive than fishes and other invertebrates.

Chronic toxicity

The chronic toxicity of silver (and most other chemicals) has not been extensively studied. A summary of the limited available data is provided below. As discussed elsewhere, the potential absence of metastable sulfides in chronic toxicity tests conducted to date has been identified as a potentially significant factor influencing chronic test results. This leads to the possibility that these studies may be overestimating the bioavailability of silver relative to worst-case, real-world conditions.

Freshwater species

Only 8 species have been evaluated for chronic silver toxicity (Chapter 2, Figure 2-6). Unlike the case for the acute data, fish species appear to be more sensitive than invertebrates. Cladocerans in particular are the least sensitive chronically but the most acutely sensitive species. In fact, the chronic sensitivity of cladocerans is less than the acute sensitivity, a surprising result. This reversal in the relative magnitudes of acute and chronic sensitivities in comparison to other metals is thought to be an artifact of the daphnid test method, as discussed in an earlier section. That is, it has been hypothesized that the reduced sensitivity in the chronic tests is due to the binding of silver to food during the test. Of course, this explanation requires that dietary silver be less bioavailable than dissolved silver, an assumption that is receiving increased scrutiny during recent years. Considering that the species that is most acutely sensitive to silver apparently has not been properly evaluated for chronic toxicity, it is clear that the chronic potential of silver has not been fully characterized.

Marine species

Pursuant to the review Chapter 2, only one marine species, *Mysidopsis bahia*, has been evaluated for chronic sensitivity to silver. Chronic values for *M. bahia* range from 15 to 87 $\mu\text{g/L}$ (139 to 806 nM).

Tissue residues

As discussed in Chapter 2, accumulated silver is not very well correlated with acute toxicity. Accumulated silver in a chronic exposure indicates silver exposure, but should not necessarily be equated with or considered predictive of toxicity. Under such exposure conditions, for example, silver accumulates on the fish gill, from where it is transported to and accumulates in the liver. In living cells, accumulated silver will tend to associate strongly with organic ligands internally, such as the thiol groups of cysteine, glutathione, or metallothionein.

Chronic toxicity and the Biotic Ligand Model

It is not known whether the Silver-Gill Ligand Model and the related silver BLM of acute toxicity, described previously, will be applicable to chronic, low-level silver exposures. It may be possible to use the existing chronic toxicity data to explore the applicability of the BLM approach to chronic toxicity. Alternatively, more chronic studies may need to be conducted to generate data that can be used to develop an analogous model for chronic toxicity. A discussion of possible features of a chronic toxicity version of the BLM is presented in Appendix 3 to Chapter 4 as part of *Use of models*.

Identification of sensitive receptors

As discussed earlier, acute species sensitivity distributions for silver in fresh water are similar to those of most metals, with invertebrates on average being more sensitive than vertebrates (i.e., fishes). Within the invertebrate group, cladocerans and amphipods are more sensitive than aquatic insects, which are more sensitive than other invertebrate groups that have been tested. Similar to the situation for freshwater species, specific groups of invertebrates (larval bivalves and planktonic crustaceans, such as copepods) are more sensitive than fishes and other invertebrates.

Only freshwater species have been evaluated for chronic silver toxicity. Unlike the acute data, fish species appear to be more sensitive than invertebrates. Considering that the species that are most acutely sensitive to silver have apparently not been properly evaluated for chronic toxicity, the potential chronic toxicity of silver has not been adequately described. To reiterate, only one marine species, *M. bahia*, has been evaluated for chronic sensitivity to silver, and chronic values for this organism range from 15 to 87 $\mu\text{g/L}$ (139 to 806 nM).

Dietary effects thresholds

Accumulation of silver through the diet occurs, but its significance varies between species. For example, in freshwater fish, uptake of dissolved silver and acute effects resulting from accumulation at the gills dominate, whereas in invertebrates, the dietary component is very important (see review in Chapter 2). Uptake of silver from the diet depends on assimilation efficiencies, and represents uptake of complexed (e.g., particulate and colloidal silver) as well as dissolved silver contained in the water. The importance of dietary uptake of silver is not well known, but subtle effects on growth and reproduction may occur.

Risk Characterization

The final phase of the risk assessment process is risk characterization. This phase of the risk assessment process makes use of data from the planning, problem

formulation, and analysis portions of the risk assessment. The risk characterization allows for conclusions about the relationship and co-occurrence of the stressor and of the ecological entity of interest. The process of risk characterization includes estimate of risk to the valued resource or entity; discussion of the significance of the estimated risk and lines of evidence supporting their likelihood; and uncertainties, assumptions, and qualifiers that are important in the overall assessment.

The conclusions of the risk assessment should be clear enough to provide a risk manager the information needed to make an informed decision about how to proceed. In some situations, it is not possible to provide a definitive statement of risk to a given entity. When this is the case, either additional data can be collected or, alternatively, the risk assessor can provide, on the basis of professional judgment supported by lines of evidence for the conclusion, guidance about the potential risk. Ideally, enough data are available to make a definitive estimate of the probability of an effect having occurred or its potential to occur.

Risk estimation

Risk estimation is the process of integrating exposure and effects data and evaluating any associated uncertainties (USEPA 1998). Further, the process uses exposure and effects profiles developed in accordance with the conceptual model and analysis plan to develop risk estimates for the valued resource.

Silver exposure characterization

The exposure data for silver in freshwater and marine settings were discussed previously in section: *Characterization of ambient Ag levels* (Figure 4-5). The exposure profiles for freshwater and marine environments describe the ranges of concentrations that have been measured for silver. These profiles are based on limited data and are not intended to be all-inclusive. In one respect, these data are considered biased on the high side because most of the measurements were made downstream of wastewater treatment plant discharges. Conversely, there may be instances where an industrial point source of silver is not first treated in a POTW before discharge, so higher cases also may exist. However, the exposure profiles are based upon actual measured data. Their use in this risk characterization is based upon the collective judgment of the workshop participants that the values are generally representative of surface water concentrations nationwide.

Based on the data of Figure 4-5, the range of silver concentrations in freshwater systems varies from less than 0.001 to 1.5 $\mu\text{g/L}$ (0.0093 to 13.9 nM; dissolved silver, less than 0.45 μm) and as high as 6 $\mu\text{g/L}$ (55.6 nM; total silver) (Figure 4-5A). Similarly, measured concentrations of silver in marine waters vary from about 0.0003 to 0.04 $\mu\text{g/L}$ (less than 0.0028 to 0.37 nM; dissolved silver) and from less than 0.001 to 0.1 $\mu\text{g/L}$ (0.0093 to 0.93 nM; total silver) (Figure 4-5B).

Silver effects characterization

The acute and chronic effects data developed in laboratory toxicity studies are summarized in detail in the *Effects assessment* section of this chapter. For purposes of this screening-level, silver risk assessment, the freshwater and marine data were presented previously as effects profiles on Figures 2-1 and 2-4 (Chapter 2), respectively. These effects profiles represent a compilation of the acute and chronic toxicity information that is available and that was screened for water-quality parameters. The data are derived from laboratory studies using standard procedures, including use of water that probably had low concentrations of dissolved organic carbon and reactive sulfide. These conditions tend to maximize the bioavailability of silver and the potential for toxicity to be expressed and are thought to represent the most conservative case.

Acute toxicity

The acute toxicity of silver to freshwater organisms has been relatively well studied with SMAVs ranging from 0.85 to 1543 $\mu\text{g/L}$ (7.88 to 14,300 nM) across 43 species tested, including species tested since 1987 (Chapter 2, Figure 2-1). The acute toxicity of silver to marine organisms has a relatively robust data set with SMAVs that range from 13.3 to 2700 $\mu\text{g/L}$ (123 to 25,000 nM) across 20 species tested (Chapter 2, Figure 2-4).

Chronic toxicity

The chronic toxicity of silver has not been extensively studied. Only 8 freshwater and 1 marine species have been evaluated for chronic silver toxicity. The chronic data set is recognized as deficient (see earlier discussion). A summary of the freshwater chronic toxicity data is presented on Figure 2-6 (Chapter 2) where the data are ranked according to their relative species sensitivity. Chronic toxicity values are within the range of 0.1 to 10 $\mu\text{g/L}$ (0.93 to 9.3 nM).

Risk characterization (water)

Risk characterization for silver in water is presented as a comparison of ambient surface water concentrations (i.e., the expected environmental concentrations [EECs]) with the available laboratory toxicity information in Figures 4-6 and 4-7, for fresh water and marine water, respectively. The continuous exposure profiles ("maximum bioavailable Ag") are based on the silver concentration data presented previously on Figure 4-5 (less than 0.2 μm data). The integration of the exposure and toxicity profiles is presented using probability distributions such that the percentage of species that potentially could be affected by a given concentration of silver in water is determined.

The integrated joint probabilities of the exposure and effects profiles indicate that 1 and 8% of freshwater aquatic species would be at risk on an acute and chronic basis, respectively (Figure 4-6). This analysis integrates both the probability of

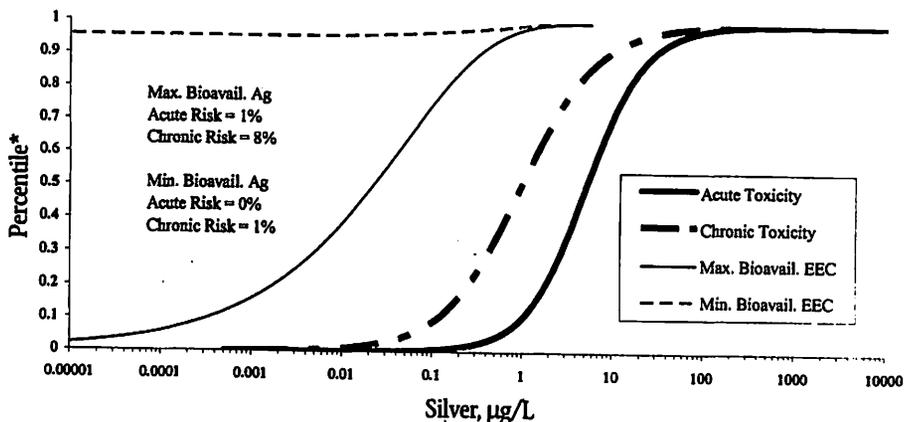


Figure 4-6 Potential acute and chronic risks to freshwater organisms based on maximum and minimum bioavailable silver

Max. Bioavail. = Maximum Bioavailable

Min. Bioavail. = Minimum Bioavailable

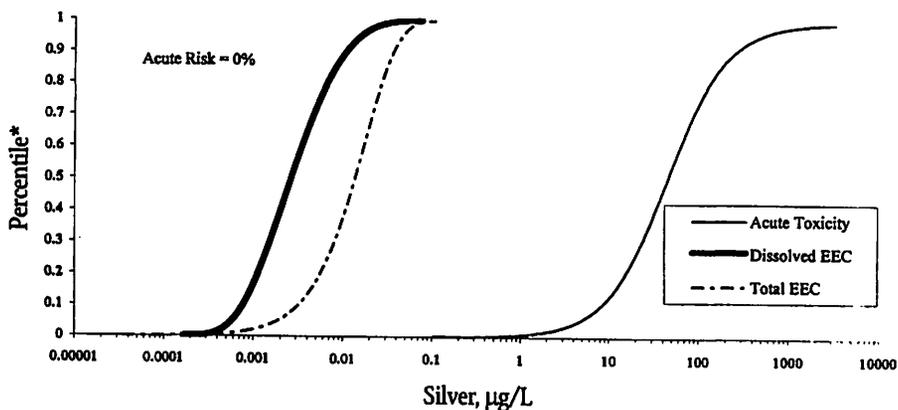


Figure 4-7 Potential acute silver risks to marine organisms (EEC), note discussion in text on limitations of data

exposure and the probability of there being an effect. The data indicate that as ambient exposure levels of dissolved silver increase to greater than 0.1 $\mu\text{g/L}$ (0.93 nM) of dissolved silver, there is an increased potential for adverse aquatic effects. Biotic ligand modeling results would be expected to indicate that the limited potential for acute effects would be essentially eliminated with even a low level of DOM in the water. It is possible that chronic effects would be similarly mitigated, but further work is needed in this area. Also, with regard to chronic exposure levels, consideration should be given to the fact that the data upon which these

*The percentile is the percentage of EECs less than or equal to a given concentration and the percentage of species expected to be affected at a given concentration.

probability distributions are based were grab sample results. Hence, the frequency of exposure to longer-term average concentrations is probably overestimated by these distributions at the higher exposure levels that are shown.

The analogous results for marine waters (Figure 4-7) indicate that the available total and dissolved (both less than 0.45 μm and less than 1 kDa) silver measurements (based on marine data of Figure 4-5) are well below the threshold level for acute effects. Hence, significant adverse acute effects due to direct exposure of aquatic life to silver in marine waters are not expected.

The above risk characterization is recognized as having several limitations that could impact the outcome of the risk analysis. These include limited water exposure data set, limited chronic toxicity information (with regard to taxa and form of silver used in testing), no assessment of dietary exposure, and no corresponding measurements of metastable reduced sulfur species in either the exposure or effects data sets (the importance of these measurements is discussed in Chapter 2). While these limitations may ultimately be shown to be important or unimportant, the above risk characterization reflects the current state of knowledge at the time of the assessment.

To further evaluate the possible significance of sulfides in surface water as potential complexation ligands, the risk to aquatic species was reevaluated with the assumptions that a minimum concentration of 10 nM reduced sulfur species co-occurred with measured silver levels in all surface water data used in this risk analysis and that the Ag-S(II) species are not bioavailable. The results of the integration of exposure and effects data following this scenario show that metastable reduced sulfur species have the potential to reduce significantly the bioavailable silver concentration (see Figure 4-6) to aquatic organisms, with a corresponding reduction in risk. The magnitude of the reduction in exposure is large and the corresponding risk is reduced to nonmeasurable (0%) on an acute basis. For chronic toxicity, the corresponding risk is estimated to potentially affect less than 1% of species, compared with 8% when sulfide is neglected. However, it should be noted that recent test results indicate that the dietary route of exposure may be important with regard to chronic effects for some organisms.

This reanalysis of the risk, using the assumed presence or complexation of reduced sulfur, shows the potential magnitude of change in the risk estimate for silver. This reanalysis of the data was done for illustrative purposes to demonstrate the potential for uncertainty in the current risk assessment. The difference in estimated risk is large enough to indicate that a better understanding of the role that reduced sulfur plays in controlling silver bioavailability at concentrations above 0.1 $\mu\text{g/L}$ (0.93 nM) of dissolved silver needs further evaluation.

Risk characterization (sediment)

An actual risk assessment of the potential for silver to cause effects on benthic organisms was not completed. However, it was previously concluded (see section: *Aquatic sediment*), based on the limited available data, that porewater concentrations of silver are low enough that direct toxicity to benthic invertebrates is unlikely. It is anticipated that in most cases the available AVS in sediment is sufficient to maintain porewater silver concentrations below acute and chronic thresholds ($0.1 \mu\text{g/L} = 0.93 \text{ nM}$). Potential accumulation of silver via the diet is recognized, but, to date, data are lacking to make an assessment of this exposure pathway. The weight of evidence to date for metals other than silver (cadmium, copper, nickel, lead, and zinc) is that in situations where the AVS exceeds the extractable metal, acute toxicity is not expressed, but measurable levels of accumulation in tissue do occur. Overall, the reactivity of silver with available ligands in sediments and pore waters is very high, the reaction times for complexation are fairly rapid, and the bioavailability of silver is therefore predicted to be very low.

Uncertainty analysis

The primary sources of uncertainty associated with the screening-level risk assessment performed for silver are as follows:

- potential for sulfide complexation to occur in natural waters (and the lack thereof in most laboratory test waters), and our ignorance regarding the bioavailability of Ag-S(II) species;
- lack of chronic toxicity data performed in water adequately characterized for sulfide content; and
- insufficient data to evaluate the dietary pathway as a source of silver in chronic toxicity tests.

Other factors contribute to the uncertainty, such as the completeness of the exposure characterization, but appear to be secondary in their importance as a source of uncertainty.

Sulfide complexation

Uncertainty is associated with the nature, concentration, and extent of binding of complexing ligands including metastable sulfides and DOM. The binding constants appear to be high, and effects of this binding on bioavailable silver in natural systems could be important (see Chapter 1). Further, the binding constants for key biotic ligands (such as the gill) also are unknown. They also are thought to be high, perhaps as high as the binding constant of silver for free sulfide. The significance of this is that if the binding affinity of silver for biotic ligands is as strong as that for metastable sulfides, the biotic ligand would be an effective competitor with sulfide. The degree to which the ligand can bind silver in the presence of sulfides (in both water and sediments) has the potential to ultimately define the bioavailability, and

hence the potential for toxicity, of silver to aquatic organisms. This relationship needs further evaluation, especially as it applies to chronic toxicity tests.

Chronic data suitability

The suitability of chronic data is another issue that increases the uncertainty of the risk characterization. Current studies appear to have been conducted in test waters with very low sulfide concentrations. The effects of sulfide on bioavailability and toxicity of silver to aquatic biota will need to be determined in future chronic studies. In this assessment, the current chronic toxicity database was used to assess effects to aquatic biota. This is a major topic of uncertainty in this assessment and it will need to be revised when additional data become available.

Significance of the dietary pathway

The significance of the dietary pathway as a source of silver toxicity to aquatic biota is an area that requires more study. The dietary pathway has different significance for invertebrates versus fishes and will likely have species-specific differences; at the present time it is thought to be most important for filter-feeding invertebrates. The dietary pathway, not considered in the present assessment, is considered to be a significant source of uncertainty in this assessment.

Mitigating issues

Although this risk assessment is considering the effects of a single stressor, silver, on aquatic systems, there are mitigating issues that need to be mentioned. First, there will almost always be other chemical contaminants present with silver. These could include other metals that are more or less toxic than silver and that are present at higher or lower concentrations than silver. Second, additive effects of metals on endpoints may need to be considered.

Although silver is likely to out-compete most other metals for sulfide, it will still necessarily compete with the other metals for binding with sulfide. Likewise, ligands other than sulfide that form complexes with silver and other metals will be present. Other chemical or physical stressors also may be present. The effects of silver on aquatic organisms have been considered at the single-species level. Population models may be needed to determine population-level responses of sensitive species identified as assessment endpoints. Often, a population will be more resistant and resilient to effects from a toxicant than would be indicated by a single species assessment.

Procedures to conduct water-effect ratios (WERs) have been developed to evaluate the significance of some of the above mentioned site-specific water chemistry effects on toxicity. More recently, the BLM has been developed to provide a computational alternative to the acute bioassay-based WER test procedure. BLM results would indicate that silver bioavailability and toxicity will be reduced to some degree in most natural water settings.

Conclusions and Recommendations

It was not the purpose of this section to provide a definitive risk assessment for silver in the environment. Rather, the standard risk assessment paradigm was adopted as a convenient way to summarize the information at hand and to identify those areas where additional information is needed. With this in mind, the results of this section lead to the formation of a number of conclusions and recommendations.

Terrestrial biota, wildlife, and human health

These areas were not covered in detail in this report. However, based on the review of available information, it is concluded that current environmental levels of silver pose a very low risk to terrestrial biota, wildlife, and humans.

Acute toxicity to aquatic life

There is little concern for acute toxicity of silver in ambient waters downstream of POTWs, whether in the presence or absence of reduced-sulfur species.

This conclusion is based primarily on the observation that ambient exposure levels of silver in both freshwater and marine settings (Figure 4-5, A and B, respectively) are usually well below reported acute toxicity levels (Chapter 2, Figures 2-1 and 2-4, respectively; exposure levels are compared to toxicity levels on Figures 4-6 and 4-7). This conclusion will be given further support if confirmatory toxicity tests conducted in the presence of reduced sulfur species indicate that silver sulfide complexes are not bioreactive. There may prove to be some exceptions to this general conclusion, such as at sites located downstream of an industrial discharge of silver that does not pass through a POTW, especially if chloride, sulfide, and DOM concentrations are low. In such cases, WER studies and/or the BLM may provide a basis for developing site-specific WQC that will be protective of aquatic life. Before use of the BLM in this manner, it is recommended that field validation of the BLM for acute toxicity of silver be performed.

Chronic toxicity to aquatic life

The potential for chronic toxicity due to direct water column exposure to ionic silver is low.

This conclusion is preliminary and is based on a comparison of observed exposure levels of silver (Figure 4-5) to effect levels measured in lab studies using clean water (Figures 4-6 and 4-7; also Chapter 2, Figure 2-6). Based on the data reported to date, reduced-sulfur species are believed to be ubiquitous in aquatic settings and are present at levels that will exceed ambient silver concentrations. If it can be demonstrated that silver-sulfide complexes are not bioreactive, then there should be a very low potential for toxicity in the presence of sulfides.

To provide confirmatory support for this conclusion, it is recommended that

- the environmental prevalence of reduced-sulfur species be characterized, including the range in concentrations across geographic areas and seasons, and their chemical reactivity towards Ag be determined; and
- the bioavailability and bioreactivity of Ag-S(II) forms need to be determined in well-designed, acute and chronic laboratory tests.

The preceding conclusions are based on direct exposure to silver via both surface water and sediment pore water. However, a remaining issue is whether the dietary route of exposure to silver is significant. This needs to be resolved. It is therefore recommended that studies should be completed to evaluate dietary pathways of silver uptake, both from food and from sediment.

APPENDIX 1 TO CHAPTER 4

HUMAN HEALTH

Exposure is a critical component in assessing health risk, and in contrast to some other environmental metals, silver is among the least encountered in terms of environmental presence (USEPA 1996a). Among metals detected at nonhazardous waste management unit sites, silver is relatively low, both in frequency of occurrence and concentration in soil. Moreover, silver is typically found within environmental regulatory limits, in contrast to other toxic metals that are frequently detected above federal or state standards (USEPA 1996a). USEPA exposure assessment (USEPA 1981), as well as recent environmental analyses (Shafer et al. 1998), confirm that silver is present at very low ambient concentrations. It is estimated that most of the total human exposure to environmental silver is through dietary sources, with ingestion of water contributing a lesser amount and inhalation of air contributing a negligibly small amount to the overall estimated daily intake (Table 4-1).

Table 4-1 Estimation of silver exposure from environmental sources

Route of exposure	Estimated intake ($\mu\text{g}/\text{day}$)	Reference
Ingestion—diet	35 - 88	USEPA 1980
Ingestion—drinking water	0.2 - 20 ^A	USEPA 1985
Inhalation (urban areas)	0.05 - 0.19 ^B	USEPA 1981

A Assumes ingestion of 2 liters water/day

B Assumes inhalation rate of 1.8m³/hour and 24-hour exposure

It is necessary to compare these estimates of daily and lifetime exposure to silver to the lowest dose known to result in an adverse effect to humans, the reference dose (RfD), to assess the human health risk to environmental silver. The endpoint upon which the RfD for silver is based is argyria, a slate-grey pigmentation of the skin or hair that is not itself associated with any other adverse physiological effects except in the most extreme circumstances (Smith and Carson 1977; Eisler 1996). These extreme cases are usually a result of occupational medical exposures.

A screening-level quantitative assessment of potential high-end estimates of exposure to silver from food, water, and air sources is summarized below. The analysis is presented in terms of the hazard index (HI), where HI is defined as:

$$\text{HI} = \text{Exposure}/\text{RfD}$$

Evaluating the RfD for argyria:

$$\text{RfD} = 0.005 \text{ mg/kg/day} \times 70 \text{ kg} = 0.35 \text{ mg/d} = 350 \text{ } \mu\text{g/day.}$$

The HI is then evaluated using the high-end estimates of average daily intake (ADI) and lifetime intake (LI) of silver to characterize the exposure to silver:

$$\text{ADI} = 88 \mu\text{g (diet)} + 20 \mu\text{g (water)} = 108 \mu\text{g and}$$

$$\text{HI}_{\text{daily}} = 108/350 \mu\text{g (RfD)} \quad 0.31 < 1$$

The high-end lifetime estimate of silver intake, assuming 100% absorption is given by:

$$\text{LI (100\%)} = (110 \mu\text{g/day})(1 \text{ g}/10^6 \mu\text{g})(365 \text{ days/year})(70 \text{ year life span}) = 2.8 \text{ g}$$

Adjusting for an assumed 10% absorption:

$$\text{LI(10\%)} = 2.8 \text{ g} \times 10\% \text{ absorption} = 0.28 \text{ g and}$$

$$\text{HI}_{\text{lifetime}} = 0.28 \text{ g}/1 \text{ g} = 0.28 < 1$$

Here, a benchmark argyria lifetime dose of 1 gram of silver is used to evaluate HI.

These results indicate that the HI for silver is less than one. Hence, as was concluded by Eisler (1996), environmental silver exposure is not expected to pose a significant risk to human health. This conclusion is supported by the fact that the analysis represents a high-end exposure scenario, being based on USEPA data that correspond to high-end estimates of exposure to silver.

In summary, silver and silver compounds are not associated with adverse effects or toxicity in humans and given the lack of inherent hazard, coupled with the very low ambient exposures to which humans are exposed, silver in the environment does not pose a risk to human health. As such, humans are excluded as important receptors in the risk assessment associated with silver in the environment.

APPENDIX 2 TO CHAPTER 4

WILDLIFE

A screening-level ecological risk assessment (ERA) was completed to assess the potential for adverse effects to wildlife as a result of environmental exposure to silver. The wildlife risk assessment is based on use of the following indicator species and food sources (HydroQual 1997):

<u>Indicator species</u>	<u>Food source</u>
Red-tailed hawk	Small mammals
Mallard duck	Aquatic macroinvertebrates and plants
Red fox	Small mammals
Mink	Small mammals and fish

Food and water intake rates by each of these indicator species were assigned in accordance with representative rates reported in the literature (Table 4-2) and are generally in conformance with rates contained in the *Wildlife Exposure Factors Handbook* (USEPA 1993a, 1993b). Dietary use factors were conservatively assumed to be unity for purposes of estimating the average daily intake of silver (Table 4-3). That is, 100% of the food and water that was consumed was from sources having elevated levels of silver. It also was conservatively assumed that 100% of the mallard's diet at an impacted site was aquatic macroinvertebrates, which typically contains higher levels of silver than would their other likely source of food, aquatic vegetation. (Hirsch [1998], in tests with agricultural plants, has shown that silver uptake by plants does not occur to a significant degree, with only slight uptake by some members of the lettuce family.)

Table 4-2 Summary of food and water ingestion rates for indicator species

Indicator species	Weight ^A (kg)	Food consumption		Water consumption	
		Specific (%/d)	Absolute (g/d)	Specific (mL/kg/d)	Absolute (mL/d)
Red-tailed hawk	1.6	25.0	400 ^B	51	81 ^C
Mallard	1.25 ^D	20.0	250 ^D	55	69 ^C
Red fox	5.0	6.4	320 ^E	100	500 ^F
Mink	1.0	15.0	150 ^C	95	95 ^F

A Charters and Kracko (1992) except as noted

B Joyce (1990) after Charters and Kracko (1992)

C Calder and Braun (1983) after Suter (1993), but with W in Kg

D Newell et al. 1987

E Samuel and Nelson (1982) after Charters and Kracko (1992)

F Based on USEPA (1987) after Suter (1993) and Calder and Braun (1983)

G Bleavins et al. (1980) after Charters and Kracko (1992) and Bleavins and Aulerich (1981) after USEPA (1993a and 1993b).

Table 4-3 Use factors for computing dose via ingestion of silver in food and water

Indicator species	Overall intake (I & I _w)		Home or feeding range (sq. miles)	Use factors (percent)		Food source
				Area	Diet	
Red-tailed hawk	Food	(g/d) 400	0.5 ^A 0.23 - 9.5 ^D	100	100	SM
	Water	(mL/d) 81				
Mallard	Food	(g/d) 250	1.1 ^B 0.14 - 5.6 ^D	100	100	AM
	Water	(mL/d) 69				
Red fox	Food	(g/d) 320	0.42 ^A 0.22 - 13.2 ^D	100	100	SM
	Water	(mL/d) 500				
Mink	Food	(g/d) 150	0.031 ^C 0.03 - 1.5 ^D	100	100	SM/Fish
	Water	(mL/d) 95				

SM = Small mammals

AM = Aquatic macroinvertebrates

A Charters and Kracko (1992)

B Belrose 1980

C Lindscombe et al. 1982 after Charters and Kracko (1992)

D USEPA (1993a)

Eisler (1996) has summarized the levels of silver in biological tissues in a comprehensive review. Based on the results of this review, the average and maximum reported levels of silver in selected biota that might serve as sources of food for the indicator wildlife species are as shown in Table 4-4.

These tissue concentrations were assigned as the concentrations of silver in food consumed by each of the indicator species. Although alternative assumptions were invoked with regard to dietary silver levels equaling either the average or maximum concentrations summarized above, the average value is considered most relevant with regard to assessing long-term, chronic effects. The maximum values

Table 4-4 Summary of silver concentrations in biological tissues (Eisler 1996) of prey

Prey	Basis	Silver in food sources (mg/kg)	
		Average of reported concentrations in food	Maximum of reported concentrations in food
Sandworm	dw	5.2	30.0
Macroinvertebrates	dw	1.2	5.5
Mollusks	dw	0.14	82.0
Fish	fw	0.25	1.9
Terrestrial mammals*	dw	0.25	(x10=) 2.5

dw = dry weight

fw = fresh weight

* Silver levels in small mammals were usually reported to be at or below the detection limit

are useful with regard to assessing the sensitivity of the analysis to extreme conditions. Silver intake from water also was considered. Based on this screening-level analysis, the primary route of exposure was a result of silver in food, with water intake accounting for 5% or less of the average daily intake. It was assumed that 100% of the dietary silver associated with food and water is bioavailable to the indicator species.

The hazard quotient (HQ) approach was used for purposes of this screening-level analysis. The HQ for each of the indicator species, was evaluated as follows:

$$\text{HQ} = \text{ADI} / \text{RfD}$$

A HQ greater than unity is, at least in principle, indicative of potential adverse effects. In practice, however, given the degree of uncertainty in this type of screening-level analysis, an HQ value in the range of 1 to 10 is considered borderline with regard to making a definitive assessment about the presence or absence of effects.

Limited data are available on the levels of dietary silver that result in effects to wildlife. Eisler (1996), in a comprehensive review of silver hazards to fish, wildlife and invertebrates, summarized much of the available information on dietary effect levels of silver on mammals and birds. Most of the results for longer-term, chronic and sub-chronic feeding studies were based on tests with laboratory rats and mice, poultry and livestock. Eisler reported effects at exposure concentrations in food and water that are well above normal environmental levels. Silver was found harmful to poultry at 200 mg total Ag/kg in their diet (about 10 to 20 mg Ag/kg body weight/day) and at lower levels in copper deficient diets (addition of copper to the diet mitigated the effects). Additionally, in an 86-day subchronic study with yearling ewes, no adverse effects were noted even at the highest dose tested of 10 mg/kg body weight/day (Younger and Crookshank 1978). Based on these results, a RfD of 10 mg Ag/kg body weight/day, is used for the HQ analysis for both birds and mammals.

Figure 4-8 presents a summary of the results of this screening-level analysis. The upper panel shows the RfD as a horizontal line at 10 mg/kg/day. The bars show the ADI of silver via food and water for each of the indicator species. The open and filled bars correspond to the dietary intake of silver with dietary silver levels at the average and maximum tissue concentrations summarized above, respectively. As shown, the ADI is well below the RfD in each case. The corresponding HQs are presented on the lower panel. With average food concentrations assigned (the open bars), the HQ ranges from about 0.001 for the hawk to about 0.03 for the mallard. That is, the estimated ADI is about 0.1% to 3% of the RfD. When the maximum reported silver concentrations in food are used to sensitize the results (solid bars), the HQ ranges from about 0.01 to 0.1, corresponding to an ADI of 1% to 10% of the RfD.

The results based on average dietary concentrations of silver are considered most relevant with regard to assessing the potential for chronic effects due to dietary

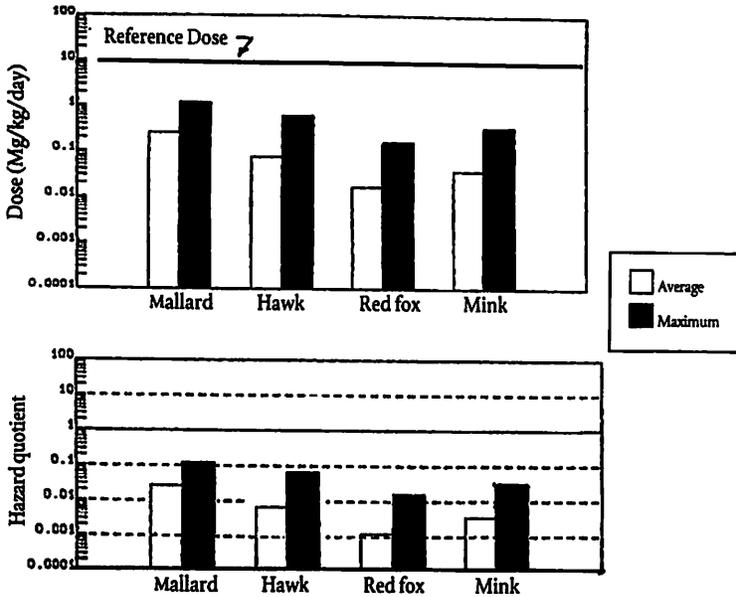


Figure 4-8 Results of screening-level analyses

exposure to silver. Considering the conservative assumptions incorporated in this analysis, a value of $HQ \leq 0.03$, corresponding to an average dietary intake of less than 3% of the RfD for silver, is low enough to indicate that significant adverse effects to wildlife are not likely to occur. The conservative assumptions include use of a NOEC for the RfD for mammals, silver in food is 100% bioavailable, area and dietary use factors equal to 1, and consideration of both average and maximum reported silver concentrations in food, and 100% of the mallard duck's diet is aquatic macroinvertebrates. However, it also should be recognized that although the review by Eisler (1996) upon which the dietary exposure levels are based was extensive in scope, it was not clear whether or not the residue data reflect silver levels in biota at highly impacted locations, such as an acid mine drainage site. To the degree the data do not reflect such conditions, the results do not necessarily represent a worst-case scenario and must be qualified in this respect. The absence of effects data for wildlife also is noted as a limitation of this analysis.

APPENDIX 3 TO CHAPTER 4

USE OF MODELS

Ecological risk assessments frequently rely on a variety of models for conducting the exposure and effects components of the analysis. This section briefly describes how fate and transport, bioaccumulation, and toxicity models may be used, and highlights some of the areas where further model development is needed, particularly with regard to their application to metals.

Fate and Transport Models

As discussed in the *Problem formation* section of this chapter, fate and transport models also are typically used to predict the concentrations of total and dissolved silver within aquatic settings over both time and space, in both the water column and sediment. Such models are useful for purposes of filling in the gaps in data that may be collected only sporadically over time and space. They also can be used in conjunction with chemical equilibrium models to evaluate the speciation of silver.

The analyses described herein have not made use of fate and transport models. This is in part because the analyses are generic in the sense that they do not focus on any single setting, such as a particular lake, stream, or other water body. Rather, the focus of the discussions has been to review data as a way of providing an overview of the current exposure levels of silver in the environment and to place in perspective the ecological significance of these levels. Although this approach serves a useful purpose, it is not generally sufficient in the context of a regulatory control setting.

Consider a water body with one or more industrial dischargers of silver and ambient silver levels that are elevated, in part in response to historical loading conditions that resulted in elevated levels of silver in sediments. Discharge permit limits must be evaluated such that applicable state and national water-quality standards for silver are not exceeded. Simple dilution calculations could be performed to set such limits, based on critical low flow conditions, but the resulting discharge limits may be either overly conservative or not protective enough, depending on conditions at the particular site. For example, USEPA WQC provide for a frequency of exceedance of once every 3 years. Development of permit limits based on a critical low flow condition such as a 7-day average low flow condition that occurs only once every 10 years on average (7Q10), may be overly protective. Alternatively, it also is possible that release of silver from sediments to the water column is the main source of silver in the water body, and failure to recognize this could result in a failure to meet WQS even after costly point-source controls are implemented.

As discussed in the Problem formation section of this chapter, fate and transport models provide a useful tool for conducting such evaluations in a more refined manner. Numerous models are available for use in this regard, and these models vary widely with regard to their level of complexity and ease of use. Model selection depends on a number of factors including, but not limited to, the characteristics of the specific problem setting, the level of detail required over time and space, and the level of experience of the analyst. Several reviews are available that provide detailed descriptions of many of the available fate and transport models, bioaccumulation models and toxicity models for organic chemicals and metals (Schnoor et al. 1987; USEPA 1997; Paquin, DiToro et al. 1999).

Models to Predict Accumulation and Toxicity

Models such as the Silver-Gill Ligand Model of Janes and Playle (1995) and the Biotic Ligand Model (BLM) of acute toxicity of silver (Paquin, DiToro et al. 1999; USEPA 1999) rely on geochemical equilibrium computations to evaluate the effect of site-specific water chemistry on silver bioavailability and toxicity. These models have been discussed briefly in earlier sections of this report and detailed descriptions are presented in the previously cited references. They have been used to illustrate some fundamental concepts with regard to factors that affect the bioavailability and toxicity of silver.

The analogous computational approach to modeling accumulation and acute toxicity has not yet been extended to chronic toxicity. How might a BLM of chronic toxicity be structured? One possible conceptual framework is illustrated on Figure 4-9 and described in the following text.

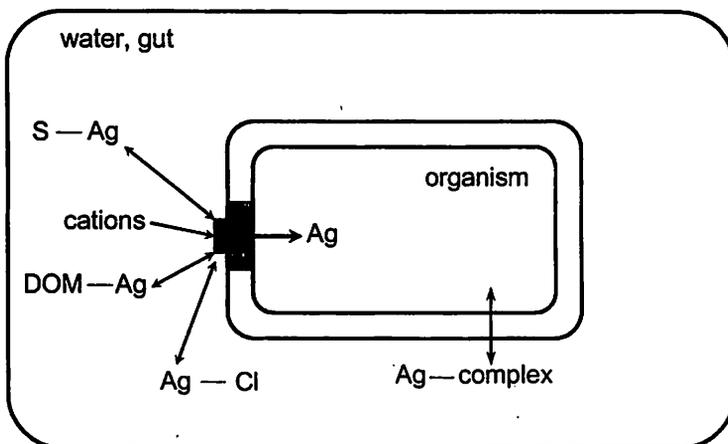


Figure 4-9 Conceptual framework for chronic toxicity model

A chronic silver BLM will have a water-organism component and a gut-organism component. The relative importance of each component is expected to vary by organism type. At low silver concentrations of less than 1 $\mu\text{g/L}$, virtually all silver will probably exist in complexed forms with various ligands, particularly with sulfide groups that bind silver strongly. These ligands may be particles or colloids. Cationic competition at silver binding sites will occur (single headed arrow on Figure 4-9); these cations will include Ca^{2+} , Na^+ , Mg^{2+} , H^+ , and possibly other metals (Simkiss and Taylor 1995; Playle 1998).

Biota bind silver strongly, presumably through sulfur-containing groups such as cysteine. Thus, even if strong Ag-S(II) complexes dominate the system, silver can still be transferred to the biota (double headed arrows on Figure 4-9). Diffusion of neutral silver species into the animal may occur at a slow rate, as may uptake of complexed silver in the gut. These processes were also described in section Silver interactions with aquatic organisms (biological membranes) and illustrated in Figure 4-2. Part of the "modeler's dilemma" is to model the important components of the system accurately enough that the model adequately reflects reality, but in a simple enough manner so that the model is robust and not too specific to a single situation.

Consider the following simple modeling exercise for a situation in which sulfide groups would be expected to control silver speciation. Assume 1 nM total silver (approximately 0.1 $\mu\text{g/L}$), either 0 or 10 nM sulfide groups with a $\log K_{\text{Ag-S}} = 11$, with an overall Ag-organism conditional binding constant of $\log K = 11$ with 0.01 nM sites. This small hypothetical number of Ag-organism binding sites will not alter much the silver speciation in the water around the organism.

Without sulfide in the external environment, the Ag-organism binding sites would be nearly 100% occupied by Ag, depending on the chloride concentration in the water, the concentration and type of DOM, and on the cations available to compete for silver at silver uptake sites. With the 10 nM sulfide in the water, 10% of the Ag-organism binding sites would still be filled. That is, given a high enough gill binding constant, the organism can compete with ambient reduced-sulfur species and some silver will still accumulate. To determine whether this silver represents bioreactive or just bioaccumulated silver would require real experimentation.

The other type of model that may prove to be useful in assessing the bioaccumulation and effects of silver is a physiologically-based pharmacokinetic (PB-PK) food-chain model such as the Thomann Model (Thomann, Aiello et al. 1995; Thomann, Mahony et al. 1995) or the Gobas Model (Gobas 1993). As discussed in the body of this report, a definitive link between silver accumulation and effects has yet to be made. However, several examples where silver has been implicated as a cause of effects have been reported (Hornberger et al. 2000; Lee et al. 2000). Application of a physiologically-based bioaccumulation model, particularly one with the ability

to evaluate organ-specific body burdens (e.g., Thomann et al. 1997), will provide a useful framework for evaluating the route of exposure (food, water, sediment, etc.) and for establishing causality between accumulation levels and effects. A more detailed description of this type of model and a discussion of their status with regard to applications to metals are presented elsewhere (Paquin, DiToro et al. 1999).

Recommendations for Further Model Development

Use of fate and transport models in the development of total maximum daily loads (TMDLs) (a watershed scale wasteload allocation) provides a quantitative basis for refining the conservative assumptions, described previously in the *Problem formation* section of this chapter, that are often incorporated in the simplest of permit development procedures. However, many of the available models were initially developed for organic chemicals and were only subsequently applied to metals. As a result, they are often limited with respect to their ability to represent some of the important physical-chemical processes that apply to metals. These limitations were highlighted as metals-related research needs at the 1996 Pellston Conference (Bergman and Dorward-King 1997). With regard to fate and transport modeling of metals (Schnoor et al. 1987), the Pellston recommendations were that, over the short term

- a probabilistic dilution modeling approach be adopted for riverine systems, rather than relying on a simple static dilution model;
- models be developed to protect both the water column and sediment; and
- discharge permits be developed on a watershed scale.

Longer term recommendations identified the need for

- development of a time-variable modeling approach for multiple loads and multiple routes of chemical exposure to biota, including the need for use of a food-chain model; and
- model testing.

Other modeling research priorities that were identified included

- testing of hypotheses that link metal exposure to effects at the target organ (i.e., testing of the utility of the Biotic Ligand Modeling approach) and incorporating the results into model algorithms;
- incorporating chemical speciation into fate and transport model frameworks; and
- developing models that include sorption or desorption kinetics and speciation.

Process and experimental research priorities in support of model development focused on sediment-water and water-particle exchange processes, including

mechanisms and rates of metal oxidation and release from in-place and resuspended sediments. Many of these topics are areas of ongoing research, and although significant progress has been made over the short period of time since the 1996 Pellston meeting, much remains to be accomplished.

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Current Regulatory Practice for Silver

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Introduction

To provide the context for the discussions further in this chapter, it is important to describe first the current approaches used to regulate discharges containing silver and silver compounds. It also is important to identify the assumptions and approximations included in these regulatory approaches.

Regulatory Approach in the U.S.

In the U.S., the Clean Water Act (CWA) sets forth the legal framework by which all pollutants, including silver, are regulated. The CWA places upon the U.S. Environmental Protection Agency (USEPA) and the states certain responsibilities to define and achieve acceptable levels of pollutants in surface waters. First the USEPA has the responsibility for developing water-quality criteria (WQC) guidance for pollutants. The states use these criteria to fulfill their responsibility for developing water-quality standards (WQS). These standards serve as binding requirements establishing the maximum acceptable amount of any pollutant in receiving waters. Additionally, the CWA requires the states to identify waters that do not meet WQS and for these waters to develop total maximum daily loads (TMDLs) for pollutants that exceed these standards. Finally, the CWA prohibits any discharge of a pollutant through a point source unless the USEPA or an authorized state develops a national pollutant discharge elimination system (NPDES) permit that both achieves WQS and implements TMDLs where developed.

The statutory steps for developing maximum allowable discharged loads can be described in 2 steps: (1) criteria development, which identifies the maximum amount of a pollutant that can exist in the water environment without causing an adverse effect and (2) exposure assessment, which through consideration of fate and transport mechanisms identifies the maximum amount of a pollutant that can be emitted into the water environment without exceeding its water-quality criterion.

Criteria

For toxic pollutants in general, the USEPA develops 2 criteria values: (1) an acute criterion that protects against short-term lethality and (2) a chronic criterion that protects against longer-term sublethal effects (Stephan et al. 1985; Stephan 1998). Acute criteria generally are developed using laboratory tests in which different species of organisms in laboratory water are exposed to various concentrations of a toxicant, which is generally the ionic form for metals. The acute criterion is defined as one-half the 95th-percentile LC50 value (i.e., the LC50 for which 95% of the species are less sensitive to the metal). This criterion is expressed as a 1-hour average concentration that should not be exceeded more than once every 3 years, whereas the chronic criterion is expressed as a 4-day average. Although the guidelines for WQC development explicitly describe a method for developing chronic criteria from a chronic toxicity database, for lack of better scientific data, chronic criteria are most often calculated by multiplying the acute criterion by an acute-to-chronic ratio (ACR). For metals, both the acute and chronic criteria are expressed as concentrations in the dissolved form (Prothro 1993). Dissolved is operationally defined as the metal in solution passing through a 0.45 μm filter.

When the states adopt these criteria into their WQS, they can modify the criteria to better reflect local species and receiving water chemistry. One way to adjust the acute and chronic criteria is through application of the water-effect ratio (WER) , (USEPA 1994). However, most states do not have the resources to modify or develop site-specific criteria and rely upon the USEPA criteria unless an NPDES-permitted facility conducts a WER.

Currently for silver, there are nationally recommended acute aquatic life criteria for freshwater and saltwater, but there are no nationally recommended chronic aquatic life criteria. The freshwater acute criterion is based on the 1980 criteria document (USEPA 1980), which uses a hardness-dependent relationship to calculate total recoverable silver criteria. The current saltwater criterion also is based on the derivation given in the 1980 document. More recently, the USEPA recommended that silver criteria be expressed as dissolved silver, rather than total recoverable silver, based on a conversion factor of 0.85 (Prothro 1993; Stephan 1998). For reference, the current freshwater acute dissolved criterion is 3.4 $\mu\text{g/L}$ based on a hardness of 100 mg/L , and the saltwater acute criterion is 1.9 $\mu\text{g/L}$ (USEPA 1999). Note that although an updated draft silver criteria document (USEPA 1987) was prepared subsequent to the 1980 document, these criteria were subsequently withdrawn (Davies 1992). Thus, the proposed criteria are not included in the current nationally recommended criteria.

Exposure

The USEPA and the states conduct exposure assessments either by directly measuring ambient water concentrations or by using water-quality models. Models are used more frequently. The models generally calculate water quality based on

conservative mass balance principles applied to pollutants in the water column. Calculations generally are not conducted for sediments because sediment quality criteria have yet to be finally adopted by the USEPA. These calculations are typically conducted using steady-state principles applied at critical environmental conditions of low dilution flow and high effluent discharge. Metal losses from the sediment to the water column can be considered, but generally are not. The models calculate metal concentrations on a total basis and estimate the dissolved fraction by assuming an instantaneous equilibrium between total and dissolved metals as a function of total suspended solids. This equilibrium approach has been described for a number of metals but not silver (USEPA 1996). When conducted for purposes of calculating a TMDL, these calculations are required by the CWA to include a margin of safety, which can be either implicitly or explicitly described. The USEPA has provided guidance on how to conduct these calculations (USEPA 1991).

Ideally, only experienced water-quality modelers, as part of a TMDL, would conduct exposure calculations. Here, the USEPA or a state will not only identify the total maximum load that the water can endure without harm but also identify what part of that total load any given facility can discharge. This part is called the wasteload allocation. However, in practice, less experienced permit writers often do these calculations as part of permit development, and these calculations pertain to that specific facility.

Currently, the states together identified approximately 21,000 waters that do not meet WQS for all pollutants. Of these, 37 waters were identified in 9 states as not meeting their WQS for silver. These states will be developing TMDLs for these waters over the next 8 to 15 years.

Regulatory Approach in Canada

In Canada, the framework for establishing environmental quality guidelines is developed in a collaborative process with the federal government ministry, Environment Canada (EC), and the provincial and territorial ministries of the environment through the Canadian Council of Ministers of the Environment (CCME). The CCME is made up of environment ministers from federal, provincial, and territorial governments. CCME members propose nationally consistent environmental standards and objectives to achieve a high level of environmental quality across the country. The CCME does not impose its suggestions on its members. It has no authority to implement or enforce legislation; each member decides whether or not to adopt CCME proposals. The provincial and territorial ministries, as stewards of the water quality in their jurisdictions, establish and implement criteria for their respective geographical areas that are intended to be consistent with the environmental quality guidelines established by EC and the CCME (Gaudet et al. 1995). The guidelines established by CCME are intended to assist in the harmonization of environmental regulations throughout Canada.

Development of the Canadian Water Quality Guidelines (CWQGs) began in 1984. In 1987, the Water Quality Guidelines Task Group of CCME and EC published the CWQGs (CCREM 1987). Originally written to protect freshwater ecosystems, the guidelines have been revised and expanded to include marine water quality, marine and freshwater sediment quality, tissue-residue quality for protection of aquatic life, and soil quality guidelines for agricultural and other uses. The CWQGs are developed to provide basic scientific information about water-quality parameters and ecologically relevant toxicological threshold values for Canadian species to protect specific water uses. The guidelines provide a numeric value or narrative statement outlining the recommended guidelines for over 100 substances. These include recommendations for chemical, physical, radiological, and biological parameters necessary to protect and enhance designated uses of water. The guidelines for water quality, sediment quality, tissue-residue quality, and soil quality define clear, scientifically defensible indicators for protecting, sustaining, and restoring aquatic and terrestrial ecosystems.

The CWQGs are derived from the available literature on the effects of the substance or physical property (e.g., temperature) on various species for the protection of the appropriate use (e.g., marine or irrigation). The technical review and summary of the substance include factors such as production and uses, physical and chemical properties, fate and behavior, bioaccumulation, and relevant toxicological data. The current CWQG for silver is 0.1 µg/L for freshwater systems (from the CCREM 1987); there is no guideline value for marine waters. As a result of the priorities established by the CCME Water Quality Guidelines Task Group, parameters for silver are currently under development.

In 1999, the CCME changed the structure to the Canadian Environmental Quality Guidelines (CEQG), which now include not only the CWQG but some additional recommendations covering such areas as air quality, wildlife, drinking water, recreation, and aesthetics (CCME 1999) (also see the CCME website at www.ccme.ca/pdfs/ceqg_rcqe/summary_table_e.pdf).

By having a national set of guidelines in place, the provincial and territorial Environmental Ministries can develop and implement standards or objectives that will take into account regional, site-specific characteristics as may be required. Guidelines are not used as a blanket value for national water quality. Environmental conditions affect water quality in different ways and guidelines may be modified according to local conditions (e.g., assimilative capacity, local species, or habitat). Site-specific water-quality objectives are established to reflect the local environment and may be adopted by a jurisdiction into legislation to become standards. The standards or objectives are implemented through provincial and territorial legislation and policy, in conjunction with the Canadian Environmental Protection Act (CEPA).

Critical Assumptions and Approximations in Regulatory Approaches

This section provides a discussion of assumptions and approximations found in regulatory approaches, primarily in the U.S., that practitioners believe occur frequently and have the greatest effect on regulatory decisions.

Analytical methods provide reliable results

Regulatory decisions to impose permit limits generally are based on measuring effluent concentrations and, after considering dilution, comparing these measurements to the appropriate water-quality criterion. Historical databases that contain metal concentrations in effluents and ambient waters below one part per million (ppm) may be invalid due to sample contamination (Bergman and Dorward-King 1997). Errors of 3 to 4 orders of magnitude have been observed when clean techniques are not used to measure concentrations below the 1 $\mu\text{g/L}$ (Bloom 1993; Shafer et al. 1993; USEPA 1997). Analytical methods for sample collection, handling, filtration, and analysis of effluent and ambient samples must use modern clean methods when the expected concentrations are lower than 1 ppm, if confidence is to be placed on the sample analysis (Bergman and Dorward-King 1997). The USEPA has published guidance that describes these techniques (USEPA 1995a, 1995b). Special care may be needed when analyzing silver samples because concentrations in laboratory tests, effluents, and ambient waters often can be below 1 $\mu\text{g/L}$. Also, recent literature suggests that silver may sorb onto Teflon bottles (Herrin et al. 2001). Silver also can be easily removed from Teflon bottles using sonication and ultraviolet irradiation (Wen et al. 1997).

Dissolved form causes acute toxicity

Dissolved metal is operationally defined as the metal in a solution that passes through a 0.45 μm filter; it comprises individual and complexed metal ions, colloids, and particulate metals that are small enough to pass through the filter. Total recoverable metal is defined as the measurement of metals after acid digestion (USEPA 1984). The USEPA recommends use of dissolved metal WQC because dissolved concentrations more accurately reflect metal toxicity in water than does total recoverable metal (Prothro 1993). In the U.S., not all states follow this approach, but instead use criteria based on the total form of metals to address sediment, food web effects, and other fate-related issues. In addition, the practice of using the dissolved metal form has raised new questions, such as whether sediment accumulation poses significant water-quality problems and whether the permitting process has become too complicated (Bergman and Dorward-King 1997). Further, although a dissolved metal-based criterion is a better predictor of metal toxicity in the water column, it may not be an appropriate predictor for a specific metal for all exposure routes.

Dilution water in laboratory tests maximizes toxic responses

In some instances, both chronic and acute toxicity tests have been conducted in very clean water to maximize toxic responses. The water used typically was treated to remove contaminants by use of such techniques as chlorination or dechlorination, activated carbon ion exchange, and reverse osmosis treatment. Unfortunately, these techniques also removed dissolved organic matter (DOM) and sulfides, which are important in modifying toxicity. This approach provides a baseline evaluation of the inherent toxicity of a pollutant to ensure that WQC based on these tests will be protective of all U.S. waters. This approach characterizes toxicity tests used by the USEPA to develop WQC for silver (Prothro 1993). More recently, however, this approach has been questioned because criteria based on this approach may instead provide an unrealistic or unnecessary level of protection for metals such as silver.

Water-effect ratio accounts for local water chemistry

The WER is the ratio of the toxicity of a metal in side-by-side tests using site water and laboratory water. It provides a site-specific methodology for adjusting national WQC to reflect local water chemistry and its effects on the toxicity of a pollutant. Although WQC for metals provide an adjustment based on water hardness, the WER is presently the only method for adjusting criteria to account for other site-specific water-quality characteristics. (If the Biotic Ligand Model [BLM] becomes adopted for criteria development, it will obviate the need for the WER.) However, there are some problems with using the WER (Bergman and Dorward-King 1997). It is a relatively complex approach that may be too costly for all facilities to use. It requires dosing samples with the ionic form of a metal until toxicity occurs, and therefore may not replicate in-stream metal chemistry. There are also some methodological uncertainties regarding the form of metal used in the study and the equilibrium time for the added metal.

Chronic effects can be related to acute effects

The USEPA generally develops chronic criteria by applying an ACR to the acute criteria. This approach implicitly considers that the significant factors governing short-term lethality to organisms also govern longer-term sublethal effects to organisms.

Sediment toxicity is addressed through equilibrium partitioning sediment guidelines

The USEPA currently interprets its WQC to be protective of organisms in the water column (Prothro 1993). The USEPA intends to address sediment toxicity through use of numeric partitioning-based equilibrium sediment guidelines (ESG) (USEPA 2000). The ESG will be based on acid-volatile sulfide (AVS) partitioning and interstitial-water metal concentration in sediments. Exposure calculations will

entail use of water-quality models that link water column and sediment metal kinetics.

Total-to-dissolved empirical relationships work in water column

In the U.S., NPDES effluent limits are required by current regulation to be expressed in terms of total recoverable metal. Furthermore, all water-quality modeling for the dissolved form of metals must consider both the dissolved and particulate fractions. The USEPA has provided guidance on translators to calculate the dissolved form of a metal as a function of the total form and total suspended solids (USEPA 1996). However, translators do pose application problems. Site-specific guidance may not be available, and thus, generic information will be used. For silver, there is no generic information available. The amount of dissolved metal in some waters may be independent of the amount of particular metals during high flow or scour events. As a result, the ability of a translator to predict reliably the amount of dissolved metal is somewhat questionable (Bergman and Dorward-King 1997).

Dilution—only significant factor affecting exposure calculations

The USEPA and the states routinely calculate ambient water-column metal concentrations using simplified models. These models typically include only mechanisms for dilution and dissolved-particulate partitioning in the water column as affecting ambient concentrations (USEPA 1991). In some limited applications, settling to and resuspension from sediments may be considered.

Overall safety factor is reasonable

Safety factors are used to account for uncertainties by reducing the amount of an allowable discharge or by using conservative assumptions when calculating the allowable discharge. Safety factors result from choices made by regulators at each step during criteria development or exposure assessment. Within the current regulatory approach for metals, safety factors arise during the choice of water for laboratory studies, in the choice of critical conditions for exposure calculations, and in the assumption that all pollutant sources will discharge at their maximum levels at the same time. The overall safety factor in the approach for metals regulation is unquantified (Bergman and Dorward-King 1997).

Water-quality standards are independently applied

The USEPA policy interprets WQC to be independently applied (Davies 1991). This policy, which is known as “independent applicability,” includes 3 concepts: (1) each of three criteria types (chemical-specific, whole effluent toxicity, and biological assessments) are considered to have equal weight when assessing water quality,

(2) WQS are considered to be exceeded if any criterion is exceeded, and (3) results of one criterion cannot be used to overrule the results of another.

The independent applicability policy prevents findings of no whole effluent toxicity or no biological impairment from overruling any predicted toxicity from comparing actual or calculated water values to the WQC for a toxic pollutant. Instead, the policy requires the USEPA or the states to investigate first the causes of any differences, which may lead to development of site-specific criteria.

These critical assumptions and approximations are designed to be conservative in order to accommodate the uncertainties of the impacts of silver in the environment. The research results presented at the Argentum meetings as summarized at Argentum VI have addressed many of the issues associated with aqueous exposures. These results should help reduce the conservatism in the regulatory system and lead to more cost-effective regulations. Work remains to refine the models to predict the impact of aqueous exposures of silver to organisms without having to perform costly site-specific toxicity studies. Additional research also is needed on other exposure pathways. Argentum VI has served not only to summarize what we know about silver in the aquatic environment but also to focus our future research efforts to address the remaining key regulatory issues.

Summary

The unique chemical characteristics of silver indicate that environmental effect assessments of silver warrant an approach that is substantially different from that of nearly all other metals. Recent acknowledgment of the apparently ubiquitous, low-level presence of reduced, complexed reactive sulfides (defined as acid-reactive sulfide [ARS]) in ambient oxic waters has raised serious questions about the completeness of the previous understanding of the fate of silver in the aquatic environment. The affinity of the silver ion to form complexes preferentially with metastable reduced sulfides has led to a questioning of the appropriateness of standard toxicity test procedures that typically include the addition of highly soluble silver salts (nitrates) to laboratory dilution water that may not contain these reduced sulfides due to conventional laboratory techniques for preparing "synthesized" bioassay water from tap water. In addition, silver can form complexes with chlorides and dissolved organic carbon, which are generally quite low in test water. This issue may be of particular significance in relation to acute toxicity given that silver-sulfide complexes are believed to be less toxic than free silver. Less is known about the chronic toxicity for these reduced silver-sulfide complexes.

These recent revelations have led to a reevaluation of the understanding of both the chemistry and the effects of silver in the environment. Many of the conclusions and recommendations in this report are based on the primary assumptions that

- ARS is consistently present in both oxic and anoxic ambient waters at concentrations greater than silver (on a molar basis), and
- the acute toxicity of silver-sulfide complexes is believed to be less than that of free silver.

Acute toxicity endpoint: Recommendations

Given the assumptions discussed above, the potential for acute toxic effects due to silver in most publicly owned wastewater treatment works (POTWs), effluents and ambient surface waters at normal pHs is low. However, acute toxicity could be of greater concern for nonbiologically treated effluents, acidic surface waters, or other waters low in reduced sulfides.

Before setting regulatory standards for acute toxicity endpoints, it is important to do the following:

- Perform acute toxicity tests on a sensitive organism such as *Ceriodaphnia* to evaluate the bioavailability and toxicity of silver sulfide complexes (proof of principle).
- Implement the BLM for the acute toxicity of silver as a method for adjusting ambient WQC on a site-specific basis. The BLM should account for the effects of reduced sulfides and other inorganic and organic ligands on the bioavailability and toxicity of silver. Current acute estimates derived from standard toxicity tests can underestimate or overestimate acute toxicity of silver in the environment. Prior to implementation, the BLM should be further validated with a wider variety of aquatic species and water-quality conditions. If the BLM is to be widely used, it is likely that default values for some input variables will need to be determined. The spatial and seasonal variability of reduced sulfide complexes also needs better documentation.
- Review methods of preparing laboratory test water and establishing modified toxicity test procedures that allow for the reintroduction of ARS, DOM, and inert particulate matter at levels consistent with those found in the natural environment. This may better reflect potential silver toxicity effects in ambient waters and many effluents.
- Perform measurements of actual chemical concentrations and species in laboratory water used for toxicology testing, rather than relying on nominal concentrations based on the amount added. Recent evidence stresses the importance of knowing the chemical speciation of a metal in interpreting toxicological responses when attempting to extrapolate laboratory results to natural waters.

Chronic toxicity endpoint: Recommendations

Chronic exposures to silver are more complex than acute in both the routes of exposures and toxic endpoints observed. The concerns with the chemistry of silver in acute tests also are relevant in the chronic tests. Most toxicity tests in the chronic

database were conducted with metastable sulfides and other complexing ligands either unmeasured or removed. While some concerns about acute silver toxicity may diminish due to recent information, this recent information also suggests that a heightened awareness of potential chronic silver toxicity effects may be appropriate. Therefore, it is important that chronic toxicity test protocols are modified to include sufficient reduced sulfide concentrations to reflect the new understanding of silver chemistry. Unlike the case for acute toxicity, levels of silver in the environment have been measured above levels observed to produce chronic toxicity in the standard toxicity tests. Therefore, chronically toxic effects to aquatic life are plausible. Given this situation, it is important that the chronic toxicity test protocols be refined and that the data set be rebuilt.

Chronic exposure pathways include dietary and waterborne uptake. Evidence also suggests that some invertebrates can assimilate silver on or in particulates and algae. The rates and relative importance of the routes vary by species and possibly between waters for the same species. Dietary pathways may be significant in causing chronic toxic effects and, thus, this factor needs evaluation.

Silver is an extremely particle-reactive metal that is quickly scavenged from the water column and incorporated into sediments. These sediments can serve as a dietary source of silver to aquatic organisms. Therefore, it is necessary to develop criteria to protect against the potentially harmful accumulation of silver in the sediment.

Before setting regulatory standards for chronic toxicity endpoints, it is necessary to do the following:

- Modify the chronic toxicity testing protocols to incorporate new understanding of issues such as sensitive organisms and endpoints, water chemistry and equilibrium issues, and dietary and other exposure routes.
- Validate any change to testing protocols before using their results to set criteria.
- Rebuild the chronic exposure database with data generated using new protocols. Given the need to develop chronic criteria quickly, it is imperative that sensitive species and ecologically relevant endpoints are concentrated on.
- Unless it can be shown that the mechanisms for acute and chronic toxicity to aquatic organisms are similar, chronic criteria should be derived from chronic data and not by the use of an ACR.
- To expedite the development of chronic exposure WQC, consider an interim approach using current chronic data with site-specific modifications for ARS. Tests need to be done to validate this approach within 3 years.
- Adopt criteria to protect organisms from silver in sediments. As a first step, use the SEM-AVS procedures with appropriate modifications (as outlined in Chapter 2, *Biological effects of silver in sediments*). Continue to evaluate other

routes of silver exposure within the sediments and modify the criteria guidelines as appropriate. Consider if this potentially new regulatory approach will increase sediment silver loadings and if so, if it will cause problems via displacement of other sediment-bound metals.

- Understand the differences between bioavailable and bioreactive fractions when assessing environmental effects.

Additional recommendations

The following are research and regulatory recommendations suggested by the conference attendees:

- The unique chemistry of silver requires the use of rigorous analytical procedures capable of measuring very low levels. The use of clean sampling and analytical procedures is critical in achieving these levels.
- The importance of reduced sulfides on the cycling and effects of silver in the environment must be documented. The ARS methodology needs to be added to USEPA and state ambient monitoring programs, and a standard (approved) analytical procedure is needed.
- The chemistry of waters used in toxicity tests needs to be fully characterized. Researchers should develop guidelines listing standard parameters of measurement and the methods to be used in all toxicity testing.
- Regulators should continue to use the USEPA's existing guidance on total or soluble relationships and WERs in assessing surface waters and in permitting discharges until new WQC procedures (e.g., BLM) are completed. At this time, ultrafiltration is not recommended for routine regulatory use because of logistical difficulties. Presently the biological relevance of particular molecular size fractions is unknown. Until these challenges have been overcome, analysts should continue to use the 0.45 μm filter when determining dissolved silver.
- The USEPA and the states should include the sediment pathways and sulfide calculations in their exposure models for metals.

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Glossary

A

ACR **Acute-to-chronic ratio**

Ratio of the acute toxicity of an effluent or a toxicant to its chronic toxicity; used for estimating chronic toxicity on the basis of acute toxicity data, or for estimating acute toxicity on the basis of chronic toxicity data (USEPA 1991).

Acute

Involving a stimulus severe enough to rapidly induce a response; note that acute means "short," not fatal (USEPA CWA Section 403).

Acute criteria also **Criteria maximum concentration**

USEPA national water quality criteria recommendation for the highest instream concentration of toxicant or effluent to which organisms can be exposed for a brief period of time without causing acute effect (USEPA 1991).

Acute toxicity

Toxicity typically elicited during or immediately following short-term exposure of a test organism to a toxicant or stimulus severe enough to rapidly induce an adverse effect; in aquatic toxicity tests, an effect is considered acute if observed in 96 hours or less for fishes and macroinvertebrates, and shorter times for organisms with shorter life spans. In aquatic toxicology or human health, an acute effect is not always measured in terms of lethality.

ADI **Average daily intake**

Long-term average estimated dose to the organism of the chemical of concern.

Adverse effects

Negative effects to the organism that result from exposure to the chemical or stressor (CREA) of concern.

Ag+

Hydrated form of Ag(I) (see Free ionic silver).

Ag(I)

All chemical forms of silver in the +1 oxidation state.

Anoxic water column

Condition in a water body where the dissolved oxygen concentration in the overlying water is zero.

APDC **Ammonium pyrrolidinedithiocarbamate**

Thiol-based complexing agent used in metal analysis and speciation (also see DDDC).

Area use factor

Ratio of the time the target organism spends at the site of contamination to the time it spends outside the site of contamination.

ARS **Acid-reactive sulfide**

Any form of sulfide that forms hydrogen sulfide readily at low pH (also see AVS).

Assessment endpoints

Explicit expression of the actual environmental value that is to be protected; an assessment endpoint includes both an ecological entity and specific attributes of that entity.

Assimilation

Conversion of food into the substances of the body; specifically, physiological absorption of constituents via the gut membrane during digestive processing of ingested diets.

ATP **Adenosine triphosphate**

ATPase **Adenosine triphosphatase**

Class of enzymes that hydrolyzes ATP to provide chemical energy for other reactions.

AVS **Acid-volatile sulfide**

Any sulfide (in liquid or solid form) that produces hydrogen sulfide readily upon acidification and can be captured for analysis in a basic solution (also see ARS).

B

BAF **Bioaccumulation factor**

Ratio of the concentration of a chemical substance in the tissue of an organism to the concentration of that chemical in the source (ambient environment or food), in situations where the organism and/or its food chain are exposed to the chemical.

Basolateral

Denotes the area of the cell that is in direct contact with the interior of the body; similar to "serosal" but usually referring to single cells in an epithelium.

BCF **Bioconcentration factor**

Ratio of the concentration of a chemical substance in an organism to the concentration of that substance in the abiotic environment from which the organism acquired the chemical; for an aqueous environment, the ratio of a substance's concentration in tissue versus its concentration in ambient water, in situations where the food chain is not exposed or contaminated. For nonmetabolized substances, BCF represents equilibrium partitioning between water and organisms.

Benchmark

Standard by which something can be measured or compared to in order to assess the likelihood of effects.

Binding site

(within organisms) Biochemical moiety such as the surface of a cell membrane that shows specificity for a certain molecule; (within chemicals) the location of a functional group or ligand in a large molecule such as humic acid.

Bioaccumulation

Process by which an organism accumulates a chemical constituent both directly from the surrounding abiotic environment and from dietary sources (trophic transfer); sometimes used to mean the accumulated concentration of a constituent in biological tissues.

Bioassay water

Water used for bioassays; may include natural or synthetic water or both (see synthetic water and dilution water).

Bioavailable

Measure of the physio-chemical accessibility of a chemical to the biological processes of an organism; bioavailability is defined by a suite of processes that determine the extent to which a constituent may associate with or cross a biological membrane. The bioavailable fraction is that fraction which is capable of associating with or crossing the membrane under a specific set of conditions.

Bioconcentration

Uptake of substances from the surrounding medium through gill membranes or other external body surfaces.

Bioreactive

Fraction of a bioavailable contaminant that may react with biochemically active sites, thereby causing toxicity.

Biospeciation

Distribution of a constituent among compartments within animal tissues; biospeciation determines trophic transfer and bioreactivity.

Bioturbation

Process where benthic sediments of a water body are physically mixed by the activity of aquatic organisms that live in the sediments.

BLM Biotic Ligand Model

Computational framework (simulation model) that assumes that the toxic effect of a metal in an aquatic system can be predicted by the accumulation of the metal at a specific binding site or by the metal-induced impairment of a specific metabolic function, given the water-quality characteristics of the water body and the amount of metal dissolved in the water.

C**CC Chronic criteria, also Criteria continuous concentration**

USEPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed indefinitely without causing unacceptable effect (USEPA 1991).

CCC Criteria continuous concentration

see CC

CCME Canadian Council of Ministers of the Environment**CCREM Canadian Council of Resource and Environment Ministries****CEPA Canadian Environmental Protection Act****CDF Cumulative distribution function**

see Probability distribution

CEQG Canadian Environmental Quality Guidelines**Clusters**

Combinations of metal ions and ligands to create molecules or ions that form polyhedral or extended structures (also see Metal-sulfide clusters).

CMC Criteria maximum concentration

see Acute criteria

Chronic

Involving a stimulus that lingers or continues for a relatively long time, often one-tenth of the life span or more; chronic should be considered a relative term depending on the life span of the organism. A chronic effect can be lethality, growth, reduced reproduction, etc. (USEPA CWA Section 403).

Chronic toxicity

Toxicity resulting from long-term exposure to a toxicant.

Clean sampling methods

Generally, any approach that strives to minimize contamination biases that would compromise the integrity of a sample. Methods are designed to control contamination from the local sampling environment, sampling equipment, sampling personnel, and chemical agents added

to samples. The degree or rigor that one applies clean sampling methods may in practice be adjusted to reflect the levels of the target trace metals. Typically the clean sampling method should be designed to limit total contamination (as measured by comprehensive field method blanks) to less than 2 to 5% of actual metal level in a sample. Specific measures that have been shown to be effective in reducing metal contamination include: strict adherence to trace-metal-compatible materials; exhaustive cleaning of bottles, filters, and sampling equipment; preparing and pre-assembling equipment and supplies in a clean-room environment; double or triple bagging in plastic all supplies; use of ultra-pure reagents; strict application of "clean-hands"- "dirty-hands" protocols; sampling within a protected enclosure; use of, and frequent change of, clean gloves; and indoctrination (training) in clean methods and development of a "clean" ethic.

Colloid

A system in which a finely divided solid is suspended in a liquid. "An aquatic colloid is any constituent that provides a molecular milieu into and onto which chemicals can escape from the aqueous solution, and whose movement is not significantly affected by gravitational settling." (Gustafsson and Gshwend 1997).

Competition

Two or more molecules contesting for the same binding site.

Complexation

The chemical binding or association of a metal with a ligand.

CWA Clean Water Act (U.S.)

CWQG Canadian Water Quality Guidelines

D

DDDC Diethylammonium diethyldithiocarbamate

A thiol-based complexing agent used in the analysis and speciation of metals like silver (also see APDC).

Depuration

Loss or efflux of a constituent from a tissue or a whole organism. Used in this context to mean physiological loss or efflux, as governed by rate constants of loss. The process of chemical elimination from an organism.

Dietary use factor

The ratio of the amount of contaminated food ingested by the target organism to the total amount of food (contaminated + uncontaminated) ingested by the target organism.

Dilution (diluent) water

(Note: not necessarily the same as synthetic or reconstituted water) Water used in aquatic toxicity tests in which organisms are exposed to defined concentrations of a toxicant, or water that is mixed in varying degrees with a test water to achieve a range of water quality characteristics. Can include both natural and synthetic waters.

Dissolved silver

Operational definition for the silver in an aqueous medium that passes through a 0.45 micron filter.

DOC Dissolved organic carbon

That organic carbon contained in the liquid fraction of a 0.45 μm filtered sample. Usually measured as milligrams of carbon per liter of sample (mg/L).

DOM **Dissolved organic matter**

All organic matter that passes a 0.45 μm filter. Often contains some trace inorganic associated matter. Usually expressed as milligrams of dry solid per liter of sample (mg/L).

Dose

Amount of toxicant that enters the organism. Dose and concentration are not interchangeable (ASTM).

dw **Dry weight**

E

EC **European Council**

ERA **Ecological risk assessment**

The process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors.

EEC **Expected environmental concentration**

Effects assessment

A component in the analysis phase of the ecological risk assessment that evaluates the ability of a stressor to cause adverse effects under a particular set of circumstances. Data on potential effects of stressor identified during problem formulation are technically evaluated and summarized as stressor-response profiles.

Elimination

Used in this volume as equivalent of depuration.

ESG **Equilibrium sediment guideline; also known as Equilibrium partitioning sediment guideline**

USEPA's recommendation of the concentrations of a substance that may be present in sediment while still protecting benthic organisms from the effects of that substance. ESGs are derived based on equilibrium partitioning (EqP) theory, which asserts that a nonionic chemical in sediment partitions between sediment organic carbon, interstitial (pore) water and benthic organisms. In some cases, the additive toxicity for specific classes of toxicants is addressed by the ESGs. The ESGs are not protective of synergistic effects of contaminants, bioaccumulative effects to benthos, or wildlife or human health endpoints (USEPA 2000).

Epibenthos

Collectively, the organisms living on the sea bottom between low tide and a depth of 100 fathoms. Epibenthic organisms live on the surface of the sediment rather than in the sediment.

Exposure

The contact or cooccurrence of a stressor with a receptor. Contact of a chemical, physical or biological agent with the outer boundary of an organism. Exposure is quantified as the concentration of the agent in the medium in contact with the receptor integrated over the time duration of that contact.

Exposure assessment

A component of the analysis phase of the ecological risk assessment that evaluates the interaction of the stressor with one or more ecological entities. Data on potential exposure to stressor identified during problem formulation are technically evaluated and summarized as exposure profiles. The determination or estimation (qualitative or quantitative) of the magnitude, frequency, duration and route of exposure; a characterization of the magnitude and variation of concentrations of the chemical of concern in the various media at the site.

Exposure pathway

(also Exposure route) The physical course a chemical or pollutant takes from the source to the exposed organism.

Exposure profile

The product of characterization of exposure in the analysis phase of the ecological risk assessment. The exposure profile summarizes the magnitude and spatial and temporal patterns of exposure for the scenarios described in the conceptual model. A characterization of the concentrations of the stressor with which the target organism comes in contact in the various environmental media (air, water, soil, and sediment).

F**Free ionic silver**

see Ag⁺

fw fresh weight

G**GFAAS Graphite furnace atomic absorption spectroscopy**

An analytical technique used for the determination of trace element constituents in sample solutions or dry samples. Unlike flame atomic absorption spectroscopy, GFAAS is a flameless technique which uses a small graphite tube at a high temperature to atomize the sample aliquot. The sample is introduced to the graphite tube which is then heated to high temperature to atomize the sample. A beam of light passes through the tube, and the amount of light absorbed by the atomized sample is proportional to sample concentration. GFAAS is a highly sensitive technique with detection limits in the upper ng/L range.

Group B metals

Also called "soft" metals. Includes metals in low oxidation states and the heavier transition metals. Cu⁺, Ag⁺, Hg⁺, Hg²⁺, Pd²⁺, Pt²⁺, Cd²⁺, Pb²⁺ are examples of Group B metals. These metals tend to react with Group B ligands of which sulfide is an example.

GSH Glutathione

An abundant thiol commonly found in the cells of organisms that protects against oxidants and electrophiles. It's main purpose, coupled with its disulfide, is to maintain the redox state of cells.

GSSG

The oxidized form of glutathione consisting of a dimer of two glutathione molecules. The molecule offers no direct protection to the cells, as does glutathione, but is recycled into two molecules of glutathione.

H**Hazard**

A measure of harm or injury of a chemical stressor; a possible harm or adverse outcome.

Hazard assessment

The identification and exploration of a hazard. The evaluation of the intrinsic effects of a stressor or the definition of a margin of safety or quotient by comparing a toxicological effects concentration with an exposure estimate.

HI Hazard index

Toxicity of a chemical mixture when assuming that the toxicity of the components of the mixture is additive.

HPLC High-performance liquid chromatography

An analytical technique used to separate mixtures of organic materials into their isolated components. The separated components are detected in a variety of ways, UV/VIS spectroscopy being the most common. Other detection techniques include refractive index, electrochemical, fluorescence spectroscopy, mass spectroscopy and infrared spectroscopy. The technique is popular due to the wide range of materials which can be analyzed.

HQ Hazard quotient

The ratio of the estimated dose to the target organism to the reference dose associated with an acceptable effect concentration.

Humic acid

A natural organic matter fraction that, along with fulvic acid and humin, comprises humic substances (the amorphous organic residue that results from the decomposition of plant and animal matter). The humic acid fraction is defined as that fraction that is solubilized at high pH (alkaline extraction and re-precipitated at low pH). Humic acids tend to be large molecules with significant acid-base and metal binding properties in aquatic systems.

I**ICP-MS Inductively coupled plasma-mass spectroscopy**

An analytical technique for the determination of trace element constituents in sample solutions. The sample is atomized and ionized in an argon plasma "flame." The ions are separated and detected using a quadrupole mass spectrometer (see Mass spectroscopy).

Indicator species

An organism considered to be representative of a class of organisms that might be exposed to contamination at a site.

Ionic strength

A measure of the total number of ions in solution; $I = 1/2 \sum C_i \cdot z_i^2$, where C is the concentration and z is the charge of each respective ion (i) in solution.

IWTU Interstitial water toxic unit

A measurement of the toxic potential of metals in the interstitial or "pore" water of sediment particles, given by $[M_i] / LC50$, where $[M_i]$ is the dissolved metal concentration in the interstitial water and LC50 is the concentration of the metal causing 50% mortality of the test species in a water-only test.

K **K_d Distribution coefficient**

The ratio of solid to operationally defined liquid concentrations of a specified substance. Usually expressed in units of liters per kilogram (L/kg).

 K_f Formation constant

The ratio of the bound metal (ML) divided by the product of the metal (M) and ligand (L) concentration or activities ($K_f = ML / M \cdot L$). K_f is the conditional formation constant, usually expressed in concentration terms.

K_{cond} Conditional equilibrium binding constant

The ratio of $[MxLy]/([M]^x [L]^y)$ at specific conditions where $[\]$ denotes concentration. In the above, $xM + yL = MxLy$.

L**LA Laser ablation**

The use of a laser to create ions that can be measured by a mass spectrometer.

LC10 Median lethal concentration 10%

The concentration of test chemical which is lethal to 10% of the test organisms in a laboratory toxicity test during a prescribed exposure.

LC50 Median lethal concentration 50%

The most commonly used term for expressing lethal concentration, being the concentration of test chemical which is lethal to 50% of the test organisms in a laboratory toxicity test during a prescribed exposure.

Ligand

An ion or functional group of a compound that binds to a metal ion or compound by donating a pair or pairs of electrons.

LI Lifetime intake

The cumulative intake of a constituent over the lifetime of the organism.

LOAEL Lowest-observed-adverse-effect level

The lowest concentration of chemical agent being tested which produces an adverse effect in the organism undergoing the test.

LOEC Lowest-observed-effect concentration

The lowest concentration of chemical agent being tested which produces an effect, either adverse or non-adverse, in the organism being subjected to the test. In a full- or partial-life-cycle test, the lowest toxicant concentration in which the values for the measured response are not statistically significantly different from those in the control (ASTM).

LOEL Lowest-observed-effect level

same as LOEC

M**Macro-particle**

A particle larger than either 0.4 or 0.45 microns.

Mass balance

A calculation that is performed with respect to a defined volume that considers the sum of the mass inputs to and mass outputs from the volume, and the transformations within it.

MATC Maximum acceptable toxicant concentration

The geometric mean between the LOEC (or LOEL) and NOEC (or NOEL).

MBMS Methylene blue measurable sulfide

An analytical method to determine that sulfide which will form hydrogen sulfide at low pH.

MCL Maximum contaminant level

Maximum allowed level for a specific pollutant in drinking water, per USEPA-published criteria for human health.

Mesocosm study

A test run outdoors under natural environmental conditions, using a small captive ecosystem with a limited number of variables being controlled, and including organisms representative of the food chain. The system permits the evaluation of chemicals being introduced.

Metabolism

The sum of all physical and chemical reactions that are used by animals and microorganisms for anabolic synthesis of macromolecules for cell assembly and catabolic degradation of macromolecules for energy production. The term is also loosely used to describe intracellular 'handling' of metals or 'biotransformation,' which is the modification of molecules by organisms in an attempt to reduce the reactivity of the molecule or facilitate its elimination from the body.

Metallothionein induction

A process whereby exposure of an organism to a chemical stimulates the production of metallothionein within the organism (also see MT).

Metal-sulfide clusters

Clusters in which sulfide is the ligand (see Clusters).

Metastable sulfide complexes

(Not an official definition, but used in the following way at the Argentum VI conference):
"Metal sulfide complexes or clusters that are resistant to oxidation and may persist for a day or longer."

Microcosm study

A laboratory test in a controlled environment with limited chemical and biological species and number of organisms; a small ecosystem that is regarded as miniature or epitome of a large world.

MM Molecular mechanics

Use of the equations of classical mechanics to describe the potential energy surfaces and physical properties of molecules.

MS Mass spectroscopy

An analytical technique used to identify unknown materials. Positive and negative ions are produced from nearly organic, organometallic or polymeric compounds. The mass/charge ratios of these ionized species and fragments of them are measured in the mass spectrometer. The resulting spectra provide molecular weight information and/or structural information about the fragments which can be used to identify the parent material.

MT Metallothionein

An important cell complex consisting of about 20 cysteine molecules which can bind Group B metals, such as silver, strongly (also see Metallothionein induction).

MW Molecular weight

The sum of the atomic weights of all the atoms in a molecule.

N**NOM Natural organic matter**

Nonspecific term to denote the total organic matter found in an aquatic system. NOM would include dissolved organic matter. Often expressed as mg (dry weight) per liter (mg/L).

NOAEL No-observed-adverse-effect level

The highest concentration of chemical agent being tested which does not produce an adverse effect in the organism undergoing the test.

NOEC **No-observed-effect concentration**

The highest concentration of chemical agent being tested which produces no observed effect, either adverse or non-adverse, in the organism undergoing the test. In a full- or partial-life-cycle test, the highest toxicant concentration in which the values for the measured response are not statistically significantly different from those in the control (ASTM).

NOEL **No-observed-effect level**

same as NOEC

NPDES **National Pollutant Discharge Elimination System**

A permit program that implements the Clean Water Act prohibitions against unauthorized discharge from a point source to waters of the U.S. A permit is required for every discharge of pollutants.

O**Oxic water column**

Condition in a water body where the dissolved oxygen concentration in the overlying water is greater than zero.

P**PC** **Phytochelatin**

Natural (including thiols) chelates found in aquatic plants and simple plants such as algae. Phytochelatins have properties similar to metallothionein and bind silver strongly.

Percentile

The probability, expressed in terms of percent, that a random variable will assume values less than or equal to a given value.

POC **Particulate organic carbon**

That organic carbon contained in the particulate fraction of a sample, as defined by being separated from the dissolved fraction by 0.45 μm filtration.

Point source

(as defined by section 502(14) of the Clean Water Act) A discernable, confined and discrete conveyance from which pollutants are or may be discharged. The term does not include agricultural stormwater discharges and return flows from irrigated agriculture.

POM **Particulate organic matter**

The particulate fraction of organic matter that is retained by a 0.45 μm filter. Usually expressed as milligrams of dry solid per liter of sample (mg/L).

Pore water/interstitial water

Water contained within the interstices of particulate material that is present in benthic sediments.

POTW **Publicly owned treatment work**

Wastewater treatment facilities that are owned and operated by municipalities. Typically wastewater is primarily domestic in origin, but the contribution from industrial sources is highly variable among all plants.

Priority pollutants

Those pollutants listed by the USEPA Administrator under Section 307(a) of the Clean Water Act.

Probability distribution

As used herein, a cumulative distribution function (CDF) that provides a particularly useful way to describe the likelihood that a variable will take on a value within prescribed limits. The value of the CDF at any point is the probability that a variable will have a value less than or equal to the associated x-axis value. The CDF is the mathematical description of the function relating probabilities with specified intervals to values, for a random variable. Ranking the values for the variable of interest, from lowest to highest, and plotting these values versus the cumulative percentile, the cumulative probability of occurrence, forms the CDF.

Problem formulation

The first phase in the risk assessment process where the assessment purpose is stated, the problem defined and the plan for analyzing and characterizing risk determined.

R**Receptor**

The individual (human or otherwise) for whom exposure is assessed; the organism that is exposed to the chemical of concern at the site.

Reconstituted water

see Synthetic water

Reverse osmosis

Process to produce ion-poor water or to concentrate solutes from water, by passing it through finely porous membranes under a high pressure greater than the normal osmotic pressure. While allowing passage of small nonpolar molecules roughly equivalent in size to water molecules, it removes a substantial percentage of monovalent polar ions and is especially effective in removing di- and trivalent polar ions, larger molecules, and suspended particles larger than 0.001 micron.

RfD **Reference dose**

The dose of the chemical of concern that is used as a basis of comparison for determining an acceptable level of exposure.

Risk

The probability of deleterious health or environmental effects.

Risk assessment

A set of formal scientific methods for estimating the probabilities and estimated magnitudes of undesired effects resulting from the release of chemicals, other human actions, or natural catastrophes. Risk assessment includes quantitative determination of both exposure and effects.

Risk characterization

This step in the risk assessment involves the integration of the exposure and effects profiles to estimate the likelihood of adverse ecological effects. It includes the description of the nature and magnitude of human or nonhuman risk, including associated uncertainty.

Risk estimation

An estimate of risk taking into account probability of occurrence, dose or exposure, and anticipated response.

S**SBAF** **Sediment bioaccumulation factor**

see BAF

Screening analysis

An approach which uses health-conservative default assumptions in the representation of variability or uncertainty in parameters and modeling. Used to identify low risk situations that do not require further detailed evaluation.

Screening-level risk assessment

A preliminary risk assessment that is performed using conservative assumptions in order to rule out those exposure scenarios where there is clearly a low level of risk to the target organism. Typically, in cases where the potential for significant adverse effects are observed in the screening level assessment, more refined analyses are performed.

SEM Simultaneously extracted metal

Operationally defined as metals extracted from sediment into solution by the AVS extraction procedure.

Silver-Gill Ligand Model

A computational framework that may be used to predict the level of accumulation of silver on the gill of a fish based on the water quality characteristics of the water body and the level of silver that is dissolved in the water.

SMAV Species mean acute value

As used in the nationally recommended aquatic life criteria methodologies (Stephan et al. 1985), the geometric mean of all the reported effect levels (available LC50 or EC50 values) of a chemical to a given living species.

SMCV Species mean chronic value

Same as SMAV above, except using chronic rather than acute effect levels.

Speciation

The different forms in which an element can exist, including different oxidation states as well as different ions or compounds of the element. Alternately, the description or categorization of the numerous chemical species that may form from the interaction between metals and ligands. Typically, chemical speciation is defined at thermodynamic equilibrium.

Species sensitivity distribution

A cumulative probability distribution of the species mean acute values for silver.

Spectrofluorometry

(now called Fluorescence or Luminescence spectroscopy) An analytical technique used to obtain quantitative and qualitative information about a class of materials which emit ultraviolet (UV) or visible (VIS) light when irradiated with ultraviolet light. The sample is held in a clear quartz/silica cell and irradiated with selected UV wavelengths. Luminescent or fluorescent materials emit light of higher wavelengths. The amount of light emitted is proportional to sample concentration. This technique is both selective and sensitive because not all materials fluoresce, and those that do emit light of specific wavelengths and produce unique emission spectra.

SQC Sediment quality criteria

Chemical-specific concentrations that are intended to be protective to aquatic life in benthic sediments.

Subchronic toxicity

Toxicity which may include growth and reproductive effects or enzymatic changes but not necessarily lethality.

Synthetic water

Water prepared for laboratory experiments such as bioassays, where natural or tap water is purified by chlorination/dechlorination, filtration, reverse osmosis, activated carbon or ion exchange treatments and/or the like, then "reconstituted" by adding back certain ions or ingredients thought to represent the natural world but excluding so-called "polluting" contaminants. Recent evidence shows that previous formulations for preparing synthetic waters may be deficient in that they have ignored certain ubiquitous substances found in almost all natural waters, including oxalic as well as anoxic, and are therefore necessary for accurate comparisons, such as sulfide, DOC, etc.

3-MPA 3-Mercaptopropionic acid

A thiol which binds silver strongly.

T**TMDL Total maximum daily load**

A written plan and analysis established to ensure that a water body or group of water bodies within a watershed will attain and maintain water quality standards. A TMDL includes wasteload allocations and load allocations, and a consideration of a margin of safety and seasonal variation.

TOC Total organic carbon

The amount of organic carbon measured analytically in a sample, usually expressed in milligrams of elemental carbon per liter. Thus $TOC = DOC + POC$.

Total silver

Measurement of all forms of silver in a sample regardless of their speciation, expressed as elemental silver.

Toxic mechanism

The physio-chemical process by which a chemical exerts a toxic effect on an organism.

Trophic transfer

The transfer of a contaminant through a food chain from the food organism to the consumer organism.

U**Ultra-clean sampling**

see Clean sampling methods

Ultrafiltration

A process for separating suspended particles in the size range of 0.001 to 0.5 microns, and solute molecules more than 10 times the size of the solvent molecule, from a liquid medium by passage through a finely porous filter under pressure. Ultrafiltration operates at lower pressure than that required by reverse osmosis.

Uncertainty

Lack of knowledge about the "true" value of a quantity or of the characteristics of the probability that should be used to represent a quantity of interest.

Uncertainty analysis

This analysis is performed to evaluate the sensitivity of the results to the uncertainty associated with the assumptions that are made in the analysis.

UV-VIS **Ultraviolet-visible spectroscopy**

An analytical technique used to obtain quantitative and qualitative information about materials which absorb incident ultraviolet (UV) or visible (VIS) light. The sample is held in a clear quartz/silica cell and irradiated with selected wavelengths of ultraviolet or visible light. The amount of light absorbed is proportional to sample concentration. Although not particularly sensitive, this technique is useful since the vast majority of organic materials absorb UV light and often produce unique absorption spectra.

V**Variability**

Heterogeneity of values over time and space or among different members of a population.

Vitellogenin

A yolk protein produced by egg-laying organisms, found in the blood, that is used for oocyte yolk synthesis; also synthesized by the fat body of insects and the liver of vertebrates. Its detection in male organisms is commonly used as a biomarker of exposure to estrogenic chemicals.

W**WER** **Water-effect ratio**

The ratio between the toxicity of a pollutant in site water compared with laboratory or reference water as determined, for example, by the ratio of LC50 values. WERs are used to calculate site-specific criteria concentrations.

WET **Whole effluent toxicity**

The aggregate toxic effect of an effluent itself or in a receiving water, measured directly by a toxicity test. Tests with aquatic organisms are generally performed using various percentages (0 to 100%) of the effluent from a discharge mixed with water from the receiving body.

WQC **Water-quality criteria**

Scientifically derived ambient limits developed and updated by USEPA, under section 304(a)(1) of the Clean Water Act, for specific pollutants of concern. Criteria are recommended concentrations, levels or narrative statements that should not be exceeded in a water body in order to protect aquatic life or human health.

WQS **Water-quality standard**

A law or regulation that consists of the beneficial designated use or uses of a water body, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular water body, and an antidegradation statement (USEPA 1991).

WWTP **Wastewater treatment plant**

see POTW

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Note: In several terms above, where slightly varying versions were being used by different scientific disciplines or publications, the editors have combined the definitions from several sources into a single definition. In a few instances the editors have supplied the definition where one had not been furnished by the authors or found in standard reference texts.

Silver in the Environment: Transport, Fate, and Effects

Edited by Anders W. Andren and Thomas W. Bober

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