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Anders W. Andren
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ARGENTUM
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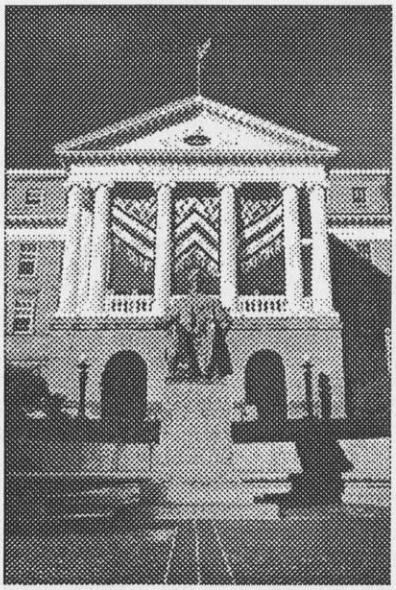
Welcome to MADISON



It is a pleasure to welcome Argentum VI partcipants to the University of Wisconsin-Madison again, the site where the Argentum international silver conference series originated and where three of our past five conferences have been held. This year's meeting may indeed represent the last of our regular Argentum series. In keeping with that sobering thought, our format this year is a substantial change from past conferences: instead of platform presentations on original scientific work, we have chosen a workshop agenda whereby the information gathered on silver over the past decade can be distilled into meaningful findings and recommendations by experts with long-term experience in these subjects. We are continuing to advance the release of new scientific information through additional poster presentations.

This year the University of Wisconsin is proud to be celebrating its sesquicentennial anniversary. In the 150 years since its founding, it has undergone a drastic transformation from a land-grant agricultural college to a major university, enjoying a world-class reputation as a pioneer in many academic fields. Of particular pride are accomplishments in the areas of environmental and multidisciplinary research.

Though the majority of environmentally related research is carried on in traditional departments, many such efforts on campus are focused and supported in the Water Chemistry Program, Center for Limnology, Water Resources Institute, Environmental Toxicology Program, the Institute for Environmental Studies, and the Sea Grant Institute. These programs all strive to put the "Wisconsin Idea" into action. That is the concept that the University serves as the research arm of the State, and as such the faculty and staff seek to quickly transfer new technologies and information to user groups. These include federal, state and local governments, industry, advocacy groups, and the general public.

In the eight years since the Argentum conferences were conceived, great strides have been made in our collective worldwide understanding of silver behavior. Some past laboratory studies had been faulted because they were designed without considering all the variables that truly influence the metal's behavior in nature. When attempts were made to extrapolate results from such laboratory simulations to the more complex natural environment, often the data did not seem to fit with actual tests and measurements made in the field. Field sampling and analytical procedures were likewise subject to criticism and review.

Of particular benefit to the international silver effort, as exemplified in this conference series, has been the gathering of scientists from various disciplines who can critique each other's project proposals and data from their own viewpoint, thus bringing valuable cross-disciplinary insight to efforts that otherwise might have been conducted strictly within the confines of a single discipline. This wholistic approach produces better experimental designs that yield more universally acceptable information, often at lower cost and without need to engage in peripheral supporting studies. It has helped eliminate the confusion that resulted from past conflicting data on silver, which made it difficult to adopt reasonable environmental standards. The advent of the new "clean" sampling and monitoring procedures has also greatly revised past thinking regarding concentrations of trace metals in the environment. These successes over the past 10 years have resulted in better and more reasonable dialogue between researchers, regulators and the regulated community. We hope to continue that process through this sixth conference.

On behalf of the organizing committee, we welcome the panelists, poster presenters and general attendees, including new faces as well as many old friends and colleagues from previous meetings. We hope you will derive benefit from the conference as well as enjoying the University and the many amenities of the Madison area.

ACKNOWLEDGEMENTS

The editors wish to note particularly the special contributions of Mr. Joseph W. Gorsuch of Eastman Kodak Company, Rochester, N.Y. and Prof. James R. Kramer of McMaster University, Hamilton, Ont., Canada, who were two of the originators in setting up the workshop format for this particular Argentum VI conference that proved to be so successful. They also made important contributions in formulating the original questions to be raised, offering valuable technical comments, critiquing the final chapters and suggesting possible changes, and helping see this project through to its conclusion.

Thanks are also due to the rapporteurs who with great dedication wrote the chapter texts and rewrote them following critiques by their fellow authors: Drs. Gaboury Benoit, Christer Hogstrand and Thomas LaPoint, and Messrs. James Pendergast and Paul Paquin. We also wish to thank Drs. Russell Bell, Peter Campbell, George Luther, Richard Playle, Chris Wood, David Armstrong, Ms. Jennifer Mitchell and Messrs. Norman LeBlanc and Kevin Brix for their invaluable postconference assistance in producing this book.

We further want to acknowledge all the distinguished and talented authors named herein who contributed their time and efforts in order to turn out a state-of-the-art technical treatise on silver in the environment, a subject that had been only cursorily addressed before this conference series began.

Also we recognize with gratitude the special assistance of the University of Wisconsin Sea Grant Institute editorial staff, including Assistant Director for Communications Stephen Wittman, Editor Elizabeth A. White, and Assistant to the Director Delphine Skinner.

Finally, we wish to thank the sponsors of this conference series and the post-meeting follow-up: the University of Wisconsin Sea Grant Institute (Madison, Wis., USA); the U.S. Environmental Protection Agency (Washington, DC, USA); Eastman Kodak Company (Rochester, N.Y., USA); McMaster University (Hamilton, Ont., Canada); Institute for Ecotoxicology, Lund University (Lund, Sweden); Photo Marketing Association International (Jackson, Mich., USA), and Photographic and Imaging Manufacturers Association, Inc. (Harrison, N.Y., USA).

Anders W. Andren
Thomas W. Bober
Editors

INTRODUCTION

Significant advances have been made in our 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, physicochemical forms, adsorption/desorption reactions, toxicology, bioaccumulation, influence of ligands, and the 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. In this book we present a synthesis of this progress on silver research in terms of its environmental chemistry, effects to biota, biological processes, risk analyses and current regulatory practices.

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 PIMA (Photographic and Imaging Manufacturer's Association, previously known as the National Association of Photographic Manufacturers, or NAPM).

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, industrial funding for silver research was used to expand ongoing efforts with other metals within the Wisconsin Department of Natural Resources. USEPA laboratories at Duluth and Narragansett 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 has also been performed by numerous universities, industries, and research institutions ranging from Scandinavia and the United Kingdom to Central Europe to North America. Most recently, funding has been awarded to Canadian and U.S. researchers through WERF (Water Environment Research Foundation).

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 three to four orders of magnitude less acutely toxic to aquatic organisms than fully dissociated ionic silver, although ionic silver (in the form of silver nitrate) has typically been used in most laboratory toxicological studies. At the same time, there were observed instances of bioaccumulation of silver in some biota, studied in natural as well as laboratory environments. Silver thus 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, 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 per 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, Wisconsin, Washington, DC and Hamilton, Ont., 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 (see *www.seagrant.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 physicochemical forms of silver in natural waters had enabled the scientific community to re-examine the environmental chemistry of silver, especially with respect to complexation. It was evident the 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 limited Argentum panel discussions had been useful but many participants agreed that insufficient time was 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.

The groups would meet individually, and also jointly once each (due to limited time) with every other group to debate points of common interest, in a "round-robin" arrangement. 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 edification of all, and to receive suggestions and criticisms.

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?

It was decided to form five groups of scientists, the first four meeting in the "round-robin" sequence and members of the fifth group free to circulate as observers among the other four groups. 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 effects of silver in marine/freshwater aquatic, sediment, 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 others: 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., $AgNO_3$, 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 BLM (biotic ligand model) 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" vs. filtered (size) vs. 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 Effects 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 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 observed 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 DOM (dissolved organic matter) 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 Web site. Editors, moderators, rapporteurs and several senior authors reassembled in February 2000 to resolve remaining text questions, with the results again posted to the Web site for further editing. Peer review and final editing produced the following compilation.

Synopsis

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 dissolved organic matter under typical, natural environmental conditions, in oxic as well as anoxic waters. We believe the time has now come to examine previous conclusions regarding metal behavior and toxicity. The mechanisms of chronic toxicity need further exploration. Additionally, laboratory toxicological practices now need to be reexamined by the regulatory and research communities in light of these new findings. We hope that this 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
Madison, Wisconsin

Thomas W. Bober
Rochester, New York

Panel Discussion

Transport, Fate and Effects of Silver in the Environment

Madison, Wisconsin, USA

Chapter 1
Group A Discussion
Environmental Chemistry of Silver

Moderator:
James R. Kramer

Rapporteur:
Gaboury Benoit

Panelists:
Karl C. Bowles
Dominic M. DiToro
Russell T. Herrin
George W. Luther III
Helen Manolopoulos
Kenneth A. Robillard
Martin M. Shafer
Joseph R. Shaw

Chapter 1
Group A Discussion

Environmental Chemistry of Silver

James R. Kramer (*moderator*), Gaboury Benoit (*rapporteur*), Karl C. Bowles, Dominic M. DiToro, Russell T. Herrin, George W. Luther III, Helen Manolopoulos, Kenneth A. Robillard, Martin M. Shafer, Joseph R. Shaw

1.1 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. Future research on the bioavailability and bioreactivity of silver needs to take into account this central fact.

1.2 ABUNDANCE OF SILVER IN AQUEOUS SYSTEMS

1.2.1 Introduction

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 1.2.1). 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-eighties must be considered suspect. Application of new analytical detection technologies, clean techniques, and, most importantly, the recognition that silver levels could be much lower than previously thought have driven the recent advances.

1.2.2 Total Levels in the Water Column

Many important conclusions can be drawn from the compilation of silver levels shown in Table 1.2.1: (1) silver is found in the environment in aqueous solution at picomolar to low nanomolar levels (1 - low 100s of ng/L); (2) even in highly impacted systems, total silver levels rarely exceed a few nM; (3) background levels of total silver in seawater range from 1-20 pM and in freshwater 5-50 pM.

Effluents from publicly-owned treatment works (POTW) 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 rapidly reduced 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.

Table 1.2.1- 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 [1]	<0.24 - 5.6 [2, 3, 4]
Open Ocean (Depth > 1 km)			10 – 23 [1]	1.9 - 54 [2, 3, 4]
Estuaries and Bays	3.2 –307 [5, 6, 7, 8]	8.1 - 1000 [4, 5, 8]		5.8 [4]
Coastal Waters	39 [7]		3 - 11 [6, 7]	4 - 17 [7]
Rivers	<0.1 – 2900 [5, 9, 11, 12, 13]	<0.1 - 4600 [5, 9, 11, 12, 13]	<0.1 – 480 [5, 10]	<0.1 - 1400 [5, 10]
Laurentian Great Lakes				2 - 5 [10]
Other Lakes			40 [12]	67 [12]

1. Martin *et al.* 1983.
2. Murozumi 1981.
3. Flegal *et al.* 1995.
4. Bloom and Crecelius 1984.
5. Wen *et al.* 1997b.
6. Miller and Bruland 1995.
7. Sanudo-Wilhelmy and Flegal 1992.
8. Benoit *et al.* 1994.
9. Benoit 1994.
10. Shafer 1995.
11. Shafer *et al.* 1998.
12. Kramer *et al.* 1999.
13. Benoit and Rozan 1999

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 three orders of magnitude higher than background levels in streams.

1.2.3 Particulate Silver

Silver is an extremely particle-reactive metal. Distribution coefficients (K_d) based on filtrate and particulate silver concentrations (Table 1.2.3) are typically $10^{4.5}$ - 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 1.2.3 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 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 - 2 nano-mol/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.

Table 1.2.3 Distribution coefficients and concentrations (on a per mass particle basis) for silver in different systems.

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

1. Benoit *et al.* 1994
2. Wen *et al.* 1997b
3. Shafer *et al.* 1998
4. Benoit and Rozan 1999

Surface complexation of silver is not limited to macroparticles (i.e., >0.4 μm). 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 and coauthors (1997a, 1997b) showed that virtually all silver in ultrafiltration (UF) retentates from the Trinity River and Galveston Bay 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 percent) 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.

1.2.4 Silver in Sediments

Table 1.2.4 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

Table 1.2.4. Partial survey of the literature on silver in sediments

Maratoto (New Zealand)	stream	0.75 - 7.0	Ward *et al*. (1977)
South Platte River (WY, NB, CO)	river	0.2 - 5.1	Heiny & Tate (1997)
Hanover Pd. (CT)	river impoundment	9.4 - 282	Benoit & Rozan (submitted)
St. Lawrence R. (Canada)	river - estuary	0.04 - 1.1	Gobeil (1999)
Quinnipiac R. Marsh (CT)	salt marsh	0.1 - 31.6	Rozan & Benoit (1999)
Puget Sound (WA)	estuary	0.015 - 0.71	Bloom & Crecelius (1987)
Long Island Sound (CT - NY)	estuary	0.006 - 25	Robertson *et al*. (1991)
San Francisco Bay (CA)	estuary	0.09 - 1.10	Luoma *et al.* (1995)
San Francisco Bay (CA)	estuary	0.04 - 0.83	Thompson *et al*. (1999)
Jordan Cove (CT)	estuary	0.06 - 1.62	Benoit *et al.* (1999)
New Haven Harbor (CT)	estuary	0.06 - 33.9	Rozan & Benoit (submitted)
Brisbane R. (Australia)	mangrove swamp	<0.6 - 2.8	Mackey & Hodgkinson (1995)
Massachusetts & Cape Cod Bays	coastal	0.03 - 6.7	Ravizza & Bothner (1996)

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 μg/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 μg/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.

1.3 SPECIATION

1.3.1 General Comments

Silver is a class B, soft metal[1] that preferentially forms complexes with sulfur(-II)-containing ligands. Silver can be expected to bind most strongly to S- and I-containing ligands and more weakly to N- and O-containing ones. The importance of this is highlighted in Table 1.3.1, which lists the strength of silver complexes with sulfur and other common ligands.

Table 1.3.1: Formation constants for silver complexes (Ag:L = 1:1) with various ligands

Inorganic sulfides	log K = 14 – 21	Refs. A, B
Organic sulfides (thiols)	log K = 12 – 15	Refs. C, D
Thiosulfate	log K = 8.2	Ref. C
N (NH_3 and amino)	log K = 3 – 6	Ref. C
I^-	log K = 6.6	Ref. C
Cl^-	log K = 3	Ref. C
O (carboxylates)	log K = < 2	Ref. C

References: A: Schwarzenbach and Widmer 1966; B: Cloke 1963; C: Smith and Martell 1997; D. Adams and Kramer 1998a. 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.

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; Kramer *et*

[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.

al. 1999; Benoit and Rozan 1997; Rozan *et al.* 1999; Theberge *et al.* 1997)[2]. Nanomolar amounts of reduced sulfur have been found in virtually all surface waters measured with suitably sensitive methods (see Table 1.3.4). The total number of sites tested is not large, but they represent a wide variety of water bodies. Furthermore, three independent analytical methods have been used: gas chromatography, voltammetry, and classical colorimetry (Cline 1969; Radford-Knoery and Cutter 1994; Rozan *et al.* 1999). 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 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 2.3.4)[3]. Measurements show that, in waters ranging from pristine streams to POTW outfalls, the reactive S(II-) concentrations typically exceed silver by three orders of magnitude (Rozan submitted; Kramer *et al.* 1999; Manolopoulos, McMaster University, personal communication; Shafer, 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(s) that are biologically available (see section 1.4). 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, 1998; 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

[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 dissolved organic matter.

[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, we henceforth refer to it as "acid-reactive sulfide," or ARS.

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 (Bell and Kramer 1999; Alberto *et al.* 1996). 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.

1.3.2 Details of Silver - Sulfide Interactions

The widespread occurrence of reduce sulfur in surface waters, and the extraordinarily high affinity of silver for reduced sulfur, imply that most aquatic silver occurs bound to sulfur. Nevertheless, specific silver-sulfur species have not been measured in nature, in part because silver generally occurs as 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 buildings 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 "bio-clusters" in laboratory experiments.

1.3.2.1 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 ($k_f > 10^8\ M^{-1}s^{-1}$) 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 $Zn_4S_6^{4-}$ is the major species (more specifically, the hydrated form $[Zn_4S_6(H_2O)_4]^{4-}$. This cluster can be a precursor to mineral formation or alternatively can form as a consequence of mineral dissolution. It has now been shown that both ZnS and CuS clusters exist in natural freshwaters (Theberge *et al.* 1999) 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 may also 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).

1.3.2.2 The Relationship of Zinc Sulfide Clusters with Silver

Freshwater and open ocean environments that contain fully saturated oxygen levels (Rozan *et al.* 1999; Theberge and Luther 1997; Theberge *et al.* 1997) 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 (Kramer *et al.* 1999b; Kuwabara and Luther 1993; Luther and Tsamakis 1989; Radford-Knoery and Cutter 1994; Rozan *et al.* 1999; Theberge *et al.* 1997), 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 (unpublished) 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 ~ 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:

$$Ag^+ + OH^- + HS^- = AgS^- + H_2O; \quad \log K = 22.8 \qquad \text{(eq.1)}$$

$$2\,Ag^+ + OH^- + HS^- = Ag_2S + H_2O; \quad \log K = 27.2 \qquad \text{(eq.2)}$$

Mass spectrometry 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, we compare complexes of similar stoichiometry. The AgS^- constant (Rozan and Luther, unpublished) 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 Zn^{2+} 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).

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 two-coordinate rather than four-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 Zn^{2+} 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 ~ 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 ever 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 and coworkers (1997a, 1997b) 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.

1.3.3 Levels of Sulfide in the Water Column

We can state with a reasonable degree of confidence across a range of aquatic environments that levels of acid-reactive, metastable sulfide (ARS; Cline 1969) nearly always exceed those of filtrate silver

(Table 1.3.4). 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 a hundred to a thousand times greater (Table 1.3.4).

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.

Table 1.3.3 - Summary of dissolved silver and ARS measurements for a range of surface water bodies and POTWs. ARS is typically three orders of magnitude greater than silver.

Ag (nM)	MBMS (nM)	log([MBMS]/[Ag])	Site	Comment	Reference
0.042	485	4.1	Cobalt, ON	river, max	(1)
<0.003	18	>3.8	Cobalt, ON	river, min	(1)
0.29	< 1	<0.5	Cobalt, ON	river	(1)
<0.003	<1	--	Cobalt, ON	tailings	(1)
0.49	<1	<0.3	Cobalt, ON	seep, max	(1)
0.18	240	3.1	Cobalt, ON	seep, min	(1)
<0.003	<1 - 190	--	Cobalt, ON	background	(1)
0.79	250	2.5	Dundas, ON, POTW	secondary clarifier	(2)
0.78	230	2.5	Dundas, ON, POTW	effluent	(2)
0.51	155	2.5	Dundas, ON, POTW	250 m downstream	(2)
0.45	137	2.5	Dundas, ON, POTW	500 m downstream	(2)
--	10 - 20	--	Sava R., Slovenia		(3)
--	30 - 60	--	Fort Erie, ON		(3)
--	30 - 60	--	Niagara on the Lake, ON		(3)
.024 - .057	34 - 78	3.2	Hammonasset R., CT	no POTW	(4)
.019 - .045	16 - 50	3.1	Naugatuck R., CT	above POTW	(4)
.21 - .80	59 - 104	2.3	Naugatuck R., CT	below POTW	(4)
.037 - .095	44 - 85	3.1	Quinnipiac R., CT	above POTW	(4)
.18 - .28	59 - 100	2.5	Quinnipiac R., CT	below POTW	(4)
.007 - .033	31 - 112	3.6	Pawcatuck R., RI	below wetlands	(4)
.005 - .02	1.2 – 6.1	2.4	9 lakes, WI, MN, MI		(5)
.005 - .04	1.1 – 19	2.7	9 rivers, WI, MN, MI		(5)
0.4 – 2	51 - 110	1.9	6 POTWs, WI		(5)

(1) Kramer *et al.* 1999.
(2) Adams and Kramer 1999b.
(3) Manolopoulos, H., personal communication.
(4) Rozan 1998 (6 sampling dates).
(5) Shafer 1999, unpublished data.

1.3.4 Macroparticles and Colloids

Due to its particle reactivity, silver occurs in natural waters predominantly bound to macro-particles (>0.45 μm) and colloids (<0.45 μm) (Shafer *et al.* 1996; Wen *et al.* 1997b). Of the so-called "dissolved" silver species (< 0.45 μm) it is now believed that colloidal particles predominate over truly dissolved species in fresh waters (Wen *et al.* 1997a; 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; Shafer *et al.* 1998; Wen *et al.* 1997b; Pham and Garnier 1998). The importance of inorganic cluster compounds should not be overlooked. (See section 1.3.2, "Details of Silver-Sulfide Interactions."), although the presence of silver or zinc sulfide clusters in Fe- 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 kDa) membrane filters (M. Shafer, 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 kDa to > 0.45 μm. 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.

1.4 BIOLOGICAL IMPLICATIONS

1.4.1 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:

$$Ag\text{-}S_1 + S_2 = S_1 + Ag\text{-}S_2 \qquad \text{(eq. 3)}$$

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 S1 and S2 and the activities (concentrations) of S1 and S2. 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 1.4.1). Of these, GSH and MT are two of the most prominent. MTs are a class of sulfhydryl-rich proteins that are thought to play a major role in the metabolism/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 Shäffer (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 (γ-glutamylcysteinylglycine) is commonly found within the cells of most tissues at high concentrations, 5-10 mM. 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-GSH complex is 1:1 (R. 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 bioreactive[4] 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/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 (Schmittschmitt *et al.* 1996; Fischer and Hook 1997; Bielmyer *et al.* 1999). PCs are derived from GSH via enzymatic addition(s) of a γ-glutamylcysteine moiety to glycine to yield $(\gamma\text{-glutamylcysteine})_n$-glycine (n=2-11). 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 three days (Maitani *et al.* 1999). The most abundant PC species was $(\gamma\text{-glutamylcysteine})_4$-glycine, and $(\gamma\text{-glutamylcysteine})_5$-glycine was also detected. These PCs would bind 4 and 5 atoms of Ag(I) per molecule, respectively.

Exchange reactions, as in eq. (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 $Ag_2S(s)$. In this experiment, silver equilibrated with FeS to form Ag_2S was spiked with 0.1 and 1 mM 3-MPA, and silver was

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

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 is also 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.

Table 1.4.1. 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.* 1999.
[c]Adams and Kramer 1999a.
[d]Mason and Jenkins 1995.

1.4.2 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, carbon filtration, or 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 $AgHS^{\circ}$(Shaw *et al.* 1997). These tests were conducted in seawater using three marine fish species, the inland silverside, topsmelt, and sheepshead minnow. For all species tested, thiols reduced acute silver toxicity. Survival approached that of controls when silver and 3-MPA were present in equimolar concentrations, and it was reduced when 3-MPA concentrations were reduced. 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 $S(II^-)$) remains unknown. This represents perhaps the greatest gap in our knowledge of the effects of silver on aquatic organisms in natural environments.

1.4.3 Proof of principle experiment - silver sulfide binding

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

$$[ARS] > 2[Ag] \qquad \text{(eq. 4)}$$

then no toxicity should occur if the resulting silver sulfide complex (Ag_2S[aq]) is not bioreactive. This is the analogous situation to the sediment toxicity prediction that no toxicity occurs if

$$[AVS] > 2[SEM\ Ag], \qquad \text{(eq. 5)}$$

where AVS is acid volatile sulfide and SEM Ag is the amount of silver that is extracted simultaneously. The reason in both cases would be the formation of Ag_2S(aq) complexes in aqueous solutions and

Ag_2S(s) (acanthite) in sediments. The latter is known to be nontoxic. It is the purpose of the proposed experiments to test the toxicity of Ag_2S(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 1.4.3a-d and consists of both negative (no Ag(I)) and positive (nonsulfide-bound Ag(I)) controls and two treatment regimes of Ag_2S(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 two-step process. First, the sulfide will be stabilized in solution using zinc to form zinc-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 Ag_2S(aq). The two Ag_2S(aq) treatments are designed to produce varying excess concentrations of nonsulfide-bound Ag(I) that bracket the predicted 50-100% mortality concentrations in tests without sulfide. Therefore, if Ag_2S(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.

Table 1.4.3a – Proposed experimental design

Treatment	1					
Ag (nM)	0	1	2	5*	10	20
Ag (µg/L)	0	0.1	0.2	0.5*	1.0	2.0
S(-II) (nM)	0	0	0	0	0	0
Zn^{2+} (nM)	0	0	0	0	0	0

* Estimated LC50 for *Ceriodaphnia*, 48-h exposure

Table 1.4.3b – Proposed experimental design

Treatment	2						
Ag (nM)	0	1	2	5	10*	20	100
Ag (µg/L)	0	0.1	0.2	0.5	1.0*	2.0	10.0
Excess Ag (nM)	0	0	0	0	10*	40	90
S(-II) (nM)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Zn^{2+} (nM)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Zn^{2+} (µg/L)	0.6	0.6	0.6	0.6	0.6	0.6	0.6

* Predicted LC50-100 for *Ceriodaphnia*

Table 1.4.3c – Proposed experimental design

Treatment	3						
Ag (nM)	0	10	20	50	100*	200	500
Ag (µg/L)	0	1.0	2.0	5.0	10.0*	20.0	50.0
Excess Ag (nM)	0	0	0	0	50*	150	450
S(-II) (nM)	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Zn^{2+} (nM)	50.0**	50.0	50.0	50.0	50.0	50.0	50.0
Zn^{2+} (µg/L)	3.0**	3.0	3.0	3.0	3.0	3.0	3.0

* Predicted LC100 for *Ceriodaphnia*

**Concentration is well below the LC50 for *Ceriodaphnia*

Table 1.4.3d - Measurements to be made in each exposure chamber. All samples filtered (0.45 mm). Sampling and analysis to be done prior to the start, at the water renewal, and after termination.

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)

1.5 ANALYTICAL METHODS

1.5.1 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 (APDC) or diethylammonium diethyldithiocarbamate (DDDC) extraction, as does ICP-MS where ambient silver levels are below 10-

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 mass spectrometry instrumentation (e.g., multicollector magnetic sector, ICP-MS, and laser ablation, LA-ICP-MS), coupling chromatography to mass spectrometry (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.

1.5.2 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. We can make no blanket recommendation other than that 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.

1.5.3 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. It is therefore 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 suggests 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 percent of filtrate silver and can represent all detectable silver in the filtrate fraction.

1.5.4 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 application of three 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 (Shafer M., 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 metal 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 were reported to be in the 1-5 nM range as late as the mid-seventies, where we now measure just a few pM. 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/controlling the more random contamination events for a particular metal is harder to quantify. Therefore the philosophy (particularly in multi-element 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 multi-acid cleaning procedures for sample bottles and filters may not be required. Relaxing strict clean sampling protocols may also be possible in certain situations. However, any such variation from comprehensive clean procedures must be thoroughly evaluated to minimize contamination biases.

1.5.5 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 percent or more of available silver. These findings are consistent with the work by Wen and coauthors (1997a) who showed that silver in samples stored unacidified for two 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.

1.5.6 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 our recommendation 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 our current stage of knowledge, the potential benefits. If, however,

future work demonstrates that colloidally associated silver within well-characterized physical/chemical fractions is or is not bioavailable, then this recommendation should be reconsidered. Using analogous reasoning, we conclude that there is no compelling reason to alter the current 0.45 μm operational cutoff for macrofiltration.

1.5.7 Sulfide Determination

We hypothesize 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. We recommend 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 (section 1.3.4). A range of simple thiols, however, are measurable via the methylene blue method when samples are first preserved/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.

1.5.8 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). Sediment-bound sulfide that reacts with cold 1 M HCl is termed acid volatile sulfide (AVS) and releases sulfide from FeS but not from FeS_2. When the metal that is simultaneously extracted by acid (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 (USEPA DATE; Ankley *et al.* 1996) 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. 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 two other approaches to the measurement of silver and sulfide that could circumvent these problems. First, 1 M H_2SO_4 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 two-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, which releases sulfide for measurement from both FeS and FeS_2 and silver associated with these phases. Another subsample can be analyzed by nitric acid digestion, which dissolves both FeS and FeS_2 (Howarth and Merkel 1984) and which will release 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.

1.6 CONCLUSIONS AND RECOMMENDATIONS

1. Silver can be assumed to be associated mainly with reduced sulfur ligands in almost all natural aquatic systems, both oxic and anoxic.
2. 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.
3. Laboratory experiments suggest that the kinetics of ligand exchange are rapid for these highly stable sulfide complexes.
4. Silver is associated with particulate and colloidal fractions ranging from less than 1 kDa to greater than 0.45 μm.
5. 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.
6. Speciation chemistry is critical to an understanding of chronic toxicity and must be considered in all toxicity tests.
7. Silver and reduced sulfides need to be measured on the same samples.
8. Sampling and analytical techniques need to be used that are appropriate to subnanomolar (<10 ng/L) silver and submicromolar sulfide concentrations.
9. Methods better than the AVS/SEM protocol are needed to measure silver and associated sulfides in sediments.
10. Analytical protocols to account for container sorption of sulfides need to be developed for samples stored more than two hours.
11. Acute and chronic toxicity tests involving silver sulfide complexes in the water phase and in sediments are urgently required.
12. Studies of the bioaccumulation of silver sulfides need to be initiated.
13. The interaction of NOM and sulfide clusters needs to be elucidated.

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Chapter 2
Group B Discussion
Biological Effects of Silver

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Biological Effects of Silver

Chris M. Wood (*moderator*), Thomas W. LaPoint (*rapporteur*), David E. Armstrong, Wesley J. Birge, Colin J. Brauner, Kevin V. Brix, Daniel J. Call, Eric A. Crecelius, Patrick H. Davies, Joseph W. Gorsuch, Christer Hogstrand, John D. Mahony, James C. McGeer, Thomas P. O'Connor

2.0 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) indicates sensitive species fall in the range of 0.85 to 3 μg/L (~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 μg/L (0.55 nM) total silver. The USEPA acute criteria for freshwater and marine dissolved silver concentrations are 3.43 μg/L (34 nM) (at a hardness of 100 ppm) and 1.92 μg/L (19 nM), respectively. Dissolved silver concentrations in U.S. publicly owned treatment works (POTW) effluents are frequently in the 0.1 to 1 μg/L (~1 to 10 nM) range (Shafer *et al.* 1998; Gill *et al.* 1997), 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 (~1 to 3 nM) dissolved silver (cf. Chapter 1). If this hypothesis for ubiquitous metastable sulfide is confirmed, then the acute water quality criteria 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 μg/L (2.1 to 68 nM) range (Hogstrand and Wood 1998). However, the chronic toxicity exposure systems were very likely conducted under laboratory conditions that eliminated metastable sulfide, as well as other naturally occurring ligands, from bioassay water and therefore 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.

2.1 APPROACH TO RATES OF SILVER EQUILIBRIUM IN NATURAL WATERS AND TOXICITY TESTING

2.1.1 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 is essentially added in its most toxic form. The added Ag(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 Ag(I) to these ligands. When conducting toxicity tests, if equilibrium of Ag(I) with silver-binding ligands is not achieved prior to test organism exposure, then the test may overestimate actual silver toxicity in the system. It is therefore 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 Ag(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 Ag(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 (Gustafsson and Gschwend 1997; Schwarzenbach *et al.* 1993; Tang *et al.* 2000; Wen *et al.* 1997). 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, and thus more sulfhydryl binding sites, than "older" NOM (such as might be derived from older terrestrial systems and lignin-containing plants) (Stevenson 1985; Steinberg and Muenster 1985; Guo and Santschi 1997; Guo *et al* 1999; 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 pre-equilibration time after addition of $AgNO_3$ 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 in which 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 would also 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 2.6). 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.

2.1.2. Research Considerations

Research is needed to:

a) identify the appropriate equilibration time between silver and all potential ligands, especially NOM, in toxicity tests,

b) understand differences among NOM types and their ability to complex with silver,

c) determine which ligand(s) is/are effective in reducing aquatic toxicity.

2.1.3 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.

2.2 EFFECTS OF SILVER ON AQUATIC AND TERRESTRIAL SPECIES

A major focus of the authors of this chapter concerned aquatic species' 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.

2.2.1 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, we focus on the state of knowledge regarding silver ecotoxicity via aqueous exposure. Dietary exposure is considered further in Chapter 3.

2.2.1.1 Acute Toxicity

The acute effects of waterborne silver on freshwater organisms have been reasonably well quantified with more than 40 organisms evaluated (U.S. EPA 1987; reviewed by Hogstrand and Wood 1998; Wood *et al.* 1999; Appendix Table 1). There is much less known about the acute effects of silver on biota in the marine environment although more than 25 organisms have been evaluated (Appendix 2.1 Table 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.

2.2.1.1.1 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 United States Environmental Protection Agency (USEPA) water quality criteria (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 µg/L (9.1 to 16,600 nM) across 41 species tested, including species tested since the 1987 USEPA data set (Appendix Table 1). In freshwater, acute species sensitivity distributions for silver are characteristic of most metals, with invertebrates more sensitive than vertebrates (i.e., fish) (Figures 2.1 and 2.2). Within the invertebrates, cladocerans and amphipods are more sensitive than are 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).

2.2.1.1.2 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 µg/L (143 to 29,000 nM) across 25 species tested (Figure 2.4; Appendix Table 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.

2.2.1.2 Chronic Toxicity

Chronic silver toxicity has not been extensively studied (as is also 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.

2.2.1.2.1 Chronic Toxicity: Freshwater

Only eight freshwater species have been evaluated for chronic silver toxicity (Figure 2.6; Appendix 2.1 Table 2). Species mean chronic values (SMCVs) range from 0.231 to 5.79 µg/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, embyro-larval studies with ten fish and amphibian species (Birge *et al.* 2000) provide supporting evidence that the limited chronic toxicity data set for silver does characterize the 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 is also 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 has clearly 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 one 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.

2.2.1.2.2 Chronic Toxicity: Marine

Only one invertebrate species, *Mysidopsis bahia*, (USEPA 1987) has been evaluated for chronic sensitivity to silver introduced as silver nitrate. Chronic values for *M. bahia* from early life cycle studies conducted in five 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 32-108 and 11-32 µg/L), respectively (or ~ 590 and 190 nM; 320-1080 and 110-320 nM, respectively). Similarly, the chronic effects of silver have been evaluated in only two marine fish species, the sheepshead minnow *Cyprinadon varigatus* and the summer flounder *Paralictys denatus* (Shaw *et al.* 1997). (Mean chronic toxicity value for sheepshead minnow was 449 microgram/L [95% conf. limits: 653 - 174]. This was calculated as the geometric mean of NOEC and LOEC for 28-day post hatch mortality and represents the mean of three tests. This is close to but less than the LC10 which should be a threshold.)

The 28-d 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% confidence limits: 410 - 660) and 1095 (95% confidence limits: 961 - 1210) µg/L, respectively. Using the same formula as that used for the sheepshead minnow above, the summer flounder chronic value would be less than 12 micrograms/liter.

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 µg/L (i.e., LC100).

These were 28-day post-hatch embro-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 µg/L; Cardin 1986). Studies evaluating the effects of silver on Na+/K+-ATPase activity in tidepool sculpins and plainsfin midshipmen, after being exposed to $AgNO_3$ (1.5 to 14.5 µg/L as silver) for 21 days demonstrated physiological mechanism disruptions (Webb *et al.* 2000).

2.2.1.3 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 sub-part-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.

2.2.1.3.1 Freshwater Studies: Regulatory Considerations

a. Based on available information, we recommend that the acute criterion be derived using the existing methodology after the toxicity database has been updated to include studies performed since 1987. We also recommend that implementation of the acute criterion include use of the BLM, as a site-specific modification (see Section 2.3.1), to reflect silver bioavailability in the aqueous environment more accurately.

b. 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. We recommend 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.

c. 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.

2.2.1.3.2 Freshwater Studies: Research Consideration

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

2.2.1.3.3 Marine: Regulatory Considerations

a. 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.

b. 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.

2.2.1.3.4 Food -Trophic Transfer Studies

Additional research is needed on food/trophic transfer versus waterborne as routes of silver exposure and uptake (See Section 2.5).

2.2.2 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.

2.2.3 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 (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 as a concern to the authors of this chapter, and thus not discussed further.

2.3 MECHANISMS OF ACUTE TOXICITY

2.3.1 The Biotic Ligand Modeling (BLM) Approach 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. 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.

BLMs 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^{++}, 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, a 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; Webb and Wood 1998; reviewed by Hogstrand and Wood 1998). This 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^{++}, a cofactor required for ATP hydrolysis (Ferguson and Hogstrand 1997).

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 should be also explored. It is assumed that if the mechanism of acute silver toxicity in saline waters is similar to that of fresh waters, the BLM model would apply. However, there is good evidence that the mechanism of silver toxicity in marine fishes 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 modeling 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.

2.3.2 Regulatory Considerations

a. The BLM should be used for site-specific modification of acute criteria in freshwater.

b. At the present time, the BLM should not be used for chronic criterion development. The possibility of alternate routes of exposure must be considered.

2.3.3 Research Considerations

a. The BLM needs further validation in a wider variety of water qualities and aquatic species.

b. There is a need to evaluate the potential applicability of the BLM in estuarine and marine systems.

2.4 MECHANISMS OF CHRONIC TOXICITY

2.4.1 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 models 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 water quality criteria for silver based on measured receiving water chemistry.

2.4.2 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 water quality criteria 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 yet be justified. Indeed, there are already some data (see below) 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 water quality criterion 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 (Rozan *et al.* 1999; Luther and Tsamakis 1989; Tang and Santschi 2000; see Chapter 1 of this volume), 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 - 300 ng/L (1 – 3 nM) range, the typical NOEC/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, we 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).

2.4.3 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; also see Chapter 3, this volume). 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 was also present from POTW wastewater discharges, comes from field studies on two marine bivalve species in the San Francisco Bay (see Chapter 3, this volume; Lee *et al.* 2000). 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 may also 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 – 96 hours, were fed to the planktonic organisms (Schmittschmitt *et al.* 1996; Fisher and Hook 1997; Hornberger *et al.* 1999; Bielmyer and Klaine 1999, and Chapter 3 this volume).

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 gets 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 fish 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 fish or amphibians (with the exception of unpublished mesocosm studies, described in Section 2.7).

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 - 2 μg/L [1 - 20 nM], added as $AgNO_3$) indicate that ionoregulatory disturbance occurs in very early life stages (Guadagnolo *et al.* 2000) 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 five-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 μg/L (10 nM) or greater (Guadagnolo *et al.* 2000). These observations on decreased growth are in agreement with the classic work of Davies *et al.* on rainbow trout (1978) 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) is 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; 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.

2.4.4 Research Considerations

a. 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.

b. A need exists to understand the mechanisms of toxicity stemming from the trophic transfer of silver.

2.5 SILVER BODY BURDEN

There are continuing disagreements concerning the importance or significance of body burdens of silver. On the subject of silver body burden (BB) in aquatic biota, there was agreement that silver residues in fish and invertebrates constitute evidence of exposure to bioavailable silver but not necessarily to bioreactive silver, nor does silver BB necessarily indicate deleterious effects on exposed organisms. The present procedure for developing regulatory criteria should not use silver BB 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 BB may be useful in: 1) distinguishing between bioavailable and non-bioavailable silver in point-source discharges, 2) confirming silver exposure of aquatic biota, and 3) estimating bioavailable Ag concentrations in the water column. Concerning the latter, silver BB 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 BB were also 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 BB measured in stoneroller minnows was in the range of 0.2 to 0.4 μg/g and calculated concentrations of bioavailable silver in suspended sediments ranged from 8 to 16 μg/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 BB 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 three-brood test with *Ceriodaphnia dubia*. Neonate production

was sharply reduced when silver BB 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 ug/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 µg/g silver. The authors concluded that this occurred because *C. dubia* avoided eating algae containing more than 1.3 µg/g silver (personal communication, J.R. Shaw, Univ. of Kentucky, April 28, 2000).

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 BB and reproductive performance deserves further attention. Further research on the physiological, toxicological, and ecological significance of silver BB 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 BB; 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.

2.5.1 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.

2.5.2 Research Consideration

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

2.6 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 three silver salts (AgNO3, Ag(S2O3)n, and AgCl) to *Hyalella azteca* in four different freshwater sediments. They found the AgNO3 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 AgNO3, whereas for AgCl the 10-d LC50 values were all >2,500 mg/kg, the highest concentration tested. For Ag2(S2O3)n, the 10-d LC50 values were >569, >648, >682, and >1,125 mg/kg (the highest concentration tested). Hirsch (1998a) found that Ag2S-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 Ag2S-amended freshwater sediment (Hirsch 1999b), the oligochaete worm *Lumbriculus variegatus* had an estimated silver bioaccumulation factor of 0.18 over a 28-d exposure. Call *et al.* (1999) obtained 10-d LC50 values of 2,750 and 1,170 mg/kg of Ag for *Chironomus tentans* tested with AgNO3-amended sediments from two lakes. In marine sediments, Berry *et al.* (1999) tested the amphipod *Ampelisca abdita* using AgNO3-amended sediments. In three different sediments, they observed high toxicity (i.e., 50-100 percent 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.

2.6.1. Application of the SEM/AVS USEPA Method

This method involves measuring the sediment-bound sulfide that reacts with cold 1 M HCl AVS while simultaneously determining the extracted metal (SEM). When [SEM minus AVS] ≤ 0, all metal is assumed to be bound as very insoluble metal sulfides, yielding negligible pore-water 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), Rodgers *et al.* (1997), Crecelius *et al.* 1997, Call *et al.* (1999), and Hirsch (1998b) 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).

2.6.2 Certain Cautionary Notes to the Application of SEM/AVS

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 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 (1998b) 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, are that the method relies on anoxia to protect oxygen-requiring organisms, that it only predicts nontoxicity, and that 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 in which AVS exceeds SEM and those in which 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.

2.6.3 Additional Sinks for Silver in Sediments

2.6.3.1 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 organic matter. This provides an additional correlative mechanism that regulates pore water 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 pore water 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 (cf., Chapter 1).

2.6.3.2 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, Wingert-Runge and Andren 1995). Recently it was shown that Fe(II) may be a somewhat unique sink for silver in sediments according to the reaction

$$Ag^{+} + Fe^{2+} = Ag^{0} + Fe^{3+}$$ (Mahony *et. al.* 1999).

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).

2.6.3.3 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.

2.6.4 Regulatory Recommendations

a. The SEM/AVS method, suitably adjusted, should be applied to silver in assessing the potential for its acute toxicity in sediments.

b. Organic matter should be incorporated into determining the ESG for silver, when a more complete set of binding constants is available.

c. Additional binding capacities of POM (particulate organic matter) and iron for silver should be applied when calculating any site-specific equilibrium sediment guidelines.

2.7 USE OF FIELD STUDIES AND MESOCOSMS IN ECOLOGICAL RISK ASSESSMENT OF SILVER

2.7.1 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 a type of test system in which population- and community-level effects of silver exposure (solely or in combination with other metals) could be studied. Such studies would encompass chronic, multiroute exposures and include trophic-level transfer.

We are aware of only two mesocosm studies (Gorsuch and Ewell 1977; Ewell *et al.* 1993; Ratte 1999) on silver thiosulfate and only two published field studies (Lee *et al.* 2000; Birge *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- and community-level responses to chemical exposure (cf., LaPoint 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 in that 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/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 fish 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 <1.0 for silver in the New River, Virginia. 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 NPDES requirements, short-term chronic experiments

were conducted with eight sets (two sets per year) of seven-day fathead minnow and seven-day ceriodaphnid studies in Colorado using 100% effluent and four serial dilutions, containing up to 33 µg/L (average 8 µg/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 1995-1998). 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).

2.7.1.1 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.

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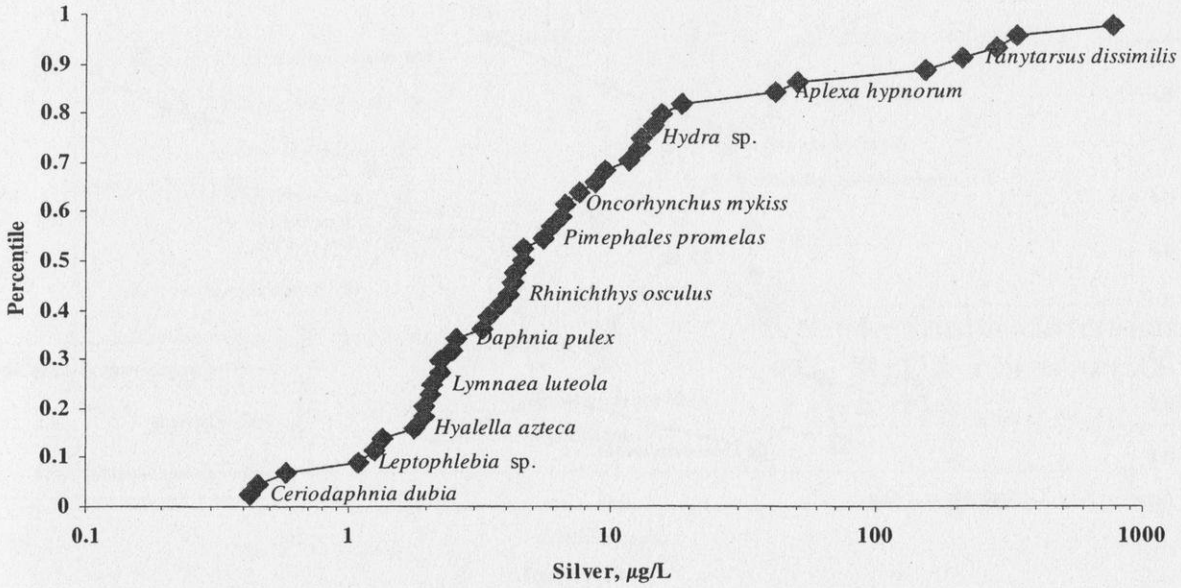
Figure 2.1 Acute sensitivities (SMAV/2) of freshwater organisms to silver.



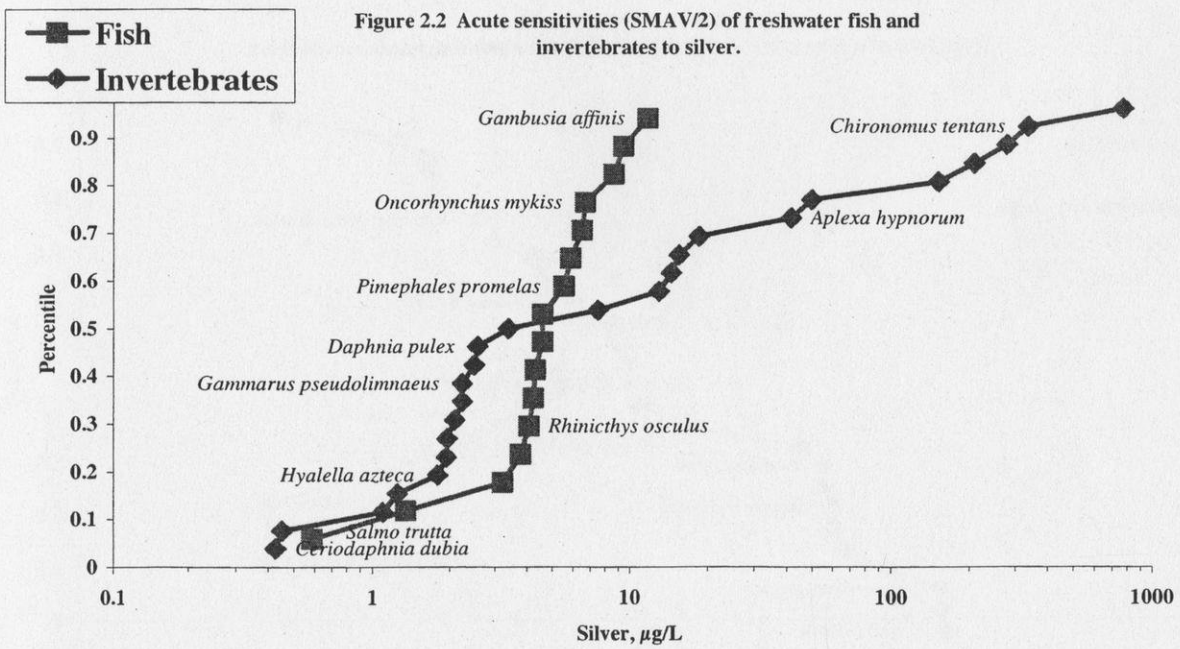


Figure 2.2 Acute sensitivities (SMAV/2) of freshwater fish and invertebrates to silver.

Figure 2.3 Acute sensitivities (SMAV/2) of different freshwater invertebrate groups to silver.

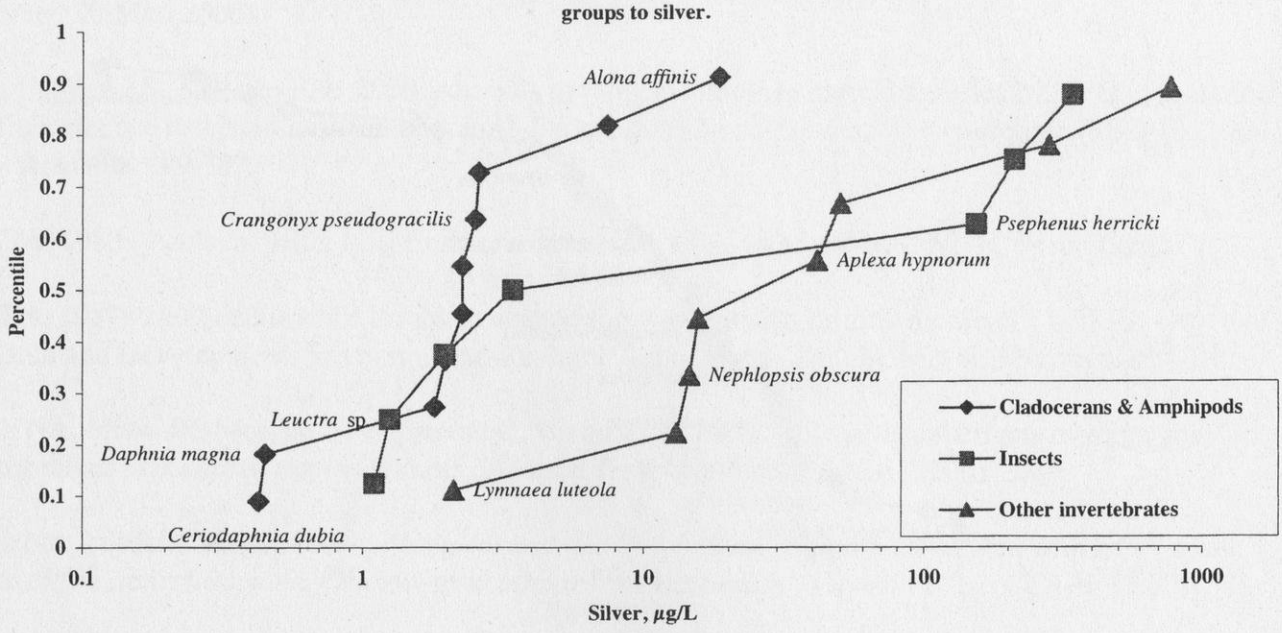


Figure 2.4 Acute sensitivities (SMAV/2) of marine organisms to silver.

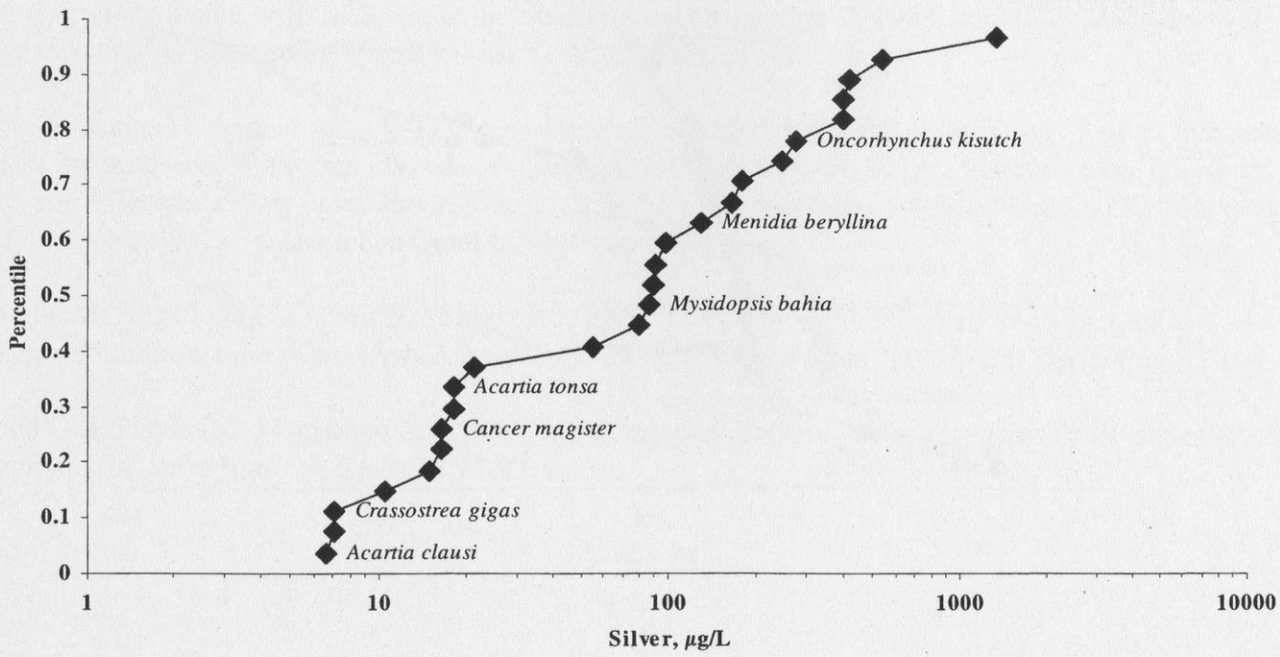


Figure 2.5 Acute sensitivities (SMAV/2) of different marine species groups to silver.

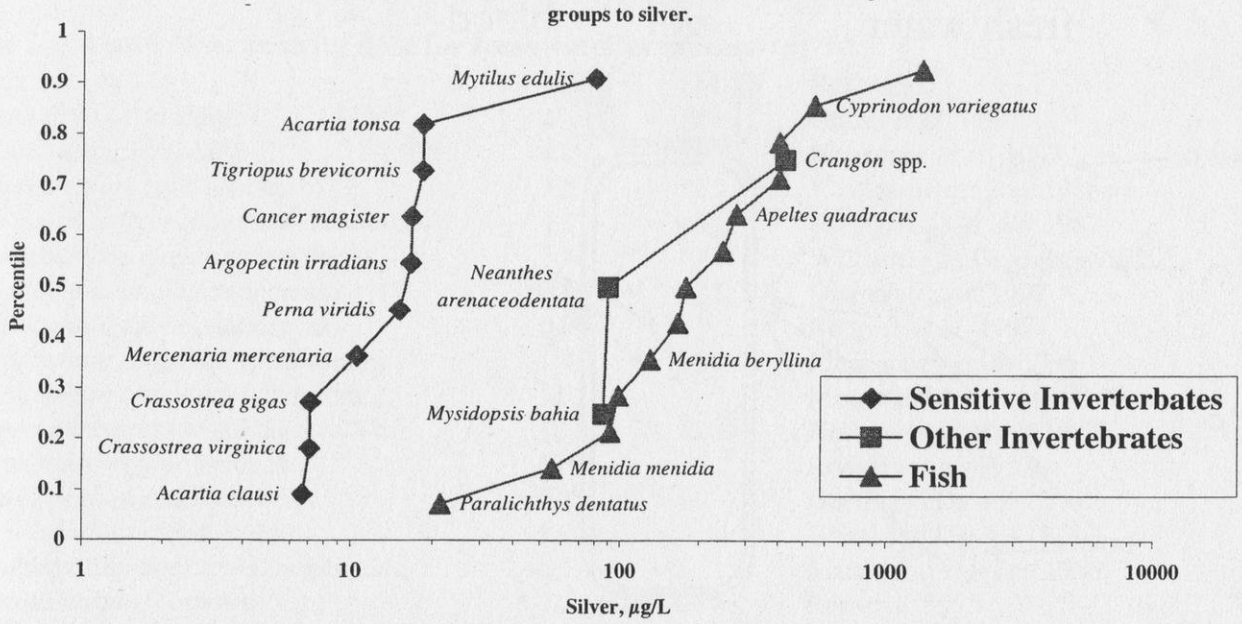
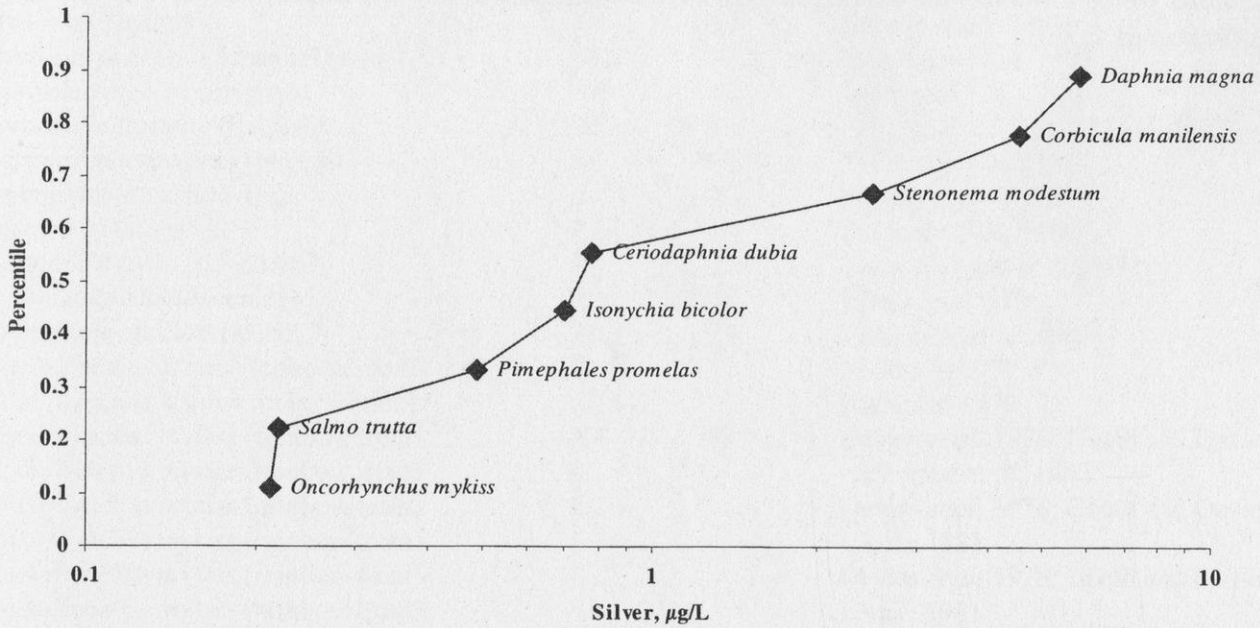


Figure 2.6 Chronic sensitivities of freshwater organisms to silver.



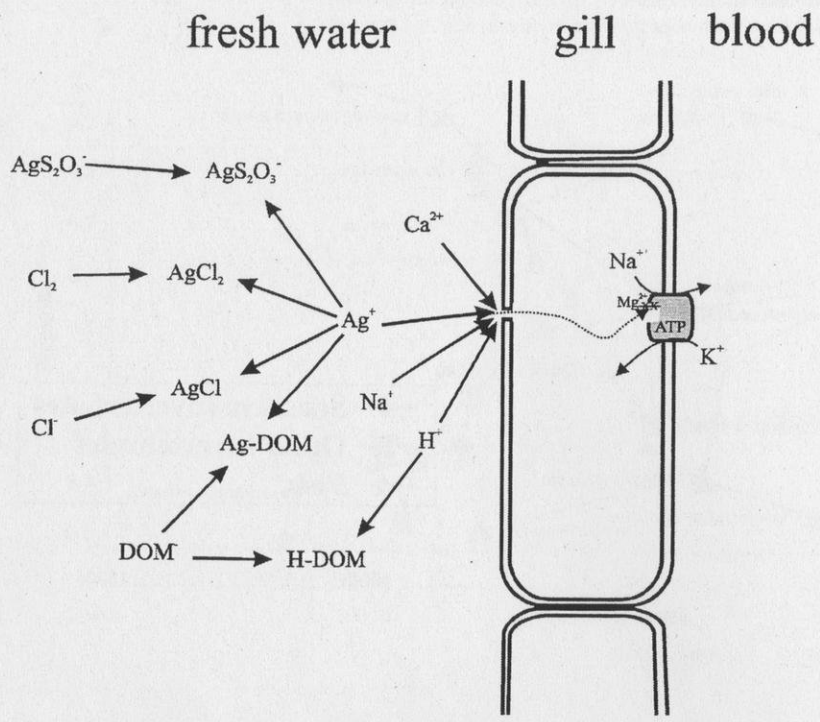


Figure 2.7. Conceptual representation of some of the anionic complexation and cationic competition reactions which influence the binding of Ag+ to a biotic ligand where it will exert its toxic effect. (From McGeer *et al.* 2000).

APPENDIX

Table 2.1 Acute silver toxicity data for freshwater organisms (μg/L).

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

Oncorhynchus mykiss (rainbow trout)	9.7		Nebeker *et al.* 1983
Oncorhynchus mykiss (rainbow trout)	11.5		Lemke 1981
Oncorhynchus mykiss (rainbow trout)	13		Davies *et al.* 1978; Goettl and Davies 1978
Oncorhynchus mykiss (rainbow trout)	14		Lemke 1981
Oncorhynchus mykiss (rainbow trout)	17.87		Lemke 1981
Oncorhynchus mykiss (rainbow trout)	240		Lemke 1981
Oncorhynchus mykiss (rainbow trout)	170		Lemke 1981
Oncorhynchus mykiss (steelhead)	9.2	9.2	Nebeker *et al.* 1983
Orconectes immunis (crayfish)	560	560	Holcombe *et al.* 1987
Philodina acuticornis (rotifer)	1400	1543	Buikema *et al.* 1974
Philodina acuticornis (rotifer)	1700		Buikema *et al.* 1974
Pimephales promelas (fathead minnow)	7.8	11.1	Erickson *et al.* 1998
Pimephales promelas (fathead minnow)	3.9		Lemke 1981
Pimephales promelas (fathead minnow)	5		Lemke 1981
Pimephales promelas (fathead minnow)	5.3		Lemke 1981
Pimephales promelas (fathead minnow)	5.6		Nebeker *et al.* 1983; Lemke 1981
Pimephales promelas (fathead minnow)	6.3		Lemke 1981
Pimephales promelas (fathead minnow)	6.7		Holcombe *et al.* 1983
Pimephales promelas (fathead minnow)	7.4		Nebeker *et al.* 1983; Lemke 1981
Pimephales promelas (fathead minnow)	9		Holcombe *et al.* 1987
Pimephales promelas (fathead minnow)	10.7		Lima *et al.* 1982; Call *et al.* 1983
Pimephales promelas (fathead minnow)	10.98		Lemke 1981
Pimephales promelas (fathead minnow)	11.1		Lemke 1981
Pimephales promelas (fathead minnow)	11.75		Lemke 1981
Pimephales promelas (fathead minnow)	16		EG&G Bionomics 1979; LeBlanc *et al.* 1984
Pimephales promelas (fathead minnow)	110		Lemke 1981
Pimephales promelas (fathead minnow)	150		Lemke 1981
Poecilia reticulata (guppy)	6.44	6.44	Khangarot and Ray 1988a
Psephenus herricki (water penny beetle)	>306	>306	Diamond *et al.* 1990
Rana hexadactyla (frog)	25.7	25.7	Khangarot *et al.* 1985
Rhinichthys osculus (speckled dace)	4.9	8.2	Goettl and Davies 1978
Rhinichthys osculus (speckled dace)	13.6		Goettl and Davies 1978
Salmo trutta (brown trout)	1.17	1.17	Davies *et al.* 1998
Simocephalus vetulus (cladoceran)	15	15	Mount and Norberg 1984
Stenonema modestum (mayfly)	3.9	3.9	Diamond *et al.* 1990
Tanytarsus dissimilis (midge)	420	420	Holcombe *et al.* 1987
Thymallus arcticus (Arctic grayling)	6.7	8.6	Nishiuchi 1979
Thymallus arcticus (Arctic grayling)	11.1		Nishiuchi 1979
Tubifex tubifex (tubificid worm)	31	31	Khangarot 1991

LC50 = Median lethal concentration
SMAV = Species mean acute value

Table 2.2 Chronic silver toxicity data for freshwater organisms (µg/L).

Species	NOEC	LOEC	Chronic Value	SMCV	Reference
Ceriodaphnia dubia (cladoceran)	1.14	0.53	0.78	0.78	Rodgers *et al.* 1997
Corbicula manilensis (Asiatic clam)	7.8	2.6	4.5	4.5	Diamond *et al.* 1990
Daphnia magna (cladoceran)	20	41	29	5.9	Nebeker 1982
Daphnia magna (cladoceran)	10.5	21.2	14.9		Nebeker 1982
Daphnia magna (cladoceran)	8.8	19.4	13.1		Nebeker *et al.* 1983; Nebeker 1982
Daphnia magna (cladoceran)	3.4	8	5.2		Nebeker *et al.* 1983; Nebeker 1982
Daphnia magna (cladoceran)	2.7	3.9	3.2		Nebeker 1982
Daphnia magna (c ladoceran)	1.6	4.1	2.6		Nebeker *et al.* 1983; Nebeker 1982
Daphnia magna (cladoceran)	1.22	0.8	0.99		Rodgers *et al.* 1997
Isonychia bicolor (mayfly)	1.56	0.31	0.70	0.70	Diamond *et al.* 1990
Oncorhynchus mykiss (rainbow trout)	0.36	0.51	0.43	0.21	Nebeker *et al.* 1983
Oncorhynchus mykiss (rainbow trout)	0.09	0.17	0.12		Davies *et al.* 1978
Oncorhynchus mykiss (rainbow trout)	0.15	0.22	0.18		Davies 1999
Pimephales promelas (fathead minnow)	0.37	0.65	0.49	0.49	Holcombe *et al.* 1983
Salmo trutta (brown trout)	0.20	0.25	0.22	0.22	Davies *et al.* 1998
Stenonema modestum (mayfly)	3.4	1.84	2.5	2.50	Diamond *et al.* 1992

NOEC = No-observable-effects concentration
LOEC = Lowest-observable-effects concentration
SMCV = Species mean chronic value

Table 2.3 Acute silver toxicity data for saltwater organisms (μg/L).

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

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

LC50 = Median lethal concentration
SMAV = Species mean acute value

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Chapter 3
Group C Discussion
Biological Processes

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Chapter 3
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Biological Processes

Samuel N. Luoma (*moderator*), Christer Hogstrand (*rapporteur*), Russell A. Bell, Gretchen K. Bielmyer, Fernando Galvez, Gerald A. LeBlanc, Byeong-Gweon Lee, Thomas W. Purcell, Robert C. Santore, Peter H. Santschi, Joseph R. Shaw

3.1 INTRODUCTION

In 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 (see this volume, 3.3.3 and 4.2.3.2; Lee *et al.* 2000; Griscom *et al.* 2000). 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 subsequent effects. Even though several of these topics are discussed in other chapters, we feel that they are important to consider in the context of pharmacokinetics and other biological and chemical processes.

3.2 HOW DOES SILVER CROSS MEMBRANES?

3.2.1 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. We may anticipate the presence of different receptors for molecules, different modes of internalization of materials, and different ways of handling molecules. 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 will usually be designated pathways for entry through transport proteins or surface receptors. However, non-essential 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, we note 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. We attempt here to develop a framework in which these data may be rationalized in chemical terms. Such a framework must also 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.

3.2.2 Low molecular weight Ag(I) compounds

Low molecular weight Ag(I) compounds are defined as "dissolved" (using the current U.S. EPA operational definition of filtration through a 0.45 μm filter) and are subdivided into two classes. One class is weakly bound Ag(I), designated $AgL_{(w)}$, 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 $AgL_{(s)}$, 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, 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 and Hogstrand 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. We can only speculate 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 $Ag_2S_2O_3$ 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 non-bioreactive 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/transporter for the purpose of tightly binding metals, wanted or unwanted, at the cell surface. Such a system would also strongly bind $AgL_{(w)}$ species, but might not have the capacity to cope with high concentrations of $AgL_{(w)}$ (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 *et al.* (1999a) 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.* 1999). 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.

3.2.3 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. We should, however, provide some comment on natural organic matter (NOM), a common aggregate to which Ag(I) binds, and note one relevant study. 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 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 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 organic matter 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.

3.2.4 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; Lee *et al.* 2000; 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 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, $Ag[S_2O_3]_n$, 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.

3.3 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, in press). These assimilation values range from 4% to 50%, depending on animal species and food type (Table 1). Lee *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 (14-28 %) 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 ~3 %. Clearly, there is a large variability in reported assimilation efficiencies for Ag(I) (Table 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 four-fold higher than control trout after 56-d feeding. However, juvenile rainbow trout (*Oncorhynchus mykiss*) provided a diet containing biologically incorporated Ag(I) (~3 mg Ag kg^{-1} or 0.03 mmol kg^{-1} 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 Ag_2S 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 found in mud snail, *Nassarius obsoletus*, from San Francisco Bay (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 and Klaine 1999) 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/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 *et al.*, unpublished). 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 by using a biokinetic model that 65-95% of accumulated Ag(I) was derived from dietary route in a marine deposit-feeding polychaete, *Nereis succinea*. Lee *et al.* (unpublished) found in a microcosm study that the deposit feeding worm, *Neanthes arenaceodentata*, obtained Ag(I) predominately from ingestion of contaminated sediments. Some marine animals could also 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 1998; Bielmyer and Klaine 1999). Schmittschmitt *et al.* (1996) demonstrated that fecundity of *C. dubia* decreased

Table 3.1 Assimilation Efficiency (%) of Ag(I) reported for aquatic invertebrates.

Animal	**Food**	**Assimilation efficiency**	**Reference**
Ciliate: *Fabrea salina*	prymnesiophyte	22	1
Copepod: *Acartia tonsa*	diatom	17	2
Copepod: *Temora longicornis*	2 diatoms natural seston	8-19 15	3
Polychaete: *Nereis succinea*	oxic sediments	16-30	4
Mussel: *Mytilus edulis*	2 diatoms, 2 chlorophytes, 2 dinoflagellates, 1 prasinophyte	4-34	5
Mussel: Mytilus edulis	oxic sediments	4-15	6,7
Oyster: *Crassostrea virginica*	prymnesiophyte	44	8
Clam: *Macoma balthica*	1 diatom, 1 prymnesiophyte	38-49	8
Clam: *Mercenaria mercenaria*	1 diatom, 1 prymnesiophyte	22-35	8
Zebra mussel: *Dreissena polymorpha*	2 diatoms, 1 chlorophyte, 1 cyanophyte, natural seston	4-16	9
Seastar: *Marthasterias glacialis*	Mussels	69	10
Clam: *Potamocorbula amurensis*	Algae sediment	18-27	11
Clam: *Macoma balthica*	Ag_2S on glass beads	14-28	12
Clam: *Mytilus edulis*	Fe-oxyhydroxides	~3	12

Fisher *et al.* 1995; (2) Reinfelder and Fisher 1991; (3) Wang and Fisher 1998; (4) Wang *et al.* 1999; (5) Wang *et al.* 1996; (6) Griscom *et al.* 2000; (7) Gagnon and Fisher 1997; (8) Reinfelder *et al.* 1997; (9) Roditi and Fisher 1999; (10) Fowler and Teyssi 1997; (11) Griscom *et al.* 2000; (12). Lee *et al.* 2000

significantly after ingestion of the algae *Selenastrum capricornutum* which had been previously exposed to Ag(I) in the dissolved phase (20 $\mu g\ L^{-1}$ or 180 nM), yielding a total Ag(I) concentration of 1.1 $\mu g\ g^{-1}$ (10 $nmol\ g^{-1}$) algae (dry weight). 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 and Klaine 1999).

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 Ag_2S 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.

3.4 WATERBORNE EXPOSURE

3.4.1 Pharmacokinetics

Published bioconcentration factors (BCF) resulting from waterborne Ag(I) exposures are relatively low in teleost fish compared to other aquatic organisms. The BCF value following a two-day radioactive pulse of ^{110m}Ag at 11.9 $\mu g\ L^{-1}$ (110 nM) Ag(I) (as $AgNO_3$) was only 2.4 for rainbow trout (Galvez *et al.*, submitted), which is comparable to a BCF of 2.4 for brown trout after 57 days of exposure to $AgNO_3$ under similar conditions (Garnier and Baudin 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 (Tehaar *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 60-70% 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; Grosell *et al.* 2000; Galvez *et al.* 2001). Exposure of European eel (*Anguilla anguilla*) or rainbow trout to Ag(I) as Ag^+ or $AgCl^0$ 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.

3.4.2 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 $AgNO_3$) is at least 10^3 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 result 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 $AgNO_3$ 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-h 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.* 1999b; McGeer and Wood 1999).

The strong relationship between Ag^+ and toxicity was also observed during physiological studies by Bury *et al.* (1999b) 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 $AgNO_3$, 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 threefold 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.

3.5 BIOAVAILABILITY AND BIOREACTIVITY OF SILVER

3.5.1 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* non-bioreactive

Ag(I). According to this model, 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 five to ten percent 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^+ (see 3.3.2.2; 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 (Fig. 3.1A; Hogstrand and Wood 1998; Wood *et al.* 1999). In contrast, waterborne Ag(I), originating as $AgCl^0$, $Ag(S_2O_3)_n$ (Ag-thiosulfate), or Ag-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 (Figs. 3.1C,D). If Ag(I) enters the gill as a stable complex it may even be present in the target cells (i.e. ionocytes) in a non-bioreactive 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 (Fig. 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 (Fig. 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 *et al.* 1999c). 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, therefore, reduce the protective effect offered by chloride. This effect can be simulated, using a BLM approach where the affinity of the site for toxicity is increased for Ag(I) and other cations.

3.5.2 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 fourfold increase in hepatic Ag(I) burden over seven 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 (fourfold increase during 7 vs. 16 days). Important here is that all accumulated Ag(I) was not equally bioreactive and at present we do not fully understand how to separate the two.

3.5.3 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 indicate that accumulated silver might be bioreactive. We have described these studies in some detail, because they are the only field studies available suggesting that silver could potentially 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 (Fig. 2). Animals were analyzed for silver, copper and zinc at near monthly intervals throughout the study period (1974-76; 1979-81; 1982–84; 1987-89). A variety of metals, grain size, and Total Organic Carbon 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 ~1.6 $\mu g\ g^{-1}$ (15 nmol g^{-1}), dry weight, in the late 1970s to 0.2 $\mu g\ g^{-1}$ (1.8 nmol g^{-1}) in the late 1990s. Concurrently, sediment copper levels dropped moderately from ~ 85 $\mu g\ g^{-1}$ (1.3 $\mu mol\ g^{-1}$) to ~ 50 $\mu g\ g^{-1}$ (0.78 $\mu mol\ g^{-1}$) Silver as well as copper tissue concentrations declined from extremely high levels (silver: ~100 $\mu g\ g^{-1}$; copper: ~300 $\mu g\ g^{-1}$) in 1975-1982 to levels about fivefold background in the late 1990's (silver: ~10 $\mu g\ g^{-1}$; copper: ~50 $\mu g\ g^{-1}$). 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 (Fig. 2; Hornberger *et al.* 1999). In one year during this period, 1982 – 1983, a reproductive cycle typical of other mudflats in San Francisco Bay (Nichols and Thompson 1983) was observed, which might be explained with a very high concurrent freshwater discharge. Reproductive inhibition was again observed in 1985 and 1987. In 1988, the year of lowest metal exposure, nearly all animals contained mature gametes in nearly all months (Fig. 2).

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 (1979–80), 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 and Jenkins 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 co-variates 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* (Luoma, S.N., 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 among 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 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 mid-estuarine sites near a military base between 1991 – 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 multi-faceted data collection. Although copper body burden co-varied 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; Hooke and Fisher 1998; Bielmyer and Klaine 1999), 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 – 80 μg g^{-1} (15 – 74 nmol g^{-1}), dry weight. 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 - 10 μg g^{-1} (46 - 90 nmol g^{-1}), dry weight.

The field results are consistent, to some degree, with laboratory results in that 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 should also 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 may also be a useful first-tier indicator of susceptible species in natural systems. For example, extremely high Ag(I) concentrations, ~100 μg 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 is also 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 (*Campostoma 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.

3.5.4 Intracellular speciation of Ag(I)

To some extent, what determines if absorbed Ag(I) is toxic or not depends on the molecular make-up 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 >17 (Hamer 1986; Kägi and Shäffer 1988) and 12.3 (Adams and Kramer 1999), respectively. Typical intracellular concentrations of these particular biomolecules are 15-60 μM for metallothionein (Hogstrand *et al.* 1996) and 5-10 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 two different salmonid cell-lines, one of which had a compromised metallothionein

expression, it was found that the cells lacking metallothionein were about five 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 two-day exposure to radioactively labeled Ag(I) (11.9 $\mu g\ l^{-1}$ or 110 nM added as $^{110m}AgNO_3$ 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 (~70%) of the ^{110m}Ag content in the liver cytosol eluted at a molecular weight characteristic of metallothionein. In comparison, the cytosolic distribution of ^{110m}Ag in gills was less specific, with binding of the metal to several fractions, including metallothionein. Only a small portion (~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.*, personal communication). 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). *M. balthica* seems to form granules to detoxify Cd and Zn, but *P. amurensis* does not use this mechanism to the same extent (W. Wallace, personal communication). The formation of non-toxic 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 four bivalve mollusks (Berthet *et al.* 1992), it was likewise 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 – 20%) was found also 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- 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, we do not understand 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.

3.6 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 requires the derivation and application of chronic values (*i.e.* >NOAEL<LOAEL). Ideally, chronic values would be derived from laboratory toxicity tests that involve exposure of the test organisms though the life cycle (chronic exposure), allowing measurement of toxicity endpoints distinct from those observed during acute exposure. Preferentially, these endpoints involve characteristics such as fecundity and scope for survival in natural environments where predators and competitors are present.

However, since full life cycle studies with fish are complex and expensive, subchronic toxicity studies are often used as surrogates. Such experiments have been performed with rainbow trout, during which effects of Ag(I) (added as $AgNO_3$) 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 water quality criteria relies heavily upon the use of Acute-to-Chronic Ratios (ACRs) to estimate chronic values for species for which 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 chemistrites 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 indicate 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 & Human Services, 1980). 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 water quality criteria, 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: (a) Exposures which incorporate as much of the organism's life cycle as is technically and practically possible; (b) incorporation of both aqueous and dietary routes of Ag(I) exposure into the study design; (c) evaluation of a variety of species representing various trophic levels; (d) use of environmentally relevant and well characterized silver speciation for exposures; (e) effects related to measured, not nominal, Ag(I) concentrations; (f) 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 could also allow for the use of ACRs among species that share common targets of chronic Ag(I) toxicity.

3.7 SILVER SENSITIVITY

3.7.1 Background

It is important to determine the most sensitive receptors of silver toxicity. This may be in terms of specific organisms, certainly, but may also 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.

3.7.2 Acute Toxicity

A large body of information is available in the scientific literature reporting on 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* spp., 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 (*cf* Chapter 2, section 2.1.1; 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 (*cf* 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; Hogstrand and Wood 1998; Ferguson and Hogstrand 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 (*cf* Section 2.2; Hogstrand and Wood 1998; Wood *et al.* 1999) There is, however, an important difference between the two 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 water quality criteria and validation of the Biotic Ligand Model (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.

3.7.3 Chronic toxicity

The most sensitive and environmentally realistic endpoint is probably a chronic one. Work by Schmittschmitt *et al.* (1996), Fisher and Hook (1998), and Bielmyer and Klaine (1999) 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 is also significant because the form in which 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 (Section 3.3.4.4), there is 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 $\mu g\ g^{-1}$ (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 only just emerging, and we recommended that research define and clarify the potential for effects in the natural environment.

3.8 SEDIMENT PROCESSES

Environmental Ag(I) concentrations are very low (<0.5 $\mu g\ l^{-1}$; 5 nM (unfiltered), *cf* 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 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 Ag_2S have been made to evaluate bioavailability and toxicity of silver sulfides. In 10-day exposures to *Hyallela azteca*, Ag_2S 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} dry weight; Hirsch, 1998a). In a similar experiment, 28-day exposures of *Lumbriculus variegatus* to sediment with added Ag_2S, had no observed effects on mortality, reproduction, or body dry weight. There were, however, higher Ag(I) concentrations measured as total body burden (80 μg Ag g^{-1} or 0.74 μmol g^{-1} dry weight) 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 (SBAF) of 0.18, which supports the idea that silver sulfide in sediments has generally low bioavailability or bioreactivity (Hirsch, 1998b). Metal spiking experiments of ionic Ag^+ (added as $AgNO_3$) 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 Ag_2S. 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 is also 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. However, the long-term field studies discussed earlier (3.3.4.4) 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 *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 United Kingdom (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, FeS_2) in anoxic sediment, thereby rendering silver bioavailable. In fact, 0.3 – 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.

3.9 GENERAL CONCLUSIONS AND RECOMMENDATIONS

3.9.1 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 (*cf* 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 over-simplifications 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.

Biotic Ligand Model (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.

Acute-to-Chronic Ratios (ACR): Do not use ACR to derive chronic water quality criteria. 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.

3.9.2 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 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 non-specifically 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). It is therefore 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 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 may also interfere with a simple relationship between total body Ag(I) and adverse effects. Thus, we recommend studies that can help to identify the intracellular localization of Ag(I) in relation to exposure regime.

Chronic toxicity studies with "chronic end-points": It cannot be assumed that end-points for acute toxicity are the most sensitive chronic endpoints. Similarly, the forms of Ag(I) that acutely are 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 sensitive species and end-points are identified, the mechanisms of chronic toxicity become unraveled, and that chronic toxicity testing is designed so that appropriate uptake pathways and effects are considered.

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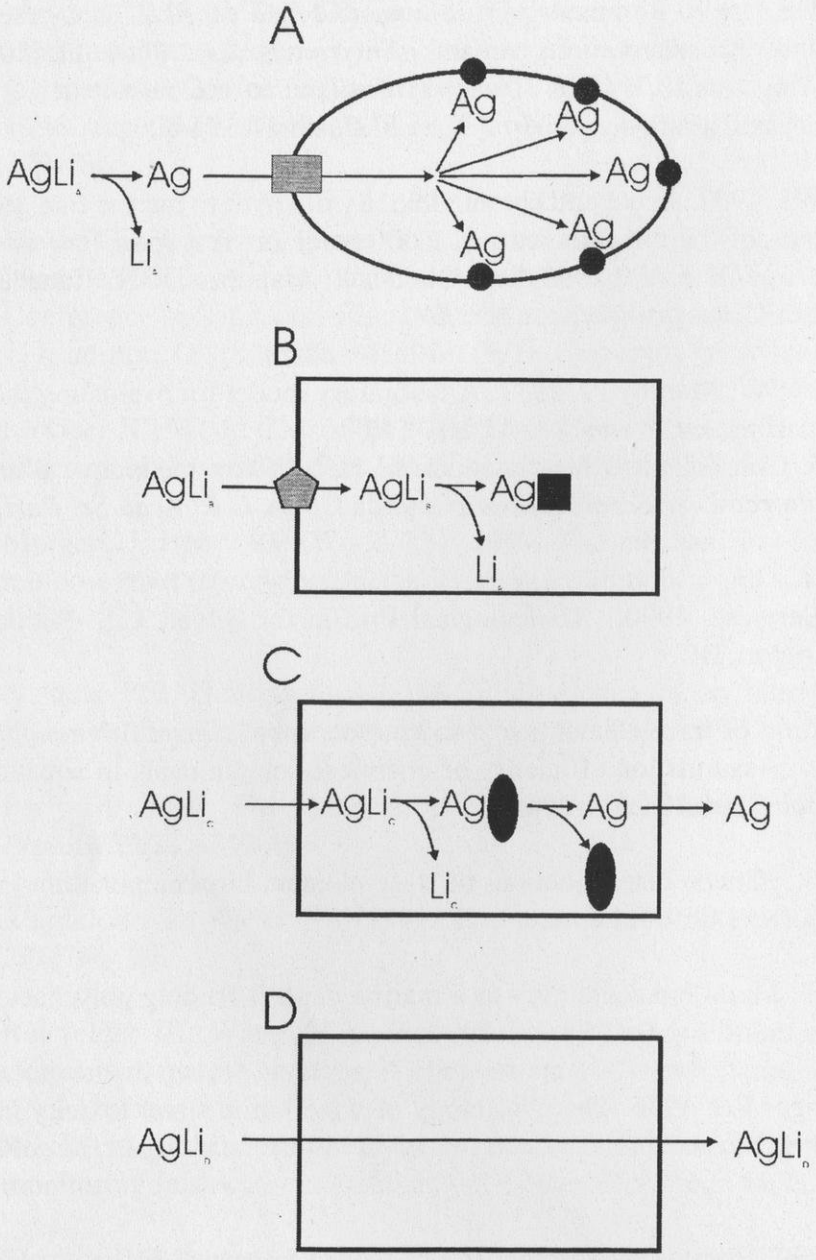

Figure 3.1. Ag(I) is bioavailable in all cases (A-D) but only bioreactive in cases A and B. **A**: Weak ligand or "free" Ag^+. Ag(I) enters through ion transporter in sensitive cell (i.e. chloride cell). Specific uptake leads to high effective dose. e.g., laboratory exposures to Ag(I) added as AgNO3 in "clean" laboratory water **B:** Moderately weak to strong ligand that is specifically transported into target cell. Ag(I) is "accidentally" co-transported and combines in the cell with sensitive target. i.e. Ag-glutathione. **C:** Moderately weak to moderately strong ligands that pass the outer cell membrane by non-specific means. The cell does not contain a sensitive target. Inside the cell, Ag(I) combines with metallothionein and relatively insensitive proteins before it may be extruded from the cell for redistribution in the body. i.e. AgCl(aq). **D:** Strong ligand that passes through the cell by non-specific means. Silver does not dissociate from the ligand and does not interact with biomolecules. i.e. Ag-sulfide complex.

Reproductive Status, M. balthica, 1979-81

Means: cu=266; Ag=90 ug/g

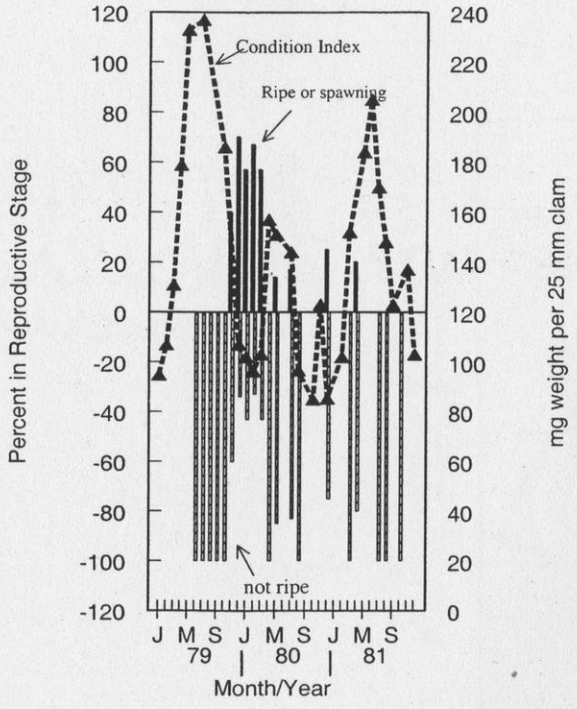

Figure 3.2a

Reproduction, PA, M. balthica, 1988-89

1988: Cu=53; Ag=20

1989: CU=35, Ag=11

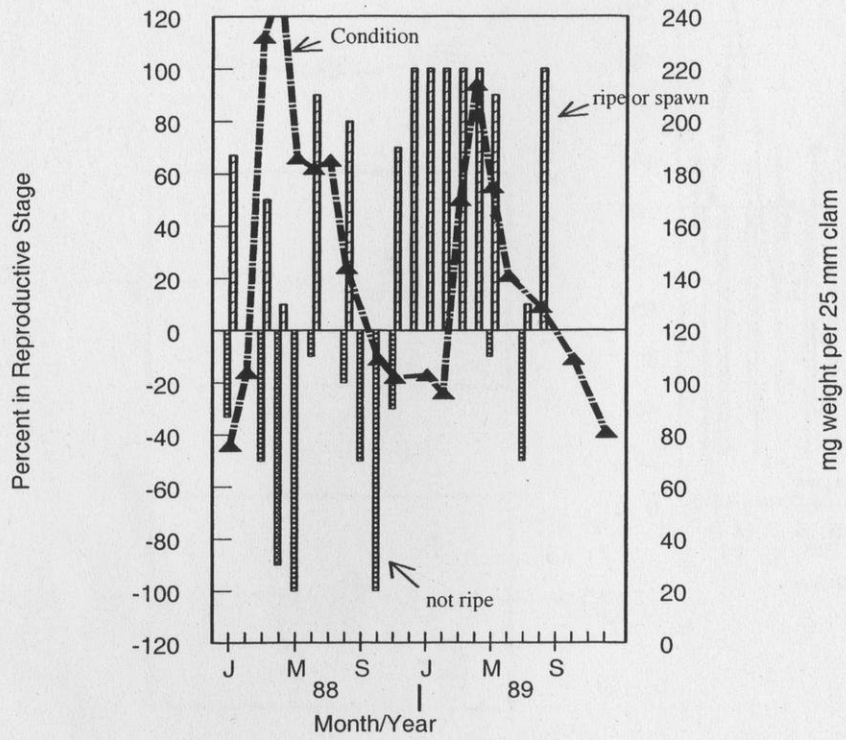

Figure 3.2b

Figures 3.2a and 3.2b. Condition and reproductive status in the clam *Macoma balthica* at a site in South San Francisco Bay in 20 near monthly samples in 1979 - 1981 and 18 near monthly samples in 1988-89. Reproductive data from 1974-76 and 1983-85 are not shown, but are consistent with trends shown here. Highest silver and copper concentrations occurred in the 1975 - 1982 period. Vertical bars on positive axis represent the percent of clams (~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. When silver and copper concentrations were highest, few animals contained mature gametes. When copper and silver concentrations declined, reproduction recovered. No other environmental variable changed unidirectionally other than pollutant concentrations over this sampling period or among the years when reproduction was studied.

Chapter 4
Group D Discussion
Risk Assessment

Moderator:
Peter G.C. Campbell

Rapporteur:
Paul R. Paquin

Panelists:
William J. Adams
Kevin V. Brix
Daland R. Juberg
Richard C. Playle
Charles J. Ruffing
Randall S. Wentsel

Chapter 4
Group D Discussion

Risk Assessment

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4.0 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, 1996). 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 to be 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.

4.1 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 photoprocessing effluents has increased in efficiency over recent years in response to both environmental concerns and because of economic demand for reclaimed silver (Eisler 1996; Purcell and Peters 1998). Nearly all photoprocessing 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, 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 (Purcell and Peters 1998; Lytle 1984).

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 section. Uncertainties and data needs will be presented. This information will be used with chronic toxicity data for aquatic biota that are presented in section 4.3 ("Effects Assessment"). 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 on the impacts of silver on these endpoints and explain why they have not been considered in further detail.

4.1.1 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).

4.1.2 Human Health

Within the assessment of potential receptors of environmental silver, it is important to consider humans and to evaluate potential health risk. 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 in that it is not associated with adverse health effects or chronic toxicity in humans and there is no evidence for carcinogenicity, neurotoxicity, or reproductive/developmental toxicity from exposure to silver. In 1991, the 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 (Federal Register 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 EPA's decision to delete the MCL. It is also important to note that the EPA reference dose (RfD) for silver, a daily exposure level deemed to be acceptable for humans, is also based on the endpoint of argyria. Argyria has not been reported for, and is not expected to occur, as a result of environmental exposures. Thus, based on our 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 A for additional discussion of exposure/risk assessment for humans).

4.1.3 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 B 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 was also 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 reference dose. 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 ≤ 0.03, corresponding to an average dietary intake that is less than 3% of the reference dose, 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 following: use of a NOEC for the reference dose 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 macroinvertebrates. However, it must also 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.

4.1.4 Aquatic life

In the remaining discussion of the implications of recent scientific findings, we will focus on direct exposure via the water column of aquatic ecosystems. 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 well be important, but unfortunately 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.

4.1.5 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.

4.1.6 Conceptual Model

The conceptual model for this risk analysis is based upon silver discharge to fresh water, where chemical speciation re-equilibration 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 are also considered.

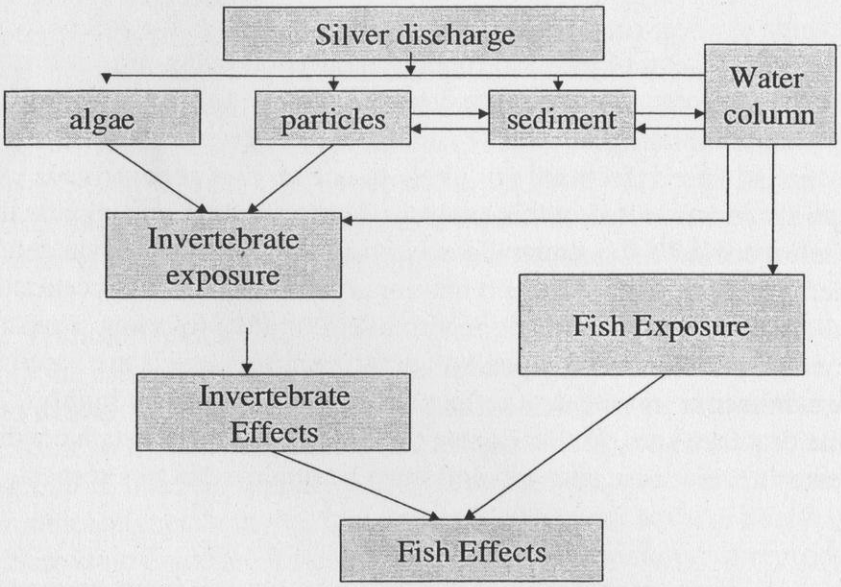


Figure 4.1. Conceptual model for silver risk analysis.

4.2 Exposure Assessment

4.2.1 Silver Speciation in Natural Waters

4.2.1.1 Background

As a type B, "soft" metal, silver(I) binds preferentially with donor atoms in the order S > I > Br > Cl ~ N > O > F. As a result of its low {charge/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), with polynuclear inorganic ligands (polysulfides), and with 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 ~ 0.5 mM (about 18 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- and di-chloro complexes.

4.2.1.2 Natural Waters

Recent measurements have demonstrated that metastable reduced sulfur species persist in oxic natural waters, despite the presence of oxygen (Luther and Tasamakis 1989; Kuwabara and Luther 1993). Reported concentrations of " reactive sulfide" (MBMS), are often greater than 10 nM (Manolopoulos and Kramer 1999); values in sewage effluents tend to be much higher (up to 100 nM) (Adams and Kramer 1998, 1999; Shafer et al. 1998). 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). 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 (J.R. Kramer, McMaster University, personal communication, August 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 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.

4.2.2 Silver Interactions with Aquatic Organisms (Biological Membranes)

The interaction of a metal with an aquatic organism involves the following steps: (i) advection or diffusion of the metal from the bulk solution to the biological surface; (ii) diffusion of the metal through an outer protective polysaccharide or glycoprotein layer; (iii) sorption/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 (iv) 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/traverse a cell membrane. The important 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.

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 (Section 4.2.1), 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 may also enter cells via route (ii) (e.g., as a negatively charged chloro- or thiosulfato-complex) or route (iii) (as the neutral $AgCl^0$ complex) – (Engel *et al.* 1981; Fortin and Campbell 1998; Reinfelder and Chang 1999; Hogstrand *et al.* 1996; Wood *et al.* 1996). 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 4.2.1 regarding the speciation of silver in natural waters, it appears unlikely that transport of chloro- or thiosulfato-complexes will be biologically significant in natural systems.

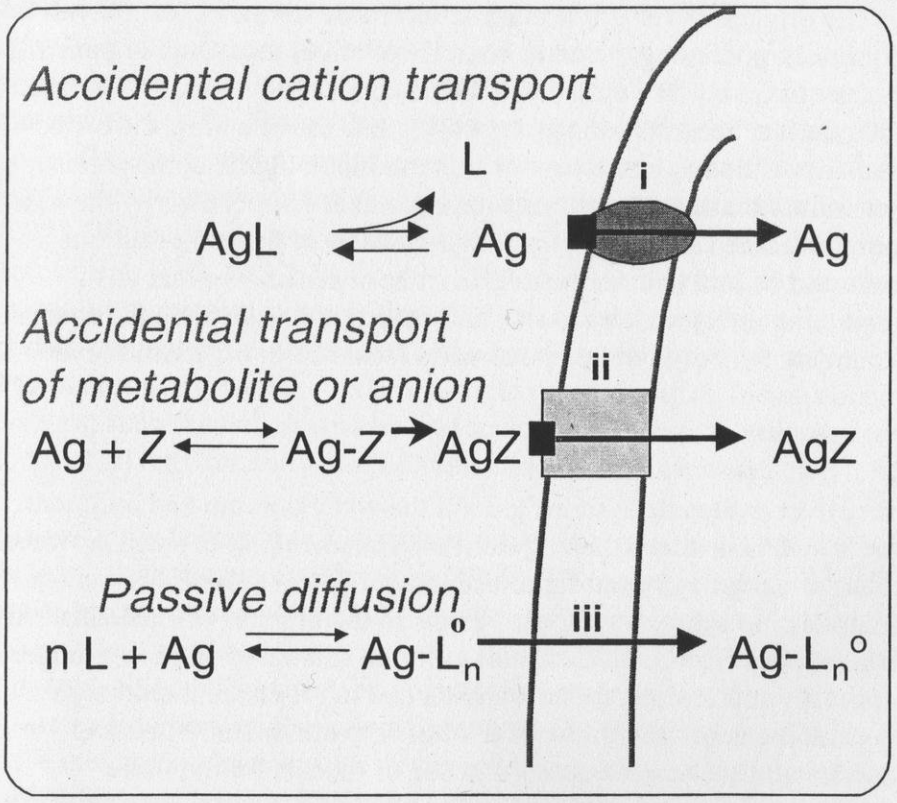


Figure 4.2. Mechanisms by which silver may cross biological membranes. For simplicity, charges are not shown for mechanisms (i) and (ii).

Acute toxicity of silver to freshwater fish involves ionoregulatory disruption through inhibition of basolateral Na^+,K^+-ATPase in the gill (reviewed by Wood *et al.* 1999, Section 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 (Richards *et al.* 1999; Lake and Hinch 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 BLM approach) demonstrated conceptually the minimal binding of silver at the fills 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 biotic ligand model (BLM) of acute toxicity (Di Toro *et al.* 1999; Paquin *et al.* 1999, as described in USEPA 1999) 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 fish and invertebrates, even in the absence of a more fundamental mechanistic understanding of these phenomena (MacRae *et al.* 1999; Meyer 1999).

4.2.3 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 are also 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 C 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.

4.2.3.1 Water Column

The conceptual model (Figure 4.1) depicts the primary exposure pathway for silver as direct uptake from water by fish and invertebrates in both freshwater and marine ecosystems.

Both pore water and surface water 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.* 1999; Lee *et al.* 2000). This pathway deserves further investigation, particularly as it relates to assessing the potential for chronic toxicity. The sediment pathway assessment (see below) also uses water (pore water) as the assessment medium. These data are summarized in the effects characterization portion of this Chapter (Section 4.4).

4.2.3.2 Aquatic Sediment

Aquatic sediments are generally 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 (Di Toro 2000). Within the deeper anoxic bedded sediment layers, the reactive silver tends to be incorporated into iron sulfide (FeS, FeS_2). 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.

It is generally 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 re-analysis 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 (upper panel), whereas it is when expressed on a dry weight basis (Figure 4.3, lower panel). 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 dry weight basis (Figure 4.4, upper panel). However, when the silver exposure level is expressed in terms of [Ag]/2 - AVS (2nd panel) or in terms of interstitial water toxic units (IWTU = LC50 based on pore water concentration / LC50 in a water only exposure; 3rd panel), it is not sediment-specific. That is, if SEM silver exceeded AVS, AVS was not measurable, IWTU > 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.

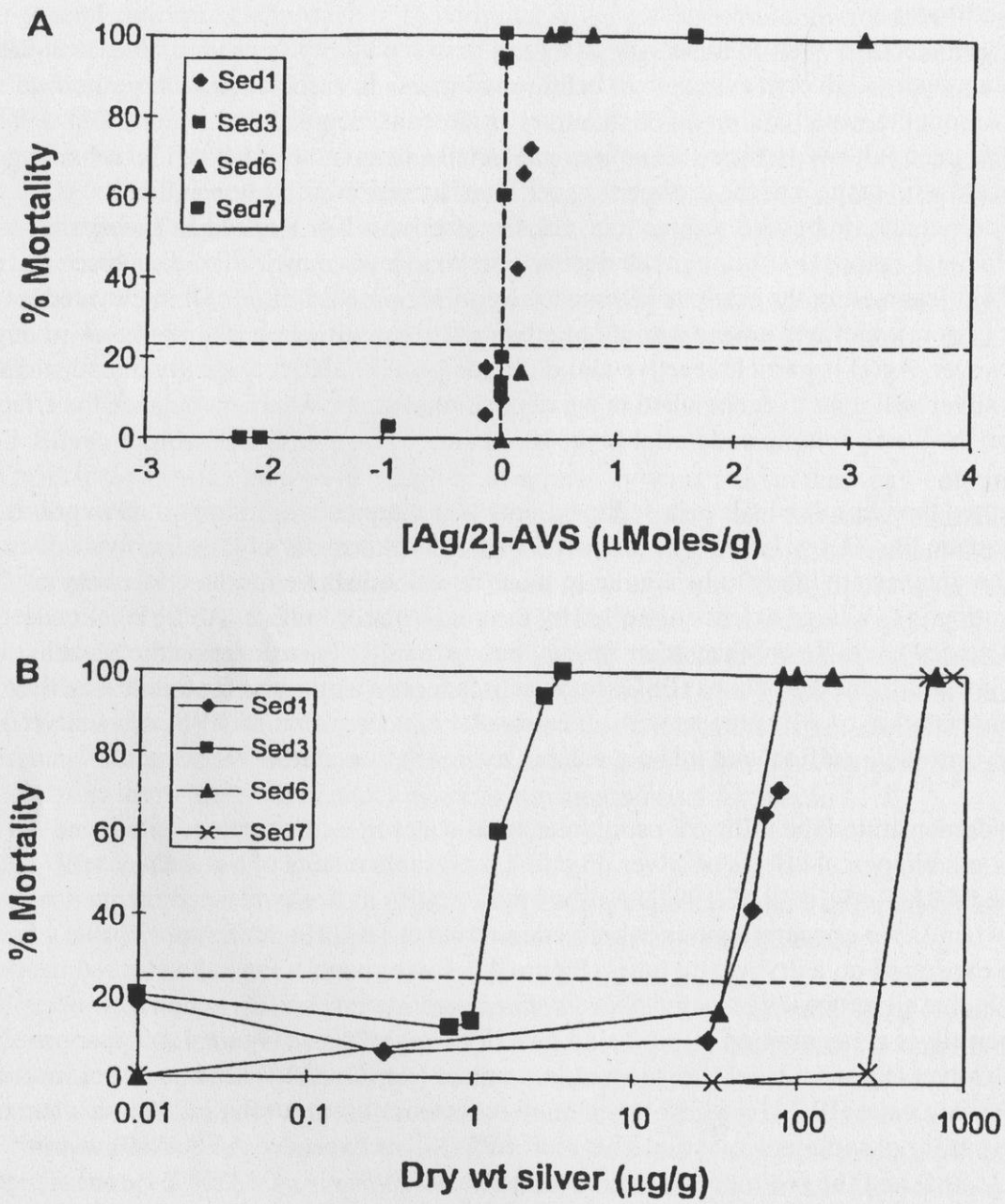


Figure 4.3. Percent mortality of the amphipod *Hyallela azteca* as a function of nominal ([Ag]/2) – AVS (A) and dry weight silver (B) in four freshwater sediments spiked with silver. (Data from Rodgers et al. 1997). Sediments below the dashed line at 24% mortality are not considered toxic. A vertical dashed line at SEM-AVS = 0 (A) indicates the predicted break point in toxicity. (From Berry 1999)

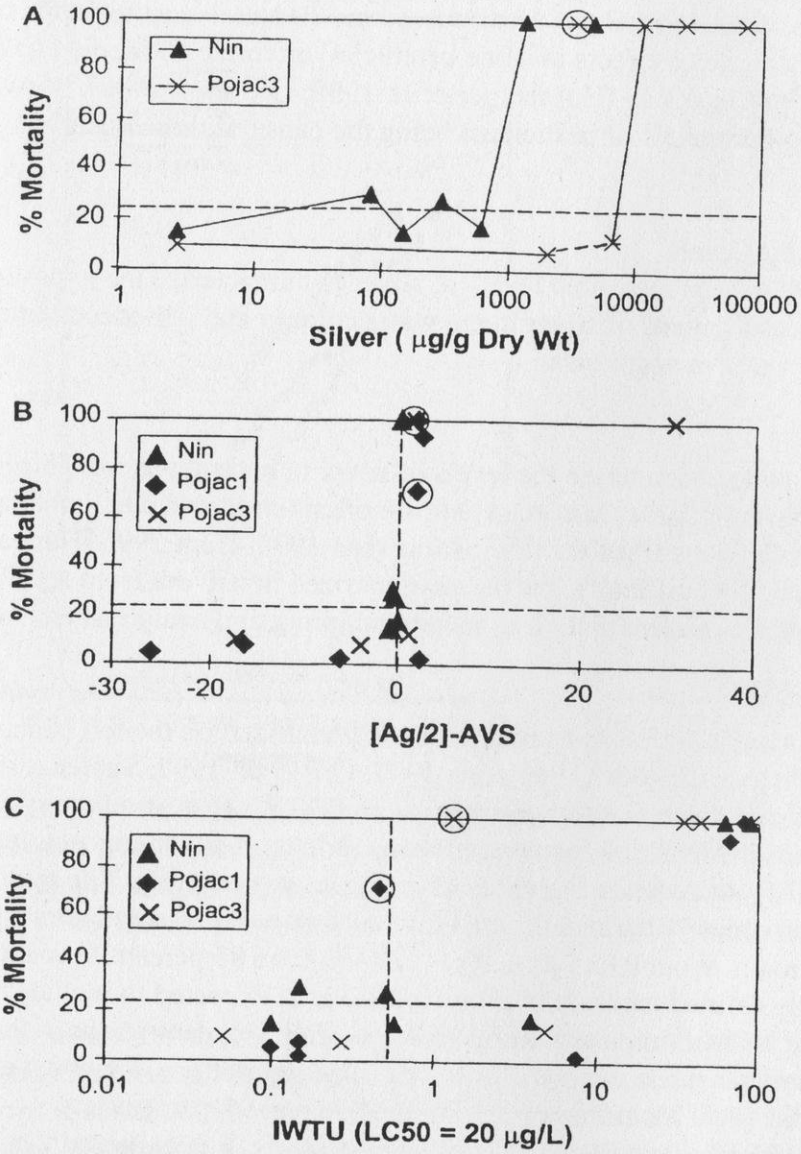


Figure 4.4. Percentage mortality of the amphipod *Ampelisca abdita* as a function of dry weight silver concentration (A), ([Ag]/2) – AVS (B), and interstitial water toxic units (IWTU) (C), in two saltwater sediments spiked with silver. Nin = Ninigret Pond sediment. Pojac = Pojac Point sediment. Sediments below the dashed line at 24% mortality are not considered toxic. Vertical dashed lines at SEM-AVS = 0 (B) and IWTU = 0.5 (C) indicate predicted break points in toxicity. Data points believed to be the result of interstitial water ammonia are included but highlighted (circled) and not connected by lines in A. (From Berry 1999.)

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 acid volatile sulfide 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 (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.

4.2.4 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.

4.2.4.1 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; Kinnerson 1995; Hunt 1995; Hunt and Lewis 1995; HydroQual 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.

Freshwater results based on different sample filtration techniques are summarized on the left panel of Figure 4.5 (Benoit 1995; Wolfe and Fitzpatrick 1996; Wen *et al.* 1997; Gill *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 95-percentile total silver concentrations of about 0.06 and 2 μg/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 μg/L (0.14 nM) and the 95 percentile is about 0.35 μg/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 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 95-percentile concentrations of about 0.004 and 0.25 μg/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 μm and < 0.1 μm) is considered to provide an upper bound estimate of the maximum bioavailable silver in these water samples.

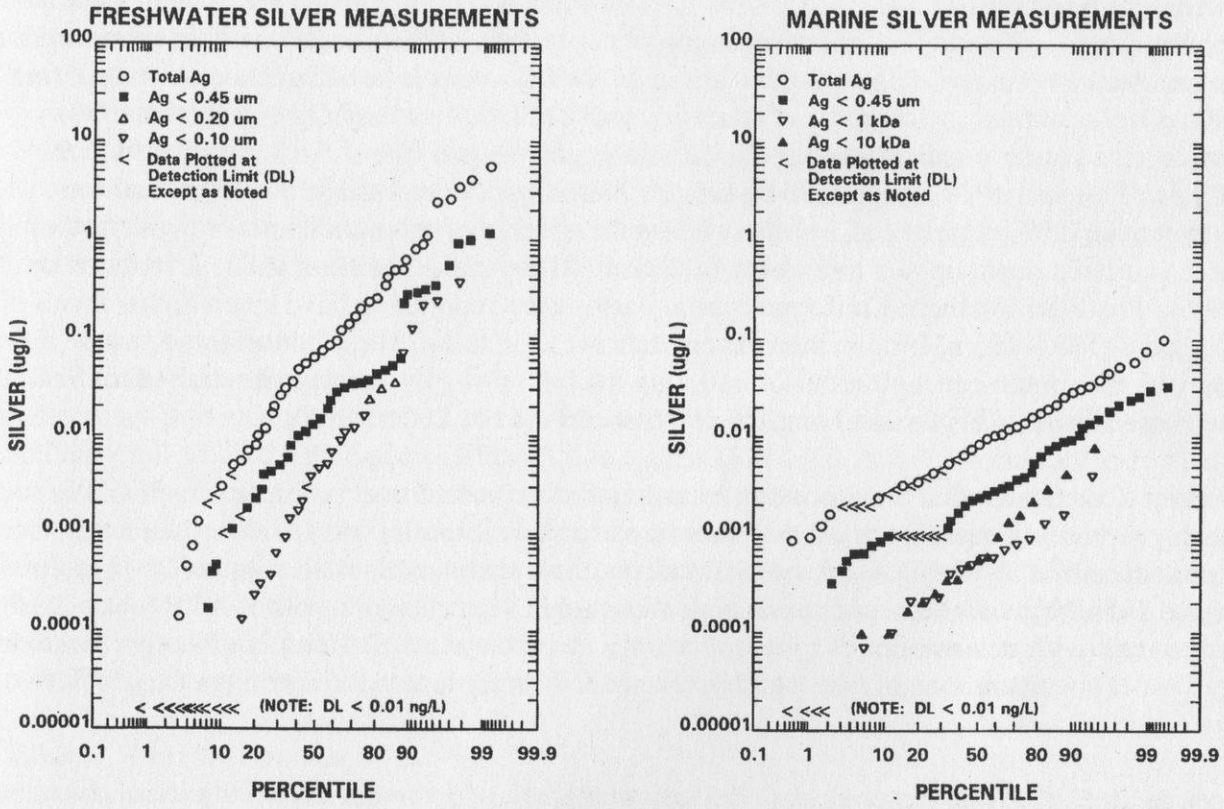


Figure 4.5. Probability Distributions of Ambient Silver Concentrations in Freshwater Settings (left panel: data from Benoit 1995; Wolfe and Fitzpatrick 1996; Wen *et al.* 1997; Gill *et al.* 1997; Shafer et al. 1998) and Marine Settings (right panel: data from Sanudo-Wilhelmy and Flegal 1992; Smith and Flegal 1993; Benoit 1994; Benoit *et al.* 1994; Sanudo-Wilhelmy *et al.* 1996; Wen *et al.* 1997)

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 95-percentile concentrations of total silver are approximately 0.008 and 0.04 μg/L (0.079 and 0.37 nM), respectively. The operationally defined 0.45 μm total dissolved concentrations, median and 95 percentile, are about 0.002 and 0.015 μg/L (0.019 and 0.14 nM), respectively. The probability distribution of data that represent the <1 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.

4.2.4.2 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 US over the last 25 years have ranged from 0.07 to 20 μg silver/gram dry weight of sediment (ug/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 - 3.5 μg/g dw (13.9 – 32.5 nM/g dw; Freeman 1979, as reported by Eisler 1996). Puget Sound sediments (0-20 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 (Bloom and Crecelius 1987, as reported by Eisler 1996). Pre-1986 Southern California coastal basins were reported to have much higher levels of 14 - 20 μg/g dw (130 – 185 nM/g dw; more recent data not available). These concentrations are consistent with maximum concentrations of >10 μg/g dw (>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., > 10,000 nM/g dw) to react with this amount of silver to form the insoluble metal sulfides that incorporate silver. Thus, pore water concentrations of free Ag are expected to be very low in most settings for which data have been reported.

For the period 1992-97, 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 < 1 μg/g dw (9.3 nM/g dw). These concentrations were measured using a two-hour extraction with weak acid (0.6 N HCl), a procedure that is similar to the EPA 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 follows then that pore water silver levels may be elevated, and the potential for toxicity via this route of exposure (i.e., Ag in pore water) exists. However, measured pore water 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 pore water 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.

4.3 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 dissolved organic matter 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 is currently 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 fish.

4.3.1 Acute Toxicity

4.3.1.1 Freshwater Species

As discussed previously (section 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 μg/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., fish) (Figure 2.1, Chapter 2). 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).

4.3.1.2 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 Section 2 (Chapter 2) a relatively robust data set is still available. SMAVs range from 13.3 to 2,700 μg/L (123 to 25,000 nM) across 26 species tested (Figure 2.4, Chapter 2). Similar to the situation for freshwater species, a specific group of invertebrates (larval bivalves and planktonic crustaceans, such as copepods) is more sensitive than fish and other invertebrates.

4.3.2 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.

4.3.2.1 Freshwater Species

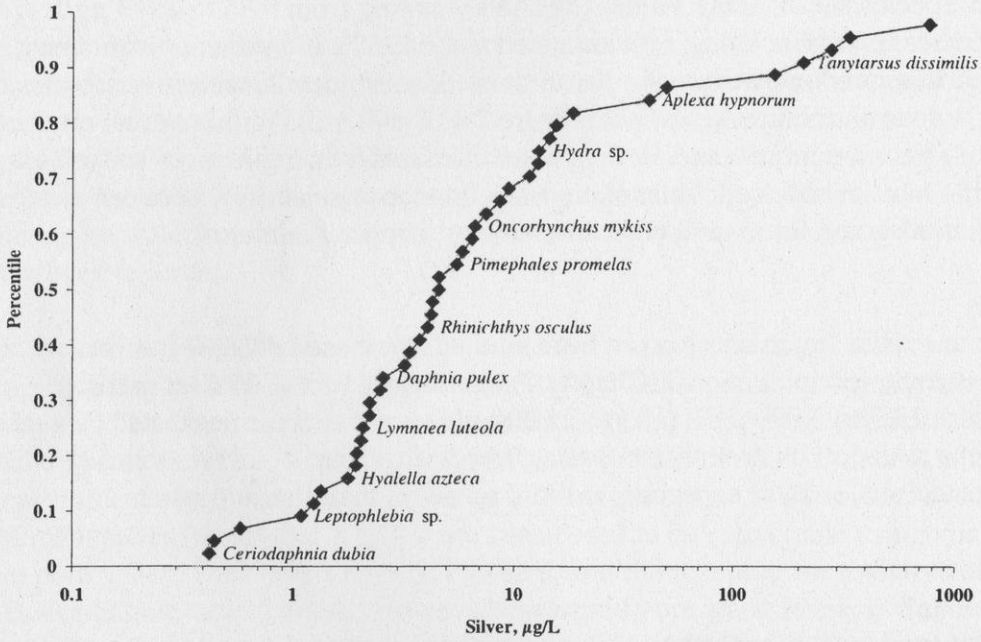
Only eight species have been evaluated for chronic silver toxicity (Figure 2.6, Chapter 2). Unlike the case for the acute data, fish species appear to be more sensitive than invertebrates. Cladocerans in particular are the least sensitive chronically, but the most acutely sensitive species. In fact, 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 has apparently not been properly evaluated for chronic toxicity, it is clear that the chronic potential of silver has not been fully characterized.

4.3.2.2 Marine Species

Pursuant to the review in section 2 (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 µg/L (139 to 806 nM).

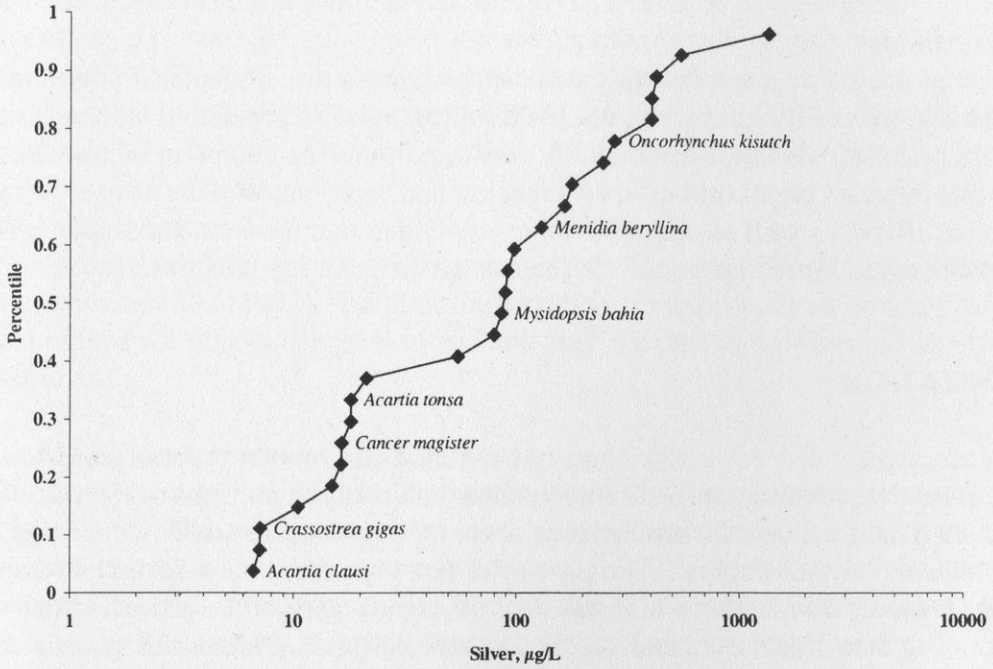
Figure 2. 1 (from chapter 2). Acute sensitivities (SMAV/2) of freshwater organisms to silver.



4.3.3 Tissue residues

As discussed previously 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.

Figure 2.4 (from chapter 2). Acute sensitivities (SMAV/2) of marine organisms to silver.



4.3.4 Chronic Toxicity and the Biotic Ligand Model

It is not known whether the silver-gill model and the related silver-BLM model 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 C, as part of "Use of Models."

4.3.5 Identification of Sensitive Receptors

As discussed previously, 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., fish). 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 fish and other invertebrates.

Only eight 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. As discussed above, only one marine species,

Mysidopsis bahia, has been evaluated for chronic sensitivity to silver, and chronic values for this organism range from 15 to 87 µg/L (139 to 806 nM).

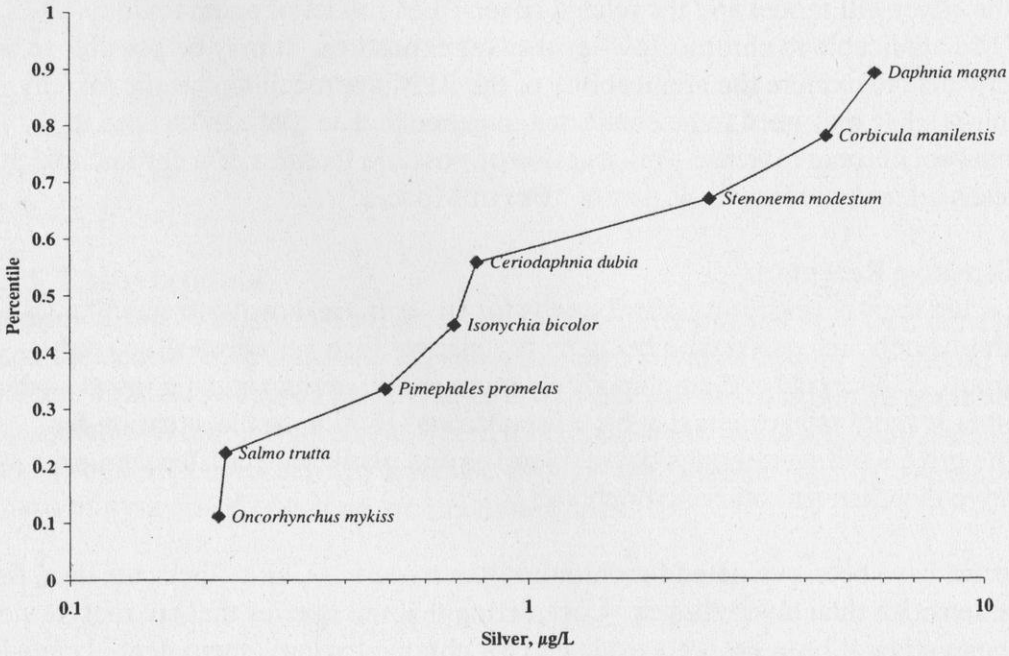
4.3.6 Dietary Effects Thresholds

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 section 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.

4.4 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 1). an estimate of risk to the valued resource/entity, 2). a discussion of the significance of the estimated risk and lines of evidence supporting their likelihood, and 3). the uncertainties, assumptions and qualifiers that are important in the overall assessment.

Figure 2.6 (from chapter 2). Chronic sensitivities of freshwater organisms to silver.



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 basisof 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.

4.4.1 Risk Estimation

Risk estimation is the process of integrating exposure and effects data and evaluating any associated uncertainties (EPA 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.

4.4.1.1 Silver Exposure Characterization

The exposure data for silver in freshwater and marine settings were discussed previously in section 4.2.4 (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 may also exist. However, the exposure profiles are based upon actual measured data. Their use in this risk characterization is based upon the collective judgement 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 μg/L to 1.5 μg/L (0.0093 to 13.9 nM; dissolved silver, <0.45 μm) and as high as 6 ug/L (55.6 nM; total silver) (Figure 4.5, left panel). Similarly, measured concentrations of silver in marine waters vary from about 0.0003 μg/L to 0.04 μg/L (<0.0028 to 0.37 nM; dissolved silver) and from less than 0.001 μg/L to 0.1 μg/L (0.0093 to 0.93 nM; total silver) (Figure 4.5, right panel).

4.4.1.2 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 (section 4.3). For purposes of this screening level silver risk assessment, the freshwater and marine data were presented previously as effects profiles on Figures 1 and 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 species mean acute values (SMAVs) ranging from 0.85 to 1,543 μg/L (7.88 to 14,300 nM) across 43 species tested, including species tested since 1987 (Figure 2.1, Chapter 2). The acute toxicity of silver to marine organisms has a relatively robust data set with SMAVs that range from 13.3 to 2,700 μg/L (123 to 25,000 nM) across 20 species tested (Figure 2.4, Chapter 2).

Chronic Toxicity - The chronic toxicity of silver has not been extensively studied. Only eight freshwater and one 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 μg/L (0.93 to 9.3 nM).

4.4.2 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 and marine water, respectively. The continuous exposure profiles ("max. bioavail. Ag") are based on the silver concentration data presented previously on Figure 4.5 (<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.

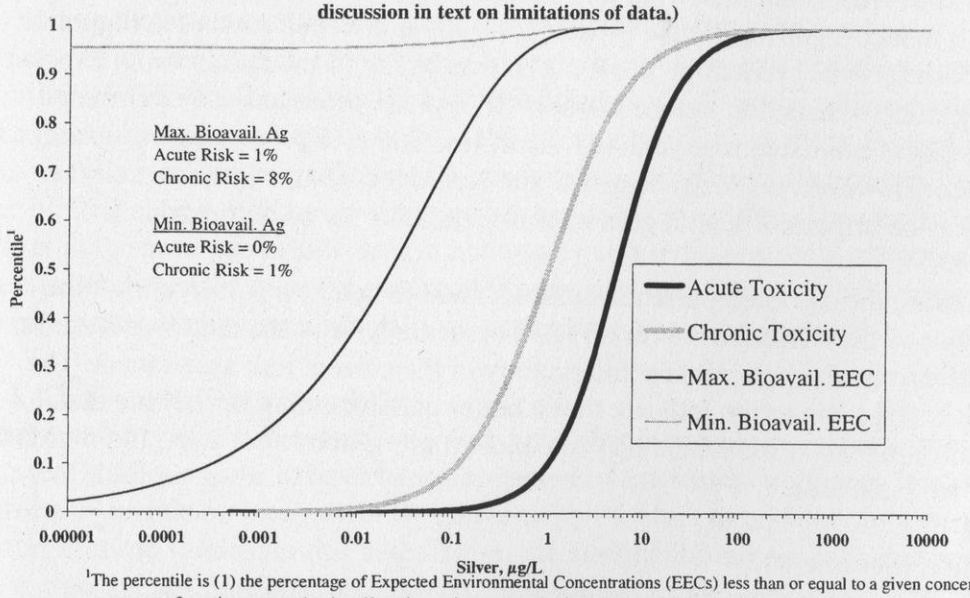
The integrated joint probabilities of the exposure and effects profiles indicate that 1% and 8% of freshwater aquatic species would be at risk on an acute and chronic basis, respectively (Figure 4.6). This analysis integrates both the probability of exposure and the probability of there being an effect. The data indicate that as ambient exposure levels of dissolved silver increase to greater than 0.1 μg/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 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 < 0.45 μm and <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 on the outcome of the risk analysis. These include: a 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 Section 2). While these

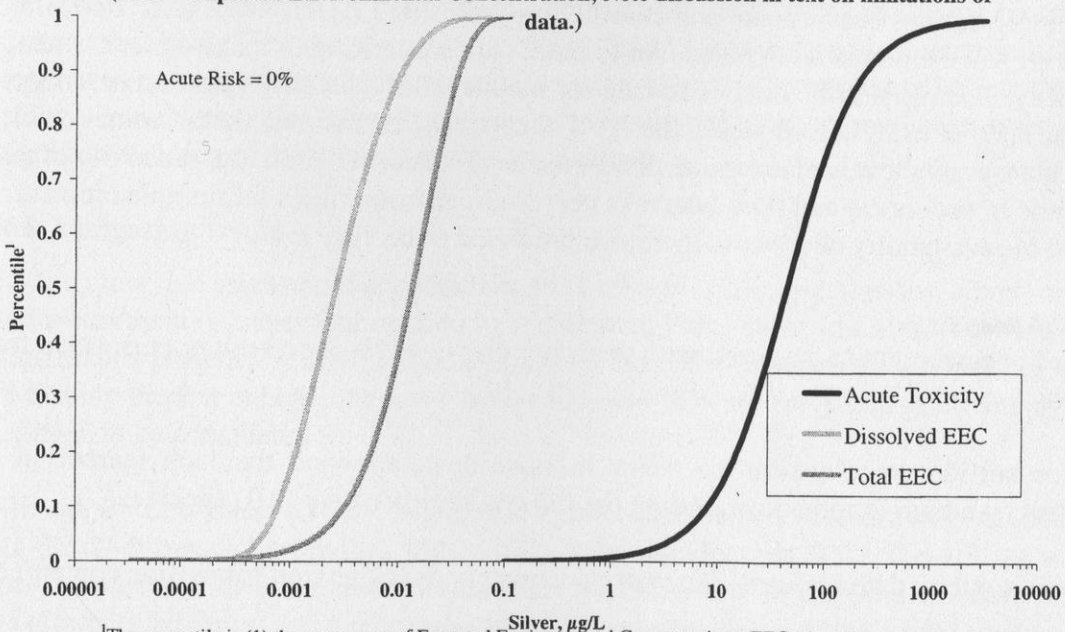
limitations may ultimately be shown to be important or unimportant, the above risk characterization reflects the current state of knowledge at the time of the assessment.

Figure 4.6. Potential acute and chronic risks to freshwater organisms based on maximum and minimum bioavailable silver. (EEC = Expected Environmental Concentration; Note discussion in text on limitations of data.)



[1]The percentile is (1) the percentage of Expected Environmental Concentrations (EECs) less than or equal to a given concentrati percentage of species expected to be affected at a given concentration.

Figure 4.7. Potential acute silver risks to marine organisms. (EEC = Expected Environmental Concentration; Note discussion in text on limitations of data.)



[1]The percentile is (1) the percentage of Expected Environmental Concentrations (EECs) less than or equal to a given concentrati (2) the percentage of species expected to be affected at a given concentration.

To further evaluate the possible significance of sulfides in surface water as potential complexation ligands, the risk to aquatic species was re-evaluated with the assumptions (i) 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 (ii) 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/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 μg/L (0.93 nM) of dissolved silver needs further evaluation.

4.4.3 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 4.2.4.2), based on the limited available data, that pore water 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 pore water silver concentrations below acute and chronic thresholds (0.1 μg/L = 0.93 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, reaction times for complexation are fairly rapid, and the bioavailability of silver is therefore predicted to be very low.

4.4.4 Uncertainty Analysis

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

1. the 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;

2. the lack of chronic toxicity data performed in water adequately characterized for sulfide content; and

3. 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.

4.4.4.1 Sulfide Complexation

Uncertainty is associated with the nature, concentration, and extent of binding of complexing ligands including metastable sulfides and dissolved organic matter. 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) are also unknown. They are also 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.

4.4.4.2 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.

4.4.4.3 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 fish 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.

4.4.5 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. 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 will be present that form complexes with silver and other metals. Other chemical or physical stressors may also 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 biotic ligand model 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.

4.5 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. They are:

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, left and right panels respectively) are usually well below reported acute toxicity levels (Figures 2.1 and 2.4, Chapter 2, 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 (Figure 2.6 in Chapter 2; also, Figures 4.6 and 4.7). 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.

- 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.

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Appendix A - 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 1996). Among metals detected at non-hazardous 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 1996). EPA 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 A1).

Table A1. Estimation of Silver Exposure from Environmental Sources

Route of Exposure	Estimated Intake (μg/day)	Reference
Ingestion – diet	35-88	USEPA 1979
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 1.8m^3/hr and 24-hr exposure

It is necessary to compare these estimates of daily and lifetime exposure to silver to the lowest dose know to result in an adverse effect to humans, the reference dose or RfD, to assess the human health risk to environmental silver. The end point 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 of 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:

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

Evaluating the reference dose for argyria:

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

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:

ADI = 88 μg (diet) + 20 μg (water) = 108 μg

and HI_{daily} = 108/350 μg (RfD) 0.31 < 1

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

LI (100%) = (110 μg/day)(1 g/10^6 μg)(365 days/yr)(70 yr life span) = 2.8 g

Adjusting for an assumed 10% absorption:

LI(10%) = 2.8 g x 10% absorption = 0.28 g

and $HI_{lifetime}$ = 0.28 g / 1 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 hazard index 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 EPA 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.

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Appendix B – Wildlife

A screening level 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):

Indicator Species	Food Source
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 B1) 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 B2). That is, 100% of the food and water that was consumed was from sources having elevated levels of silver. It was also 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 B1. 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[g]	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]NYSDEC (1987); [e]Samuel and Nelson (1982) after Charters and Kracko (1992); [f]Based on ECAO (1987) after Suter (1993) and on reference (c), above; [g]Bleavins, Aulerich and Ringer (1980) after Charters and Kracko (1992) and Bleavins and Aulerich (1981) after USEPA (1993).

Table B2. Use Factors for Computing Dose Via Ingestion of Silver In Food and Water

Indicator Species	Overall Intake (I, & I_w)			Home/Feeding Range (sq. miles)	Use Factors(%):		Food Source[a]
					Area	Diet	
Red-tailed hawk	Food	(g/d)	400	0.5[b]	100	100	SM
	Water	(mL/d)	81	0.23 – 9.5[e]			
Mallard	Food	(g/d)	250	1.1[c]	100	100	AM
	Water	(mL/d)	69	0.14 – 5.6[e]			
Red Fox	Food	(g/d)	320	0.42[b]	100	100	SM
	Water	(mL/d)	500	0.22 – 13.2[e]			
Mink	Food	(g/d)	150	0.031[d]	100	100	SM/Fish
	Water	(mL/d)	95	0.03 – 1.5[e]			

[a]SM: Small Mammals; AM: Aquatic Macroinvertebrates; [b]Charters and Kracko (1992); [c]Bellrose, 1976; [d]Lindscombe et al., (1982) after Charters and Kracko (1992); 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 B3.

Table B3. Summary of Silver Concentrations in Biological Tissues[a] of Prey

		Silver in Food Sources (mg/kg)	
Prey	Basis	Average of Reported Concentrations in Food	Maximum of Reported Concentrations in Food
Sandworm	Dry wt	5.2	30.0
Macroinvertebrates	Dry wt	1.2	5.5
Mollusks	Dry wt	0.14	82.0
Fish	Fresh wt	0.25	1.9
Terrestrial Mammals[b]	Dry wt	0.25	(x10=) 2.5

[a] Eisler 1996

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

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 are

useful with regard to assessing the sensitivity of the analysis to extreme conditions. Silver intake from water was also 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 approach was used for purposes of this screening level analysis. The hazard quotient (HQ) for each of the indicator species, was evaluated as follows:

$$HQ = \text{Average Dietary Intake / Reference Dose} = ADI / RfD$$

A hazard quotient 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 sub-chronic 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 reference dose of 10 mg Ag/kg body weight/day, is used for the hazard quotient analysis for both birds and mammals.

Figure B1 presents a summary of the results of this screening level analysis. The upper panel shows the reference dose as a horizontal line at 10 mg/kg/day. The bars show the average daily intake (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 reference dose in each case. The corresponding hazard quotients 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 reference dose. 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 reference dose.

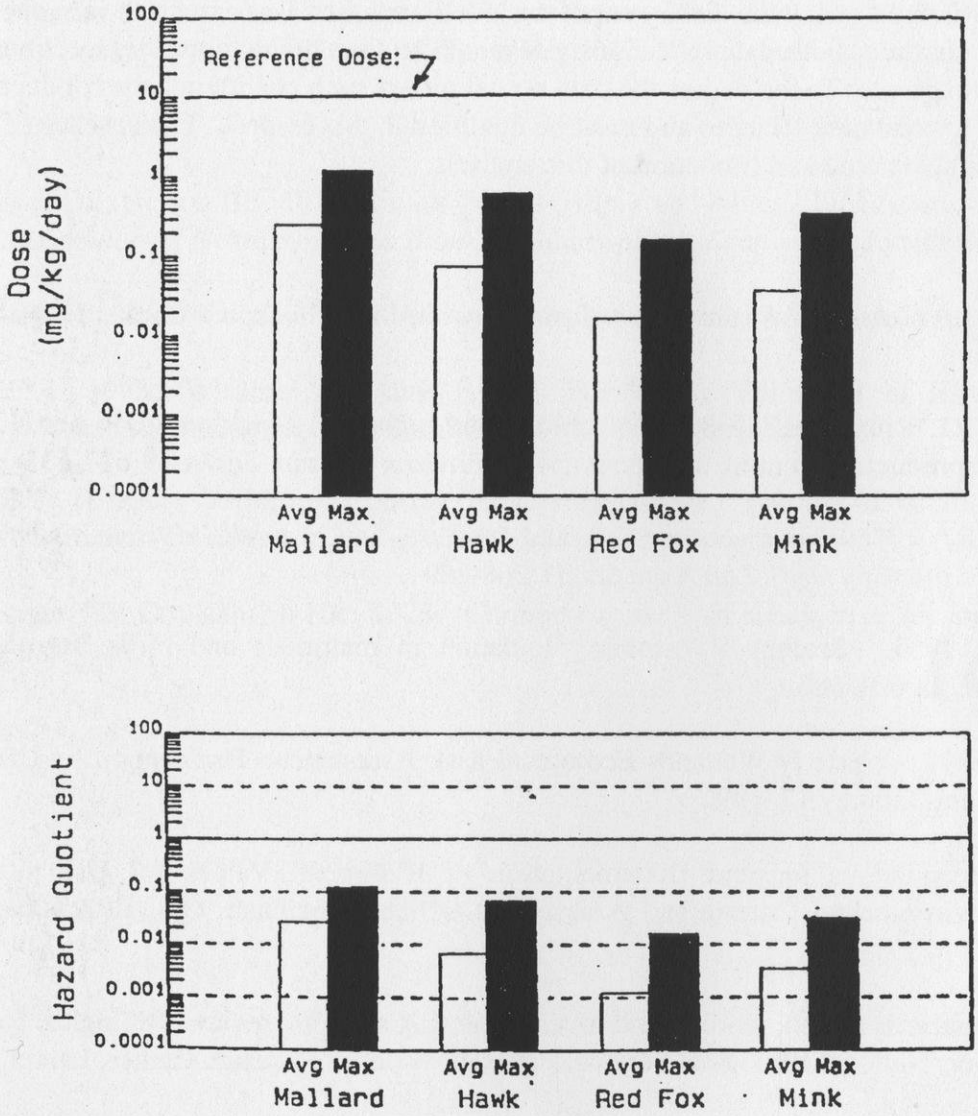

Figure B-1. Results of screening level analyses

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 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 reference dose 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 reference dose for mammals, silver in food is 100% bioavailable, area and dietary use factors = 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 should also 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 is also noted as a limitation of this analysis.

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Appendix C - 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.

C1.1 Fate and Transport Models

As discussed in Section 1, fate and transport models are also 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 can also 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, EPA WQC provide for a frequency of exceedance of once every three 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 ten years on average (7Q10), may be overly protective. Alternatively, it is also 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 Section 1, 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; EPA 1997; Paquin *et al.* 1999)

C1.2 Models to Predict Accumulation and Toxicity

Models such as the silver gill accumulation model of Janes and Playle (1995) and the biotic ligand model (BLM) of acute toxicity of silver (USEPA 1999; Paquin *et al.* 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 biotic ligand model of chronic toxicity be structured? One possible conceptual framework is illustrated on Figure C1 and described below.

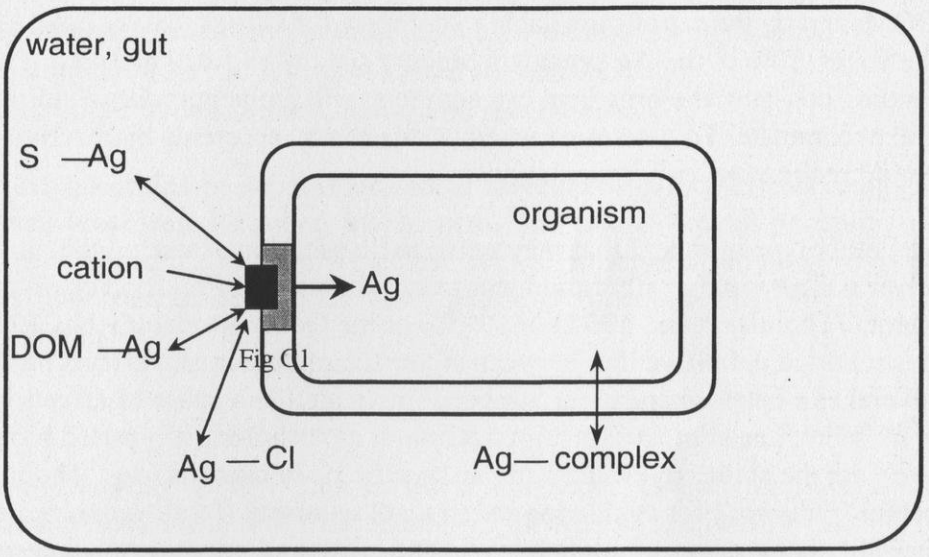

Figure C-1. Conceptual framework for chronic toxicity model

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 μg/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 C1); these cations will include Ca^{2+}, Na^{+}, Mg^{2+}, H^{+}, and possibly other metals (Playle 1998; Simkiss and Taylor 1995).

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 C1). 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 4.2.2

and illustrated on 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 (~0.1 μg/L), either 0 or 10 nM sulfide groups with a log K 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 pharmaco-kinetic (PB-PK) food chain model such as the Thomann Model (Thomann *et al.* 1995a or 1995b) 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.* 1999; 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 *et al.* 1999).

C1.3 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 Section 1, 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 1997 Pellston Conference (Bergman and Dorward-King 1997). With regard to fate and transport modeling of metals (Schnoor *et al.* 1997), 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;
- 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/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 1997 Pellston meeting, much remains to be accomplished.

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Chapter 5
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Chapter 5
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5.1 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 is also important to identify the assumptions and approximations included in these regulatory approaches.

5.2 REGULATORY APPROACH IN THE UNITED STATES

In the United States, the Clean Water Act (CWA) sets forth the legal framework by which all pollutants, including silver, are regulated. The CWA places upon the United States Environmental Protection Agency (USEPA) and the states certain responsibilities to define and achieve acceptable levels of pollutants in surface waters. The USEPA has the responsibility for developing water quality criteria guidance for pollutants. The states use these criteria to fulfill their responsibility for developing water quality standards. 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 water quality standards 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 EPA or an authorized state develops a National Pollutant Discharge Elimination System (NPDES) permit that both achieves water quality standards and implements TMDLs where developed.

The statutory steps for developing maximum allowable discharged loads can be described in two steps. The first is criteria development, which identifies the maximum amount of a pollutant that can exist in the water environment without causing an adverse effect. The second is 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.

5.2.1 Criteria

For toxic pollutants in general, the EPA develops two criteria values, an acute criterion that protects against short-term lethality and a chronic criterion that protects against longer-term sublethal effects (Stephan *et al.* 1985, Stephan 1997). Acute criteria are generally 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 ½ 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 one-hour average concentration that should not be exceeded more than once every three years, whereas the chronic criterion is expressed as a four-day average. Although the guidelines for water quality criteria 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. 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 water quality standards, 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 is also based on the derivation given in the 1980 document. More recently, the EPA 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 1997). For reference, the current freshwater acute dissolved criterion is 3.4 μg/L based on a hardness of 100 mg/L, and the saltwater acute criterion is 1.9 μg/L (USEPA 1999a). 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.

5.2.2 Exposure

The EPA and the states conduct exposure assessments either by directly measuring ambient water concentrations or by using water quality models. Models are more frequently used. The models generally calculate water quality based on conservative mass balance principles applied to pollutants in the water column. Calculations are not generally 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 EPA 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 water quality standards for all pollutants. Of these, 37 waters were identified in nine states as not meeting their water quality standards for silver. These states will be developing TMDLs for these waters over the next eight to fifteen years.

5.3 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, 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 Environment Canada 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 the Canadian Council of Ministers of the Environment and Environment Canada published the Canadian Water Quality Guidelines (CCREM 1987). -Originally written to protect freshwater ecosystems, they 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 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).

5.4 CRITICAL ASSUMPTIONS AND APPROXIMATIONS IN REGULATORY APPROACHES

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

5.4.1 Analytical methods provide reliable results

Regulatory decisions to impose permit limits are generally 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 may be invalid due to sample contamination (Bergman and Dorward-King 1997). Errors of three to four orders of magnitude have been observed when clean techniques are not used to measure concentrations below the 1 mg/l (Adeloju and Bond 1985; Berman and Yeats 1985; Bloom 1993; Shafer *et al.* 1993). 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 one part per million, 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 can be often below 1 µg/l. Also, recent literature suggests that silver may sorb onto Teflon bottles (Herrin *et al.* 2001). Silver can also be easily removed from Teflon bottles using sonication and ultraviolet irradiation (Wen *et al.* 1997).

5.4.2 Dissolved form causes acute toxicity

Dissolved metal is operationally defined as the metal in a solution that passes through a 0.45 um 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 water quality criteria 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 Doward-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.

5.4.3 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 was typically treated to remove contaminants by use of such techniques as chlorination/dechlorination, activated carbon ion exchange, and reverse osmosis treatment. Unfortunately, these techniques also removed dissolved organic matter and sulfides, which are important in modifying toxicity. This approach provides a baseline evaluation of the inherent toxicity of a pollutant to ensure that water quality criteria based on these tests will be protective of all U.S. waters. This approach characterizes toxicity tests used by the U.S. EPA to develop water quality criteria 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.

5.4.4 WER 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 water quality criteria to reflect local water chemistry and its effects on the toxicity of a pollutant. Although water quality criteria 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 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.

5.4.5 Chronic effects can be related to acute effects

The USEPA generally develops chronic criteria by applying an acute-to-chronic ratio (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.

5.4.6 Sediment toxicity is addressed through equilibrium partitioning sediment guidelines

The USEPA currently interprets its water quality criteria to be protective of organisms in the water column (Prothro 1993). The USEPA intends to address sediment toxicity through use of equilibrium partitioning-based numerical sediment guidelines (ESG) (USEPA 1999b). 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.

5.4.7 Total-to-dissolved empirical relationships work in the water column

In the United States, 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).

5.4.8 Dilution is the 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 re-suspension from sediments may be considered.

5.4.9 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).

5.4.10 Water quality standards are independently applied

USEPA policy interprets water quality criteria to be independently applied (Davies 1991). This policy, which is known as "independent applicability," includes three concepts: each of three criteria types (chemical-specific, whole effluent toxicity, and biological assessments) are considered to have equal weight when assessing water quality; water quality standards are considered to be exceeded if any criterion is exceeded; and 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 water quality criteria for a toxic pollutant. Instead, the policy requires the EPA 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 is also 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.

5.5 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 acknowledgement of the apparently ubiquitous, low-level presence of reduced, complexed reactive sulfides (defined as acid-reactive sulfide) 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 of the chronic toxicity for these reduced silver-sulfide complexes. These recent revelations have led to a re-evaluation 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:

Acid-reactive sulfide is consistently present in both oxic and anoxic ambient waters at concentrations greater than silver (on a molar basis).

The acute toxicity of silver-sulfide complexes is believed to be less than that of free silver.

5.5.1 Acute Toxicity Endpoint: Recommendations

Given the assumptions discussed above, the potential for acute toxic effects due to silver in most publicly owned treatment works (POTW) 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:

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 biotic ligand model (BLM) for the acute toxicity of silver as a method for adjusting ambient water quality criteria 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 under- 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 establish modified toxicity test procedures that allow for the reintroduction of acid-reactive sulfide, dissolved organic matter, 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.

5.5.2 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 are also 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. It is therefore important that we modify chronic toxicity test protocols 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 we refine chronic toxicity test protocols and rebuild the data set.

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:

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 we concentrate on sensitive species and ecologically relevant endpoints.

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 water quality criteria, consider an interim approach using current chronic data with site-specific modifications for acid-reactive sulfide. Tests need to be done to validate this approach within two 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 Section 6, Chapter 2). 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.

5.5.3 Additional Recommendations

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. We need to add the acid-reactive sulfide methodology to EPA and state ambient monitoring programs, and we need a standard (approved) analytical procedure.

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 EPA's existing guidance on total/soluble relationships and WERs in assessing surface waters and in permitting discharges until new water quality criteria 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 filer when determining dissolved silver.

The EPA and the states should include the sediment pathways and sulfide calculations in their exposure models for metals.

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Glossary

Transport, Fate and Effects of Silver in the Environment

Madison, Wisconsin, USA

GLOSSARY

ACR (Acute-to-chronic-ratio) – The ratio of the acute toxicity of an effluent or a toxicant to its chronic toxicity. It is used as a factor 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 Sec. 403).

Acute criteria (also called Criteria maximum concentration, CMC) – The EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed for a brief period of time without causing an acute effect (USEPA 1991).

Acute toxicity – Toxicity typically elicited during or immediately following relatively 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 typically considered acute if observed in 96 hours or less for fish and microinvertebrates, and shorter times (e.g., 48 hours) for organisms with shorter life spans. When referring to aquatic toxicology or human health, an acute effect is not always measured in terms of lethality.

ADI (Average daily intake) – The 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 of concern.

Ag+ – The 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 (i.e., the water column) is zero.

APDC (Ammonium pyrrolidinedithiocarbamate) – A thiol-based complexing agent used in metal analysis and speciation (also see DDDC).

Area use factor – The 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 – An 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. For example, salmon are a valued ecological entity; reproduction and population maintenance of salmon form an assessment endpoint.

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

ATPase (Adenosine triphosphatase) – A 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).

BAF (Bioaccumulation factor) – The 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.

BB (Body burden) – The concentration of a constituent within the body tissues of an organism. In some specific circumstances this is used to mean the mass of constituent in the organism (i.e., total content) rather than the mass per unit weight (i.e., concentration).

BCF (Bioconcentration factor) – The 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 – A standard by which something can be measured or compared to in order to assess the likelihood of effects.

Binding site – (Within organisms): A 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 – The process by which an organism accumulates a chemical constituent both directly from the surrounding abiotic environment (i.e., water, air, soil) 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 – A 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 – The fraction of a bioavailable contaminant that may react with biochemically active sites, thereby causing toxicity (i.e., interruption of normal function).

Biospeciation – The distribution of a constituent among compartments within animal tissues (e.g., cytoplasm, cytosol, granules; within the cytoplasm, subcompartments include macromolecules that may contain biochemically active sites, associations with metallothioneins, organelles, membranes, etc.). Biospeciation determines trophic transfer and bioreactivity.

Bioturbation – The process whereby the benthic sediments of a water body are physically mixed by the activity of aquatic organisms that live in the sediments.

BLM (Biotic Ligand Model) – A 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 (i.e., the site of action of toxicity to the organism, called the "biotic ligand") or by the metal-induced impairment of a specific metabolic function (such as ATPase activity), given the water quality characteristics of the water body and the amount of metal dissolved in the water.

CC (Chronic criteria, also called Criteria continuous concentration, CCC) – The EPA 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 – see CC

CCME – Canadian Council of Ministers of the Environment

CCREM – Canadian Council of Resource and Environment Ministries

CDF (Cumulative distribution function) – see Probability distribution

CEPA – Canadian Environmental Protection Act

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. Chronic means "long-term." (USEPA CWA Sec. 403).

Chronic toxicity – Toxicity resulting from long-term exposure to a toxicant.

Clean sampling methods – In the most general sense, 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 2-5% 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 (e.g., polyolefins, Teflons); exhaustive cleaning of bottles, filters, and sampling equipment; preparing and preassembling 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.

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

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. L&O 42(3), 519-528).

Competition – Two or more molecules contesting for the same binding site.

Complexation – The chemical binding or association of a metal with a ligand or ligands.

CWA – U.S. Clean Water Act

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

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) – The exposure concentration(s) that is(are) predicted to occur at a site under a given set of conditions. It may be determined on the basis of field monitoring data and/or modeling.

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(s) identified during problem formulation are technically evaluated and summarized as stressor-response profiles.

Elimination – Used in this volume as equivalent of depuration (which see).

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.

ESG (Equilibrium Sediment Guideline; also known as Equilibrium Partitioning Sediment Guideline) – EPA'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 (e.g., metal mixtures or polycyclic aromatic hydrocarbon mixtures) 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).

Exposure – The contact or co-occurrence 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(s) 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).

Free ionic silver – see Ag+

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 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 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. Its 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.

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 reprecipitated at low pH. Humic acids tend to be large molecules with significant acid-base and metal binding properties in aquatic systems.

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 (e.g., a red-tailed hawk for the class of raptors).

Ionic strength – A measure of the total number of ions in solution; $I = 1/2\ \Sigma C_i * z_i^2$, 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 $[M_d]$ / LC50, where $[M_d]$ is the dissolved metal concentration in the interstitial water and LC50 is the concentration of the metal causing 50 percent mortality of the test species in a water-only test.

K_{cond} (Conditional equilibrium binding constant) – The ratio of $[MxLy]/\{[M]^x\ [L]^y\}$ at specific conditions(e.g., constant pH and ionic strength) where [] denotes concentration. In the above, xM + yL = MxLy.

K_d (Distribution coefficient) – The ratio of solid to operationally defined liquid concentrations of a specified substance (e.g., silver). 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 * L). K_f' is the conditional formation constant, usually expressed in concentration terms.

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 (e.g., 96-hr. LC10).

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 (e.g., 96-hr. LC50).

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-Effects Level) – The lowest concentration of chemical agent being tested which produces an adverse effect in the organism undergoing the test.

LOEC (Lowest-Observed-Effects Concentration) – The lowest concentration of chemical agent being tested which produces an effect, either adverse or nonadverse, 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

Macroparticle – 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 EPA-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 (e.g., volume) 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 (i.e., toxicity) 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.

NOAEL (No-Observed-Adverse-Effects-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-Effects-Concentration) – The highest concentration of chemical agent being tested which produces no observed effect, either adverse or nonadverse, 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

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

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 United States. A permit is required for every discharge of pollutants.

Oxic water column – Condition in a water body where the dissolved oxygen concentration in the overlying water (i.e., the water column) is greater than zero.

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 storm water 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 works) – 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 (e.g., concentration), 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.

Receptor – The individual (human or otherwise) for whom exposure (i.e., contact under a defined set of conditions to a contaminant of concern) 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 divalent 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.

Screening analysis – An approach which uses health-conservative default assumptions in the representation of variability/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 accumulation 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 LC50s or EC50s) 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 oxic 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.

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.

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 (Ultra-violet 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.

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 on male organisms is commonly used as a biomarker of exposure to estrogenic chemicals.

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 LC50s. Water-effect ratios 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 EPA, 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).

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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.

Poster Session

Transport, Fate and Effects of Silver in the Environment

Madison, Wisconsin, USA

Silver Uptake and Depuration in Rainbow Trout in the Presence and Absence of Thiosulfate

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The gills are an important target of waterborne metals and have many vital physiological functions, including gas transfer and ionoregulation (Playle, 1998). On a molar basis silver (Ag) is one of the most toxic metals to fresh water fish, primarily when it is in its free cationic form (Ag^+, Janes and Playle, 1995). The main toxic effect of Ag exposure is interference with Na^+ and Cl^- uptake at the gill (Wood *et al.*, 1996). There are a number of negative binding sites on the gill epithelium, which are competed for by Ag^+ and other cations. The amount of Ag^+ available in the water can be reduced by complexation with a variety of naturally occurring and synthetic ligands (Le Blanc *et al.*, 1983). Once Ag^+ is bound to the gill it can enter the plasma, become associated with plasma proteins and may then eventually be stored in the liver (Wood *et al.,* 1999).

One of the main sources of Ag in the aquatic environment is silver thiosulfate from photoprocessing effluents (Purcell and Peters, 1998). Thiosulfate is an anionic synthetic photoprocessing fixer used in film developing (Purcell and Peters, 1998). Complexation of Ag^+ with thiosulfate can prevent Ag^+ from binding to the gill (Janes and Playle, 1995) and thus exert a protective effect against Ag toxicity. Thiosulfate in water could theoretically act as a sink in depuration of Ag accumulated by a fish or may even be able to pull bound Ag from the gills. Silver in the plasma may be quickly pulled into the liver which may itself act as a sink for Ag. The purpose of our experiments was to determine the effectiveness of thiosulfate in reducing the interactions of Ag^+ with the gills, and its role in reducing the physiological effects of Ag on the fish. We also examined the ability of thiosulfate to act as a sink for Ag depuration, and therefore its ability to increase Ag depuration rates in fish previously loaded with $AgNO_3$.

For the Ag uptake experiment rainbow trout (*Oncorhynchus mykiss ~50 g*) were acclimated to ion-poor (soft) water for one week. For each treatment, 25 fish per replicate were exposed to ~0.1 μM Ag (as $AgNO_3$) with or without ~5 μM $Na_2S_2O_3$ in a flow-through system. At each sample time, five fish were sampled per duplicate exposure after four h, 24 h and one week exposure using MS222 as an anaesthetic. The gills and livers were removed by dissection and blood was drawn using the caudal puncture technique. Plasma Ag was measured using graphite furnace AAS, and digested gills and livers (5x weight in 1 N HNO_3) were diluted (10x with E-pure water) and analysed for Ag also using graphite furnace AAS. Water and plasma Cl were assayed using Sigma reagents, while plasma and water Na and Ca were measured with flame furnace AAS. Hemoglobin, hematocrit, and lactate were also measured.

For the Ag depuration experiment, soft water acclimated rainbow trout (~50 g) were first exposed to ~0.1 μM $AgNO_3$ for one week. The trout were then placed in silver-free soft water with or without ~5 μM $Na_2S_2O_3$. For each replicate, five fish per group were sampled at zero, one, two, four, six, eight and 13 days of depuration or until no fish were left for that treatment. Blood, gills, and livers were sampled and analysed in the same manner as in the Ag uptake experiment.

At all sample times the fish exposed to $AgNO_3$ alone had significant accumulation of Ag on the gills (Figure 1A, asterisks). In contrast, at no sample time did the $AgNO_3$ plus thiosulfate exposed fish have significantly more gill Ag than the control fish. The Ag plus thiosulfate exposed fish usually had a significantly lower gill $AgNO_3$ concentrations than the Ag only exposed fish (crosses). These results indicate that thiosulfate had strong protective effect against Ag binding to the gills by complexing Ag^+.

No significant differences in plasma Cl were seen at the four and 24 h sampling times between any of the groups (Figure 1B). However, at one week both the $AgNO_3$ alone and the $AgNO_3$ plus thiosulfate fish had lower plasma Cl concentrations than the control fish (asterisks). In addition, the $AgNO_3$ alone exposed fish had significantly lower plasma Cl than the Ag plus thiosulfate exposed fish, indicating that the presence of thiosulfate reduced the ionoregulatory effect of the $AgNO_3$ but did not completely prevent it. There were very few mortalities observed among the Ag exposed fish (80% $AgNO_3$ only, 87% $AgNO_3$ plus thiosulfate, vs 100% survival for the control fish) indicating that the ionoregulatory effect of the added $AgNO_3$ was stressful but not very severe.

Trout exposed to $AgNO_3$ for one week had between four and 10 nmol Ag/g wet tissue on their gills (Figure 2A, day 0). These fish did not lose Ag from their gills over one week in Ag-free water, with or without thiosulfate present. All fish in the $AgNO_3$ treatment groups at Day 0 were exposed to the same conditions and were randomly assigned to the treatments, therefore, there should not have been a difference in gill Ag levels between the two treatments at day 0 (crosses). By day eight, the two treatments had similar gill Ag concentrations and it is apparent that the thiosulfate did not increase the depuration of Ag off the gills.

No significant differences in plasma Cl were seen at any sampling time between all treatment groups (figure 2B). The fish loaded with $AgNO_3$ for one week exhibited an increase in plasma Cl concentration as the depuration period went on. This may have indicated a recovery of the Na/K ATPase enzyme activity which is affected by Ag^+ binding to the gill. However, since gill Ag concentrations did not decrease it appears the fish were able to reverse the ionoregulatory effects of $AgNO_3$ without necessarily removing Ag from their gills.

Plasma Ag concentrations in the fish loaded with Ag were similar for the fish of both treatments from day 0 until day four (figure 3A), and were usually significantly greater than control fish throughout the depuration period (asterisks). At day four the fish held in Ag-free water with thiosulfate had significantly lower plasma Ag concentrations than did fish held in Ag-free water alone (crosses). However, the final plasma Ag concentrations in fish from both treatments were not significantly lower than initial values. Therefore, there was a hint - but not strong evidence - that thiosulfate increased depuration rates of Ag from the plasma.

Except at time zero, there was no significant difference in liver Ag concentrations between the Ag-loaded fish held in Ag-free water with or without thiosulfate (figure 3B). The concentrations of Ag in the livers did not decrease over the depuration period and even increased slightly. This increase was most likely attributed to the redistribution of plasma Ag into the liver as seen in previous studies (Wicklund *et al.*, 1988). The presence of thiosulfate during depuration did not increase the elimination of Ag from the liver.

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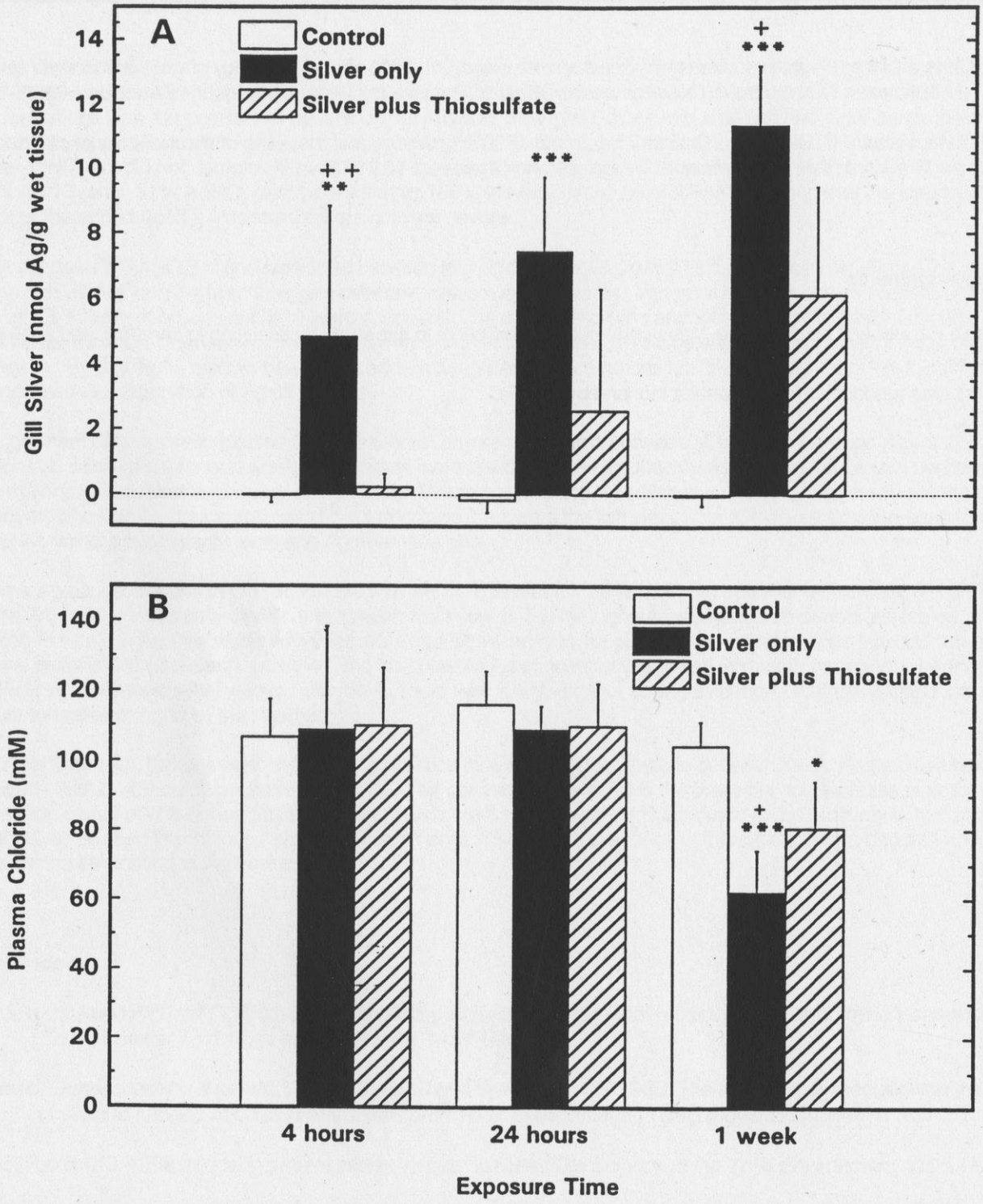

Figure 1. Thiosulfate was able to partially protect against Ag uptake at the gills over the entire week exposure to 0.1 μM $AgNO_3$(A). Thiosulfate was not able to prevent a drop in plasma Cl after the week exposure to $AgNO_3$(B). For all figures, asterisks indicate significant difference between the treatment group and the control group at that sample time (ANOVA *=P<0.05, **=P<0.01 and ***=P<0.001). Crosses indicate significant differences between the two $AgNO_3$ groups of fish.

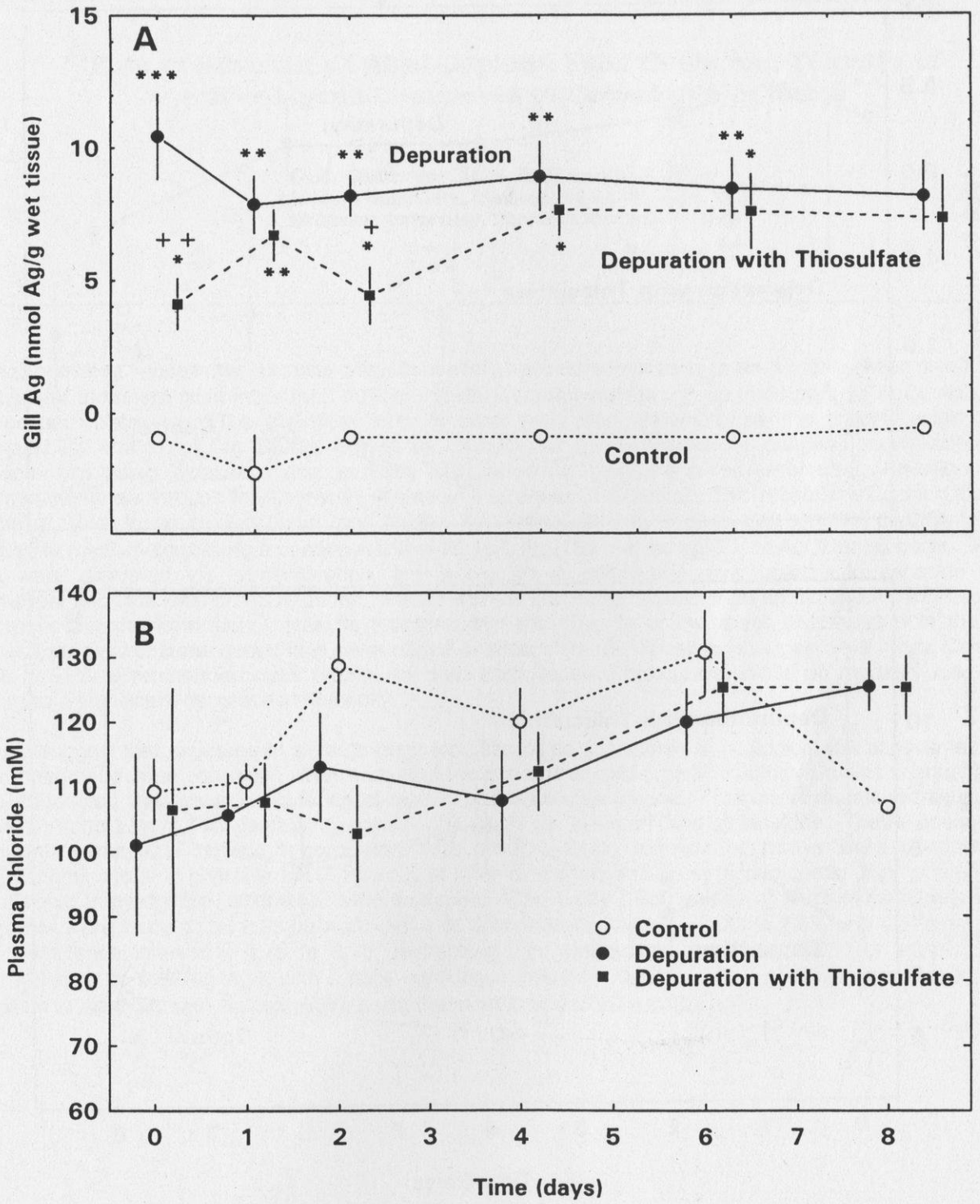

Figure 2. Thiosulfate was not able to increase the rate of depuration of Ag from the gills (A). Although not statistically different, there was a tendency for plasma Cl to increase at the end of the depuration period (B). This increase in plasma Cl may indicate the ability of the fish to recover ionoregulatory function once in Ag-free water without necessarily removing Ag from the gills.

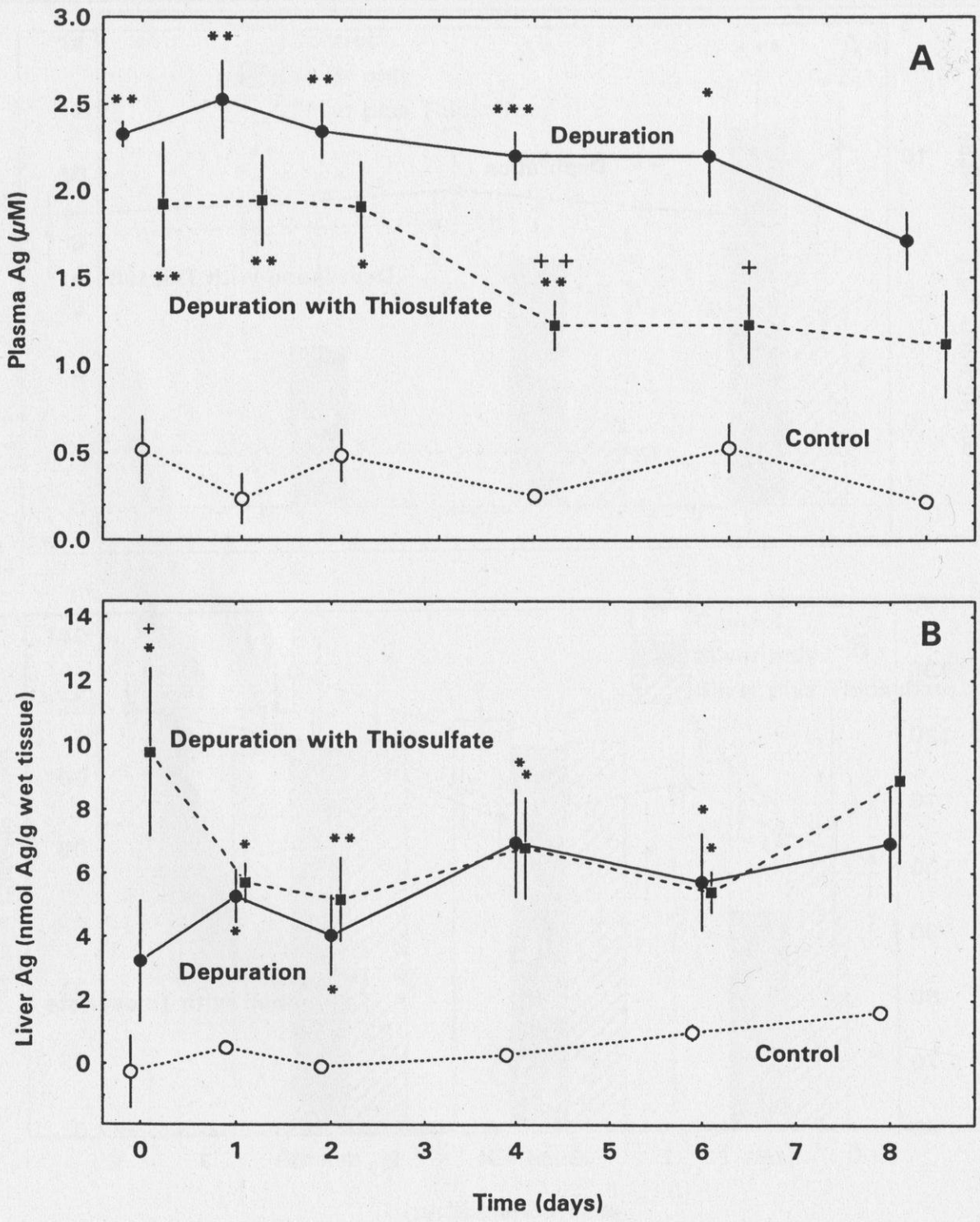

Figure 3. Thiosulfate slightly increased the removal of Ag from the plasma compartment of the fish (A). However, there is no statistical difference between the initial and final Ag concentration in the plasma of the fish in either depuration condition. Thiosulfate did not increase Ag removal from the liver (B). The Ag concentration in the liver showed a slight increase over time, indicating that some Ag from the plasma was being relocated to the liver for storage.

Fate of Silver in an Algal-Daphnid Food Chain and Toxicity of Silver-Ligand Complexes to *Ceriodaphnia dubia*

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Photoprocessing wastewater contains silver thiosulfate concentrations as high as 5 mg/L. After going through a wastewater treatment plant more than 90% of the silver is removed leaving up to 50 µg/L as AgCl, AgS, colloidal Ag, or particulate Ag. The chemical form of silver has been shown to be the critical factor controlling bioavailability and toxicity to aquatic organisms. Toxicity of silver to aquatic organisms has traditionally been characterized using bioassays that evaluate only aqueous exposure of silver nitrate. Aquatic organisms accumulate metals through food consumption as well as aqueous exposure. This research examined the fate and effects of silver in an algal-daphnid food chain. The unicellular green alga, *Selenastrum capricornutum*, was cultured in medium containing a concentration of 20 µg/L Ag (185 nM) as $AgNO_3$ or AgCl for 96 hours. Harvested cells were disrupted via ultrasonication, and subjected to differential centrifugation to separate cell wall, organelles, and total membrane fractions. Pellet fractions and supernatants were analyzed for silver using atomic absorption spectrophotometry. Results indicated that the majority of the silver accumulated in the cell wall fractions for algal cultures dosed with silver nitrate or silver chloride. Silver-laden algae were fed to *Ceriodaphnia dubia* at EPA-recommended rates during standard static renewal bioassays. While no mortality was observed there was a significant decrease in fecundity.

Results suggest that exposure of silver through food should be considered in ecological risk assessments. In the environment and in an organism, silver complexes with inorganic and organic ligands. Studies in aquatic systems have suggested that silver is transported mainly in the colloidal phase. Transition metals, particularly silver, interact strongly with thiol-containing compounds such as cysteine and glutathione. These environmentally important mercaptans form 1:1 complexes with silver. Static renewal bioassays were conducted using *Ceriodaphnia dubia* to characterize the toxicity of silver cysteinate and silver glutathionate. Both acute (mortality) and chronic (reproduction) endpoints were measured. The 8-day LC50 values of silver glutathionate and silver cysteinate were found to be 2.23 µg Ag/L with a 95% confidence interval = 1.73 to 2.88, and 4.67 µg Ag/L with a 95% confidence interval = 3.76 to 5.78, respectively. In comparison, the 8-day LC50 of silver nitrate was determined to be 0.32 µg Ag/L with a 95% confidence interval = 0.19 to 0.54. All three compounds caused a reduction in fecundity with Ag cysteinate being the most toxic (LOEC = 0.002 µg/L).

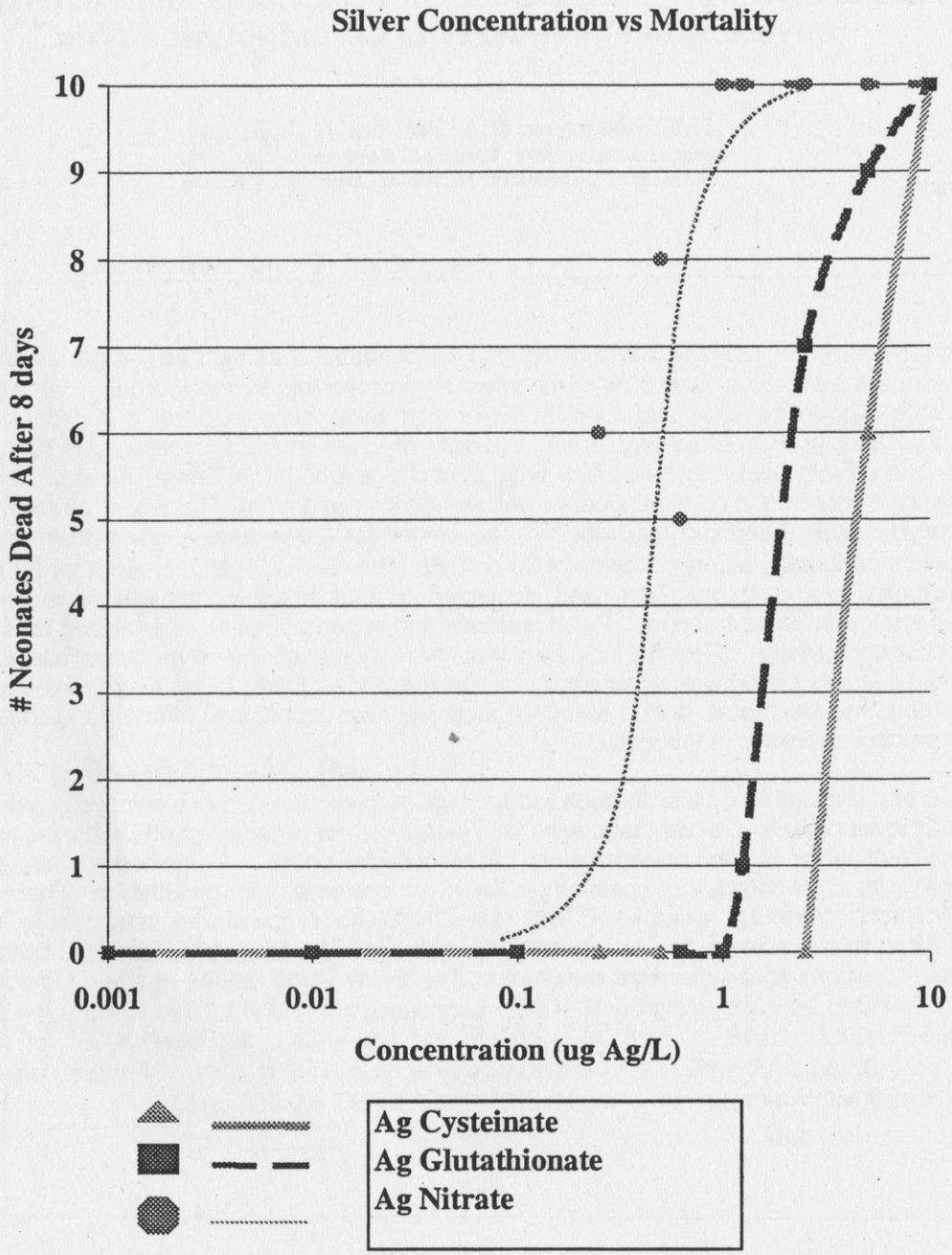

Figure 1. Dose response curve for Ag exposures to *C. dubia*

Table 1. The LC50 values of Silver Compounds at 48 hours, 96 hours, and 8 days

Ag Compound	**48h LC50**	**96h LC50**	**8d LC50**
Ag NO_3	0.5±1.2 µg/L	0.5±1.2 µg/L	0.3±0.2 µg/L
Ag glutathionate	_____	2.5± 0.7 µg/L	2.2±0.5 µg/L
Ag cysteinate	4.7±0.9 µg/L	4.7±0.9 µg/L	4.7±0.9 µg/L

Table 2. NOEC and LOEC values for silver compounds

Ag compound	**NOEC (µg/L)**	**LOEC (µg/L)**
$AgNO_3$	0.001	0.01
Ag glutathionate	0.1	0.6
Ag cysteinate	*	0.001

* Significant decrease in fecundity observed at lowest concentration tested (0.001µg/L)

The Effect of Varying Water Quality Conditions on Acute Silver Toxicity to *C. dubia* and *P. promelas*

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Introduction

Water quality criteria (WQC) are derived from results of acute and chronic toxicity tests with aquatic organisms exposed to a concentration gradient of the chemical (Stephan et al. 1985). These laboratory tests are typically conducted with waters low in suspended solids and organic carbon concentrations in order to maximize chemical bioavailability and provide a conservative estimate of concentrations at which effects occur (*ibid.*). For silver, with the exception of hardness, the WQC do not account for the variations in water quality characteristics. However, silver toxicity in ambient waters can differ substantially from toxicity in laboratory waters due to differences in pH, hardness, alkalinity, chloride, sulfide, suspended solids, and dissolved organic carbon (DOC) (Bury et al. 1999, Erickson et al. 1998, Janes and Playle 1995). In addition to factors influencing metal bioavailability, it is now recognized that for most metals, the ionic form of the metal is the dominant toxic moiety and so metal speciation is also important (Allen and Hansen 1996). Currently, WQC for metals in most areas of the United States are based on dissolved rather than total recoverable metal concentrations to provide a better estimate of the bioavailable fraction (Prothro 1993).

In this study, we determined the effect of varying pH, DOC, and chloride on the acute toxicity of dissolved silver to *Ceriodaphnia dubia* (daphnids) and *Pimephales promelas* (fathead minnows). Acute toxicity was determined in laboratory water, river water, a 1:1 mixture of river water and municipal effluent, and whole municipal effluent.

Materials and Methods

Four solutions of varying organic carbon content were evaluated in this study: a synthetic laboratory water (<1.0 mg/L DOC), a river water sample (2 to 4 mg/L DOC), a municipal effluent (7 to 9 mg/L DOC), and a 1:1 mixture of effluent and river water (4 to 9 mg/L DOC). The synthetic laboratory water was prepared according to U.S. EPA (1993). The municipal effluent was a grab sample collected from the City of Spokane POTW, in eastern Washington. The river water sample was a surface grab sample collected from the Spokane River approximately 100 meters upstream of the POTW outfall. The effluent and river water samples were shipped on ice, stored at 4 °C in the dark, and used for testing within 48 hours of collection.

Toxicity tests were conducted with the cladoceran, *Ceriodaphnia dubia* and the fathead minnow, *Pimephales promelas*. *C. dubia* were obtained from laboratory stock cultures and were ≤ 24 hours old at test initiation. *P. promelas* were purchased from Aquatic BioSystems, Fort Collins, Colorado, and were seven days old at test initiation. The static acute tests were conducted according to standard U.S. EPA guidelines (U.S. EPA 1993). The test chambers were 30 ml polypropylene cups for *C. dubia* and 250 ml HDPE beakers for *P. promelas*. Four replicates were used in each concentration, and each replicate contained either five (*C. dubia*) or 10 (*P. promelas*) organisms. Test organisms were not fed during the 48-hour exposure. Water quality parameters (dissolved oxygen, pH, and temperature) were measured daily in one replicate from each test concentration in each experiment.

Nominal silver test concentrations were prepared by spiking silver into the test waters using a silver stock solution made from $AgNO_3$ (CAS #7761-88-8). The dissolved silver concentrations were determined at test initiation and LC50s are expressed in terms of the measured dissolved metal concentration.

As this study evaluated the influence of pH as well as organic carbon sources, each water and effluent solution was tested at pH 6.5, 7.5, and 8.5. Conducting the tests in gas-tight chambers and adjusting atmospheric carbon dioxide concentrations within the chambers controlled the pH. This was accomplished similar to the methods described by Mount and Mount (1992). The gas-tight chambers used were 36 x 28 x 4 inch glass boxes sealed with silicone. The 38 x 30 inch removable top was sealed to the walls of the chamber via a silicone gasket. To lower the pH to 6.5 and 7.5, a measured amount of CO_2 was injected into the chamber via a 2 L Hamilton® gas-tight syringe. To raise the pH of each water and effluent solution to 8.5, the pH was initially adjusted to 8.5 using 1N NaOH and then maintained at this pH by flowing CO_2-free air (21% O_2/ 79% N_2) through the chamber at a rate of 25 ml/second. The continuous injection of CO_2-free air in the pH 8.5 tests was necessary because bacteria and test organisms are continuously generating CO_2 in the test system. The pH of each test was monitored daily, and each time the chambers opened, the air inside was purged and then either the appropriate amount of CO_2 was injected or the CO_2-free air flow was re-initiated. Test solutions were allowed to equilibrate for approximately 2 hours prior to testing organisms being introduced to the test system.

Subsamples of each test concentration were collected and filtered at test initiation. The dissolved silver concentrations were determined by inductively coupled plasma-mass spectrometry using EPA Method 1638 (U.S. EPA 1996b). Detection limits varied depending on the method used, but were always at least a factor of three lower than the lowest concentration tested, excluding the control.

In addition, the dissolved concentrations of total solids (EPA Method 160.1); organic carbon (EPA Method 415.1); bicarbonate (EPA Method SM 2320B); chloride and sulfate (EPA Method 300); sulfide (EPA Method 376.2); and sodium, calcium, potassium and magnesium (EPA Method 6010) were measured in each site water, effluent solutions, and the laboratory control water (U.S. EPA 1983, U.S. EPA 1996a). Duplicate and matrix spike samples were analyzed at a minimum five percent frequency.

Results

Water quality conditions for all tests met test acceptability requirements. Temperature ranged from 19.4 to 20.2 °C, and the mean dissolved oxygen concentration (± s.d.) was 8.39 ±0.59 mg/L across all tests. Measured pH in the test waters closely approximated the nominal pH (6.5, 7.5, 8.5), with an average pH (± s.d.) for all tests of 6.51 (± 0.10), 7.30 (± 0.14), and 8.41 (± 0.17), respectively. Table 1 summarizes water quality characteristics for each of the dilution waters.

Silver 48-h LC50 values are presented in Figures 1 and 2. Silver LC50 values for *C. dubia* ranged from 0.441 to 7.090 µg/L, while *P. promelas* exhibited a greater sensitivity to changes in water quality characteristics with LC50 values ranging from 0.545 to 26.420 µg/L. Toxicity testing with *C. dubia* in whole effluent at pH 6.5 and 7.5 resulted in significant mortality in every concentration tested (excluding the controls) and LC50 values could not be calculated for either test.

Discussion

Overall, differences in toxicity test results between the various dilution waters were what would be expected given their differing water quality characteristics. Silver LC50 values increased with increasing organic carbon content, again consistent with previously published studies. The effects of pH on acute silver toxicity, however, were somewhat variable. In general, silver was most toxic at pH 6.5 and least toxic at pH 7.5. Relative toxicity at pH 8.5 was quite variable, ranging from the least toxic for a given dilution water, to comparable in toxicity, to the test conducted at pH 6.5 (i.e., the most toxic) with the same water. No specific pattern to the pH-dependent effect at

pH 8.5 (e.g., type of water) was discernable. Previous studies have indicated a general decrease in silver toxicity with increasing pH over the range of 6.5 to 8.5 (Erickson et al. 1998). These results are counterintuitive as competition between H^+ and silver at gill binding sites should result in reduced, not increased toxicity at lower pH. Further study of this phenomenon is needed to resolve the causative mechanism.

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Table 1. Water Quality Characteristics of Dilution Waters (mg/L).

Parameter (Dissolved)	Laboratory Water	River Water	1:1 River / Effluent	Effluent
Sulfide	<0.005	<0.005	<0.005	<0.005
Bicarbonate	58-68	36-41	84-93	120-150
Chloride	2.2-4.1	1.4-2.1	35-37	60-77
Sulfate	83-97	7.2-7.4	18	24-29
Calcium	16-17	11-12	27-28	37-43
Magnesium	13-15	4	12	16-20
Potassium	2.3-2.8	<1.0-1.2	4.1-5.3	8.6-9.6
Sodium	31-35	3.5-6	33-36	50-67
Alkalinity	70	38	82	130
Hardness	90	44	112	180
DOC	0.01-0.6	2.2-4.2	4.4 - 8.8	6.91-8.8

Figure 1. LC50 (µg/L dissolved Ag) for *Ceriodaphnia dubia* as a Function of pH

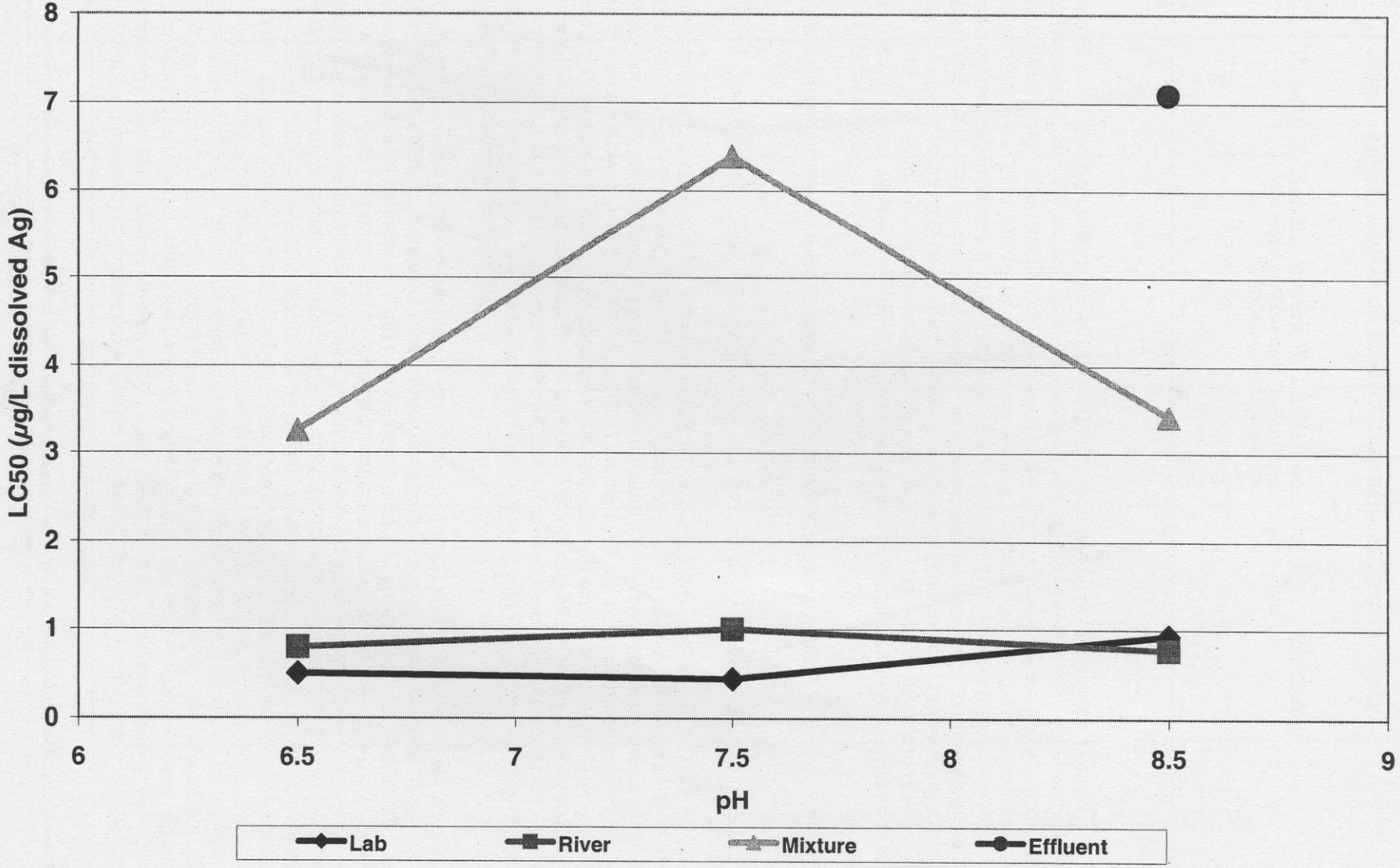
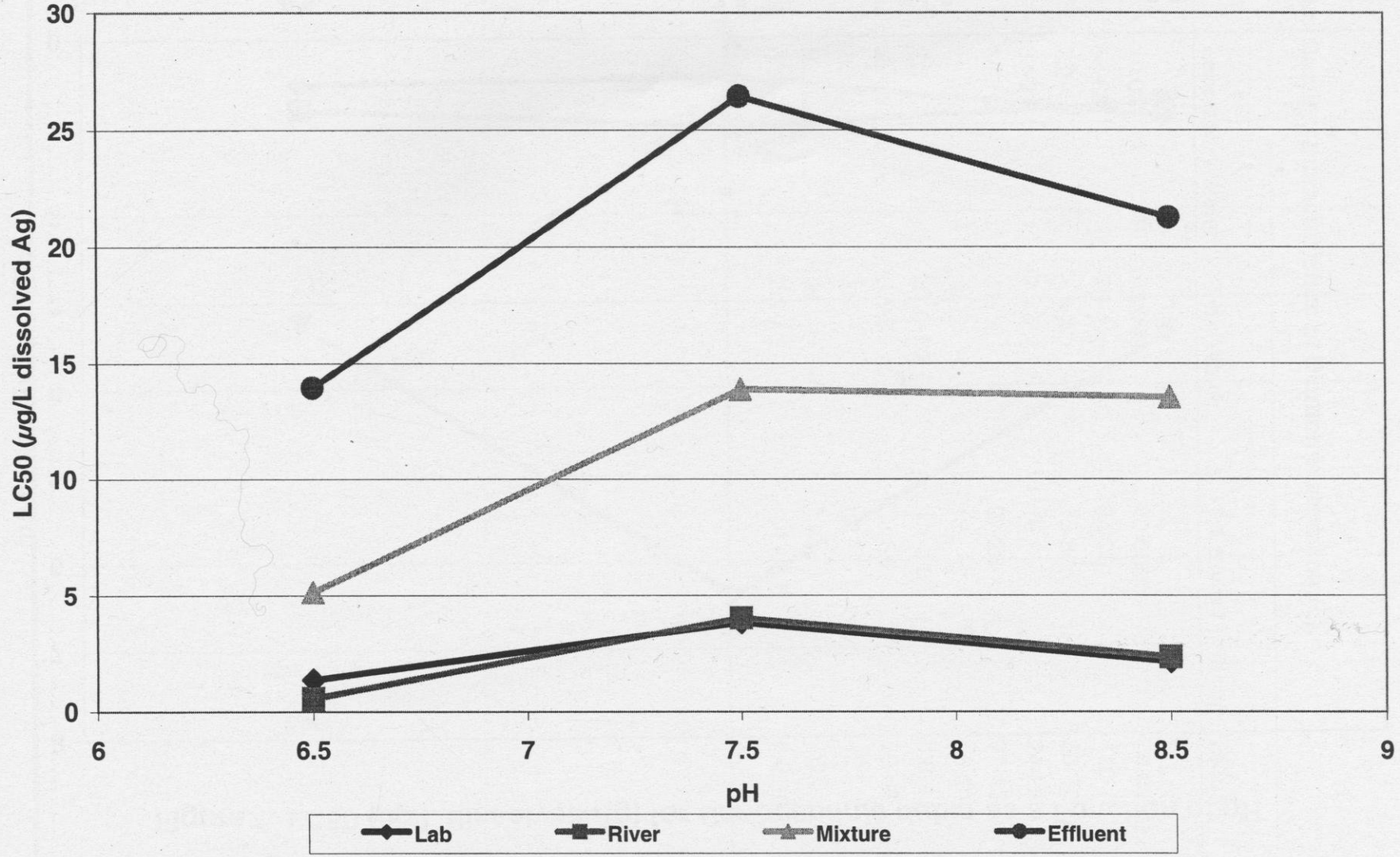


Figure 2. LC50 (μg/L dissolved Ag) for *Pimephales promelas* as a Function of pH



Binding Strength of Silver Ligands in Fresh Water

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Strong associations of silver ion (Ag^+) with dissolved and colloidal ligands may have dramatic effects on its availability to aquatic organisms. Recent studies of Ag in natural waters, both pristine and impacted by human activities, have shown that a significant fraction of "dissolved" (< 0.4 μm) Ag in surface waters is associated with colloids (Shafer et al. 1998; Wen et al. 1997(a)). In addition, indirect evidence has pointed to association of Ag with strong, reduced sulfur ligands (Benoit and Rozan 1997; Santschi et al. 1997), either colloidal or truly dissolved. Relatively few studies, however, have been performed to directly determine the strength of Ag^+ association with colloids and dissolved ligands. Our work has focused on addition of a competing ligand to an aqueous sample in order to use the competition between the added ligand and natural ligands to determine the strength of Ag complexes in fresh waters. We have emphasized three different techniques in order to study a spectrum of natural ligand binding strength.

Competing ligand techniques are based upon competition between the following reactions:

$$Ag^+ + nL \Leftrightarrow AgL_n \qquad (1)$$
$$Ag^+ + mAL \Leftrightarrow AgAL_m \qquad (2)$$

where L is a ligand present in a water, and AL is the ligand added to the system. As long as a small concentration of free Ag^+ is present in the system, these two reactions will lead to an equilibrium state in which the concentration of AgL_n and $AgAL_m$ will depend on the formation constants of the two reactions and concentrations of the two ligands. If the concentration and formation constant for equation (2) are known and equilibrium has been achieved, mass action laws can be used to determine the strength and extent of Ag binding by ligands present in the system. This method also requires the assumption that virtually all of the Ag is present as Ag(I). Both strength and concentration cannot be determined from this single set of reactions. To separate the effects of natural ligand strength and concentration, the system must either be titrated with Ag^+, or studied with different ligands and/or different experimental techniques.

The model of competing-ligand techniques presented in equations (1) and (2) is clearly a simplified one. In a surface freshwater or POTW effluent, in either of which a host of ligands including organic matter functional groups and stabilized sulfides may be present, Ag(I) may be associated with several ligands of different strength and concentration. A single ligand concentration and single formation constant for Ag^+ with ligands present in solution will, therefore, be artificial but potentially useful. If titration with Ag^+ is used to determine ligand concentration and stability constant simultaneously, however, only ligands with formation constants within an order of magnitude to either side of the added ligand will be detected (Miller and Bruland 1995). It is potentially more informative to use a strategy in which different ligands and techniques are used to look at a broader range of complexing strength and capacity. We have adopted this strategy. Very strong Ag ligands (e.g., those with Ag-ligand formation constants of 10^{10} or greater) are studied using diethyldithiocarbamate (DDC) as the competing ligand. The Ag-DDC complex is hydrophobic, and is separated for analysis by extraction into chloroform. This is similar to the work of Miller and Bruland (1995) and Adams and Kramer (In Press). Relatively weaker ligands, and ligands with extremely slow dissociation kinetics, are studied using 24-hour batch equilibration with Chelex resin; the functional group on the resin (iminodiacetate, IDA) is the competing ligand in this case. Ligands that

are weaker but that may be kinetically nonlabile on a shorter timescale are studied using short-term contact with Chelex (i.e., flow through a column of Chelex at a rate of 6-7 mL min^{-1}). Comparison of results from these tests should provide an informative picture of the speciation of Ag in a water body.

The behavior of dissolved DDC as a competing ligand can be modeled effectively using the results of Miller and Bruland (1995) and Adams and Kramer (In Press). Short-term sample-Chelex contact time as a method for study of weak but kinetically nonlabile species has also been explored (Donat et al. 1994). To interpret the results of 24-hour batch experiments with Chelex, however, it was necessary to determine the behavior of Chelex when it competes with a dissolved ligand. This behavior was studied in simple laboratory solutions using cyanide ion (CN^-) as a ligand to compete with Chelex (log K ($AgCN_2^-$) = 20.48). Experiments of this sort gave reproducible results, and showed that the behavior of Chelex as a competing ligand was only slightly influenced by effects of pH (over a range from 8 to 10), Ag concentration (0.46 to 4.6 nM), and resin counterion (Na^+ and Ca^{2+}). The results of these experiments indicated that Chelex could be modeled as a dissolved ligand with a log formation constant for Ag complexation of 7.5 (Fig. 1). Kinetics experiments indicated that solutions containing Chelex, Ag and CN^- were at or very close to equilibrium in 24 hours. In all laboratory tests and subsequent analyses, trace-metal clean techniques were used. The same was true for all field-sampling expeditions.

Experiments using these three techniques have been performed on two systems to date: Black Earth Creek (BEC), a small stream near Madison, Wisconsin whose only anthropogenic impacts are from agriculture; and effluent from the Madison Metropolitan Sewerage District (MMSD effluent). In both cases water was filtered at 0.4 μm before experiments were performed. BEC was sampled twice; the first sampling showed that Teflon bottles were unacceptable for use as a result of the fact that the majority of Ag^+ (as $AgNO_3$) spiked into unacidified solutions sorbed to Teflon bottle walls. This effect was noticed by Wen et al. (1997(b)) after two months of unacidified storage, but our work indicated that major sorption losses are observed after 12 hours. This effect was not observed in laboratory samples. It is therefore likely that Ag ligands in BEC water had some hydrophobic character and that these Ag complexes sorbed to the hydrophobic Teflon surface. Glass containers sorbed Ag to a lesser, in fact almost negligible, degree, providing more evidence of a hydrophobic interaction. Glass containers also provided acceptable Ag mass balances in samples on which the Chelex and DDC speciation techniques were used (80-115 percent Ag recovered). Glass containers were used in a second BEC sampling, and in the MMSD effluent sampling.

All three competing ligand tests showed similar results for BEC water and MMSD effluent. In both cases, greater than 90 percent of detectable Ag associated with DDC ([DDC] = 1 mM), indicating that very strong Ag ligands were not present or were present at very low concentration. For example, equilibrium speciation modeling indicates that as little as 0.2 μM of a ligand with a Ag formation constant of 10^{12} should compete to a detectable degree with 1 mM of DDC. In Chelex batch tests, greater than 85 percent of Ag remained unassociated with Chelex in both BEC water and MMSD effluent. This indicates that Ag ligands strong enough to compete with Chelex are present in these two systems. Chelex column experiments showed results similar to those of batch experiments, indicating that weak but slow-to-dissociate ligands were not important to Ag speciation in either system. The results of these tests – along with selected characteristics of the streams tested and comparisons to CN^- competition with Chelex – are summarized in Table 1. Equilibrium speciation indicates that a CN^- concentration of 100 nM or greater would be required to show detectable competition with DDC for Ag. Thus, the 40-47 nM equivalent CN^- concentrations calculated for natural ligands using the Chelex batch technique does not conflict with the DDC results.

When BEC water and MMSD effluent were oxidized, using ultraviolet light and hydrogen peroxide before performing Chelex tests, much more Ag associated with the resin. After oxidation, greater than 90 percent of Ag in BEC water associated with Chelex, and in oxidized MMSD effluent, 50-80 percent of Ag associated with Chelex. It is clear that the majority of Ag ligands in these systems are oxidizable by this process and, therefore, probably are organic or reduced sulfur species. It is not as clear why ligands remained in the MMSD effluent; it

may be that certain ligands are not oxidizable by this process, or that they were stabilized with respect to oxidation by high metal concentrations in MMSD effluent.

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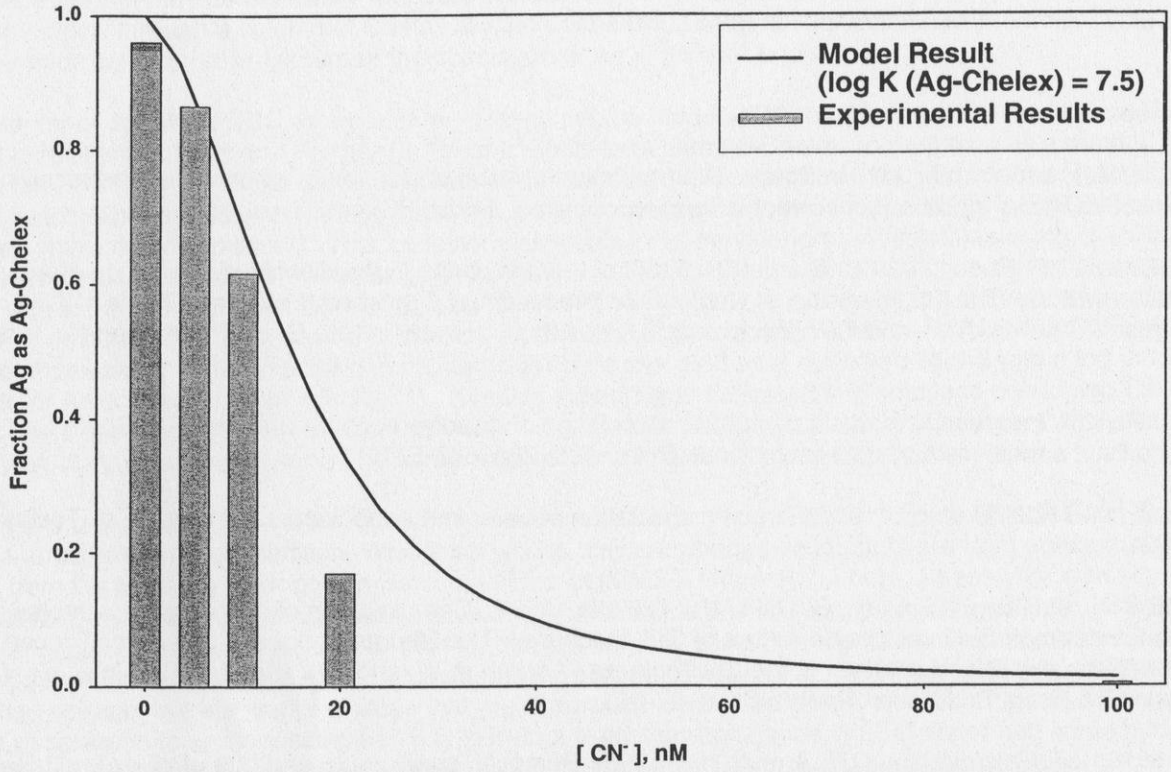

Fig. 1: Fraction Ag associated with Chelex as a function of CN concentration. Experimental results are based on average results of three experiments in which Ag- and Chelex-containing samples were titrated with CN. Total concentration of Ag ranged from 0.46 nM to 4.6 nM.

Table 1: Characteristics of streams sampled

Sample	DOC (mg/L)	pH	Conductivity ($\mu S\ cm^{-1}$)	$[Ag\text{-}Chelex]/[Ag]_{tot}$ [a] batch samples	Natural Ligand Equivalent $[CN^-]$ [b] (nM)	$[Ag\text{-}DDC]/[Ag]_{tot}$ [c]
BEC water	2.5	8.1	620	0.1	40	0.92
MMSD effluent	6.0	7.8	1730	0.07	47	1.07

[a] average of batch (24-hour) tests. BEC water: n=2. MMSD effluent: n=4, s=0.04.
[b] Represents concentration of CN^- that, according to the model in Fig. 1, would lead to the same mean fraction of Ag associated with Chelex as was caused by the ligands in the given water.
[c] Average of analyses. For both systems n=2.

New Data on the Accumulation of Silver by Mosses, Soil and Sediments in the South Baltic Region

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Questions regarding the distribution of silver and its compounds and the possibility of their accumulation in bottom marine sediments, terrestrial soil and plants are very important, due to the discharge of silver from a number of sources into the atmosphere and world's oceans. Research sampling was done in the Baltic Sea area by Dr. A. Blazchishin, notably bottom sediments from the Gotland Depression of the Baltic Sea (east of Gotland Island), and also terrestrial soil and mosses from the Curonian Spit of the Kaliningrad District by the authors.

Atomic Adsorption Spectrometry (AAS) was used to estimate the concentration of silver. Samples taken from the surface of the bottom organogenous silts, in the central part of Gotland Depression at the depth of 243 m, showed silver concentrations varying from a minimum range of 0.057-0.061 ppm to a maximum range of 0.10-0.13 ppm. Higher silver concentrations, beyond this maximum, were measured in the transition area between anoxic and upwelling zones. Some differences were observed in the distribution of microelements (V, Zn, Pb, Cu, Mo, as well as Ag) between the west and east slopes of Gotland Depression (profile: station K-2619 to station K-2628). The reason is apparently the discharge of large amounts of metal-organic compounds from point sources in the North Baltic. The concentration of silver in the silt upper layer (0-5 cm), taken from the slope of the Gotland Depression at a depth of 91 m (station K-2628), increased to 0.10 ppm. The primary cause appears to be wastewater and industrial effluents from coastal sources.

The moss harvesting technique as means of surveying atmospheric heavy metals deposition was used to determine silver concentration in three-year-old plants. The samples of mosses were collected from protected sand dune areas of the Curonian Spit during four seasons: from autumn 1997 to summer 1998. Unfortunately, we do not have any experimental control for comparison. Since mosses have a high capacity to retain several heavy metals, the common species of terrestrial mosses could be used as bioindicators of their contamination.

It was found that the capacity of these mosses to accumulate silver differ. The most accumulative of them is *Pleurosium schreberi*. In spite of this, the values of silver content in these mosses are closely correlated. Seasonal variation of silver concentration for three species of mosses were negligible. Silver content in mosses varied from 0.10-0.12 ppm, 0.35-0.36 ppm, and 0.42-0.44 ppm for *Hylocomium splendens*, *Scleropodium purum* and *Pleurosium schreberi*, correspondingly. The silver distribution had such features: the silver concentrations in the centers of the dune spit had minimums of 0.085 ppm and 0.340 ppm for *Hylocomium splendens* and *Scleropodium purum*, respectively. In the south and north parts of the spit the concentrations of silver in mosses were a little larger, with an average annual silver uptake of 0.02-0.05 ppm. Samples of the upper layer of soil, directly beneath the mosses, were taken. There, the concentration of silver ranged from 0.18 to 0.22 ppm.

The occurrence of silver in marine sediments, terrestrial soil and mosses indicates some accumulation of the metal. All of them could be used as indicators of silver contamination.

A Unique Sink for Silver in Sediments

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The role of acid volatile sulfide (AVS) as well as sediment organic carbon, for binding toxic heavy metal ions by forming very insoluble compounds or by chemisorption to humic material, has been demonstrated. These processes have also been related to sediment toxicity because the extent to which AVS and organic carbon are present establishes the amount of heavy metal that may be present in pore water which, in turn, is related to the potential for biological effects. These same two binding phases have been shown to apply to silver in addition to copper, cadmium, nickel, zinc and lead.

However, the high reduction potential for Ag^+ (0.80 v) provides a unique third sink for the removal of silver ions from pore water. Because Fe^{2+} is present in virtually all sediments it provides the corresponding redox couple for the reduction of Ag^+ to Ag^0 (silver metal).

Thus:

	$\mathcal{E}^0$
$Ag^+ + 1e^- \rightarrow Ag^0(s)$	+0.80 v
$Fe^{2+} - 1e^- \rightarrow Fe^{3+}$	-0.77 v
	+0.03 v

Because of the insolubility of Fe^{3+} the hydroxide is readily formed at sediment pH and this is subsequently converted to iron (III) oxyhydroxide.

$$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3(s)$$

$$Fe(OH)_3(s) \rightarrow FeOOH(s) + H_2O$$

While the standard potential for the redox couple is only slightly positive, the reaction potential, $\mathcal{E}$, for the reaction

$$Ag^+ + Fe^{2+} \rightarrow Ag^0 + Fe^{3+}$$

is actually much larger when environmentally realistic activities for Fe^{3+} and Fe^{2+} (0.1 ng/L and 10 mg/L, respectively) are used in the Nernst equation:

$$\mathcal{E} = \mathcal{E}^0 - \frac{RT}{nF} \ln Q$$

The following experiments have been performed to verify these conclusions.

Experiment 1:

The reaction

$$Ag^{+} + Fe^{2+} \rightarrow Ag^{0} + Fe^{3+}$$
$$Fe^{3+} + 3OH^{-} \rightarrow Fe(OH)_3(s)$$

In black vessels, 100 μmol of $FeSO_4$ were combined anaerobically with 100 μmol of $AgNO_3$ in 100 ml of water buffered to pH = 7.0 using 3-(N-morpholino) propane-sulfonic acid (MOPS). After 45 minutes, 95% of the soluble silver and iron were removed. In approximately five hours the reaction was complete. In addition, a black residue of what appeared to be metallic silver and a yellow residue of iron (III) hydroxide were observed.

Experiment 2:

Examination of the displacement vs the redox process

In black vessels, 100 μmol of freshly prepared FeS(s) and 200 μmol of Ag^{+} were combined in 150 mL of pH = 7.0 buffered water under anaerobic conditions. After 30 hours, the results showed no soluble silver or iron. Thus

$$2Ag^{+} + FeS(s) \rightarrow Ag_2S(s) + Fe^{2+}$$

could not be the only reaction occurring. The presence again of black and yellow residues suggests that the reaction

$$Ag^{+} + Fe^{2+} \rightarrow Ag^{0} + Fe^{3+}$$

also occurred although the sequence was not clear.

Experiment 3:

Anaerobic titration of FeS in the presence of excess Fe^{2+} with Ag^{+}

In black reaction vessels, 20 μmol of freshly prepared FeS was combined with 30 μmol of Fe^{2+} in a total volume of 100 mL of water buffered to pH = 7.0. Silver ions were added in 5 μmol increments over a period of five hours. The results are shown in Figure 1. Based on the stoichiometric quantities of reacting species present the following seems to be the order in which reactions are occurring as silver ions are added to the system.

In Segment 1, the amount of Fe^{2+} drops from the original 30 μmols to 0 μmols as 30 μmols of Ag^{+} are added, according to the reaction

$$Ag^{+} + Fe^{2+} \rightarrow Ag^{0} + Fe^{3+}$$

followed by

$$Fe^{3+} + 3OH^{-} \rightarrow Fe(OH)_3(s).$$

Segment II represents the reaction of 20 μmols of Ag^{+} with the 20 μmols of FeS initially present. The reaction would appear to be

$$Ag^{+} + FeS \rightarrow Ag^{0} + Fe^{3+} + S^{2-}$$

followed by

$$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3(s)$$

as well as, possibly,

$$2Fe^{3+} + 3S^{2-} \rightarrow 2FeS(s) + S^0 \quad (I).$$

In Segment III, if the reaction

$$2Ag^+ + S^{2-} \rightarrow Ag_2S$$

were occurring exclusively, then the Ag^+ concentration should not increase until 90 μmols have been added, as shown by the green dashed line. The fact that the actual Ag^+ concentration gradually increases after 55 μmols have been added suggests that (I), a known reaction may be occurring, thereby making the $Ag_2S(s)$ formation a heterogeneous reaction and consequently slower. This would account for the elevated Ag^+ concentration beyond 55 μmols.

Experiment 4:

Addition of Ag^+ ions to sand cores without AVS or organic carbon

The previous experiments were conducted as suspensions or solutions. This last series of experiments was conducted using sand as the bedded sediment and adding Ag^+ ions to the overlying water. In this case, the only binding phases present are sand and iron (II) carbonate ($FeCO_3(s)$).

In 2.5 inch diameter Lucite core tubes, 6 cm of clean sand was placed. Freshly prepared $FeCO_3(s)$ was completely mixed with the sand under anaerobic conditions on a 2% weight/weight basis. One-hundred fifty mL of pH = 7.0 buffered water was added. The system was then placed in the dark and continuously aerated. A schematic representation is shown in Figure 2.

Separate systems were set up, one with an initial Ag^+ concentration in the overlying water of 90 mg/L, the other with an initial concentration of 7 mg/L. The overlying water was sampled with time. Both systems were run in duplicate with duplicate controls containing sand but no $FeCO_3(s)$. The results are shown respectively in Figures 3 and 4. The limit of detection for Ag^+ was 0.1 mg/L using flame atomic absorption spectroscopy. A black, as well as a yellow, residue was observed to increase in depth with time. The shapes of the curves are consistent with the increase in distance that Ag^+ must diffuse into the sand to react with $FeCO_3(s)$.

The results clearly indicate the ability of Fe^{2+} ($FeCO_3(s)$) to remove Ag^+ by the reaction

$$Ag^+ + Fe^{2+} \rightarrow Ag^0 + Fe^{3+}$$

followed by

$$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3(s)$$

The controls show the absorption of Ag^+ to the sand.

The pH of the overlying water in the $FeCO_3(s)$ containing cores gradually increased from 6.7 to approximately 9 over the course of the experiment. No increase was observed in the controls. This was most likely due to the release of CO_3^{2-} as Fe^{2+} is oxidized.

Conclusion:

Within the framework of the present study, it appears that there is an important sink for the removal of silver ions from pore water, as well as overlying water in contact with sediments when iron (II) is present. This mechanism operates in the absence of both AVS and organic carbon. In fact, it may very well be the primary removal pathway.

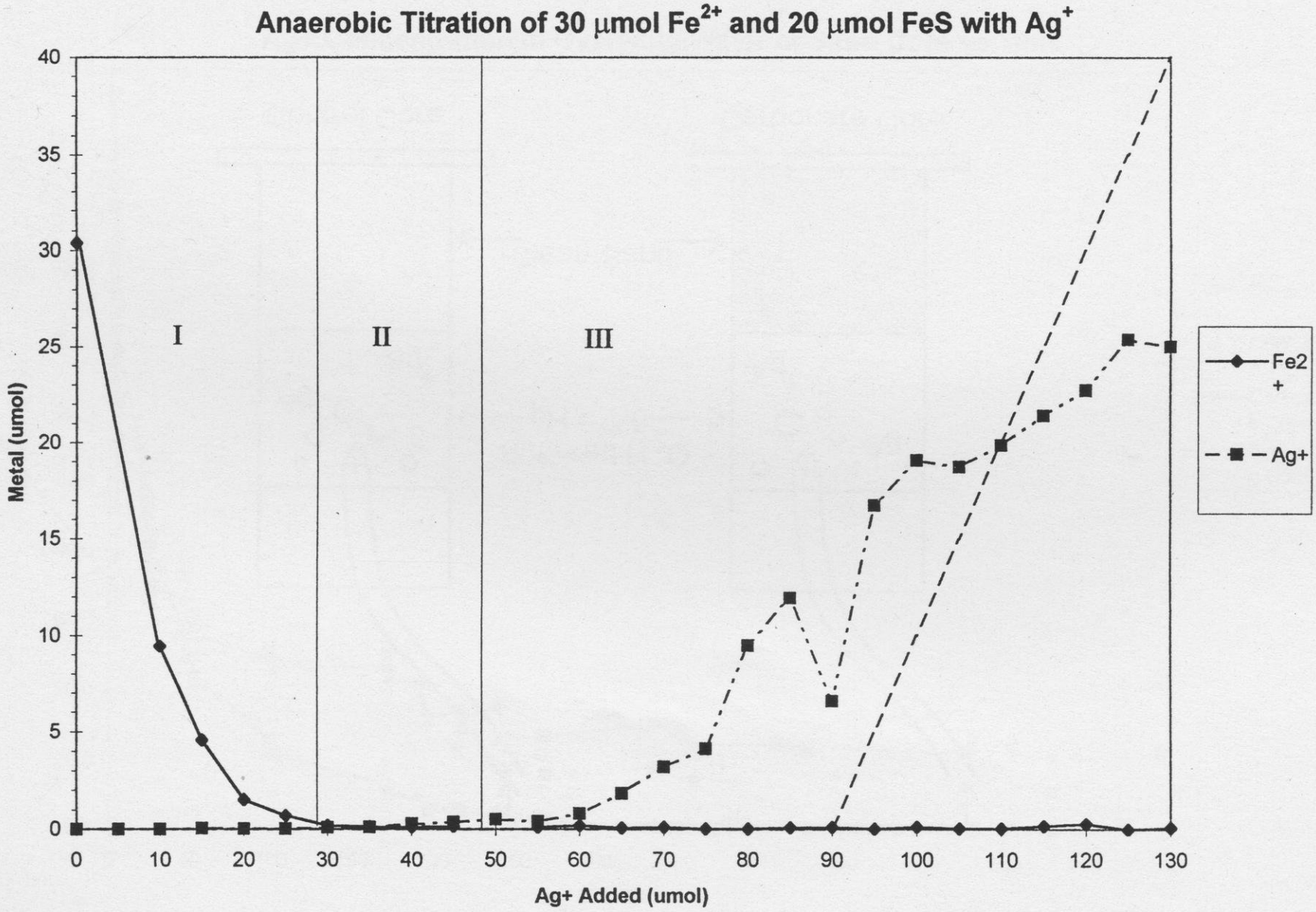
Anaerobic Titration of 30 μmol Fe2+ and 20 μmol FeS with Ag+
Metal (umol)
Ag+ Added (umol)
I
II
III
Fe2+
Ag+
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
60
70
80
90
100
110
120
130

Figure 1

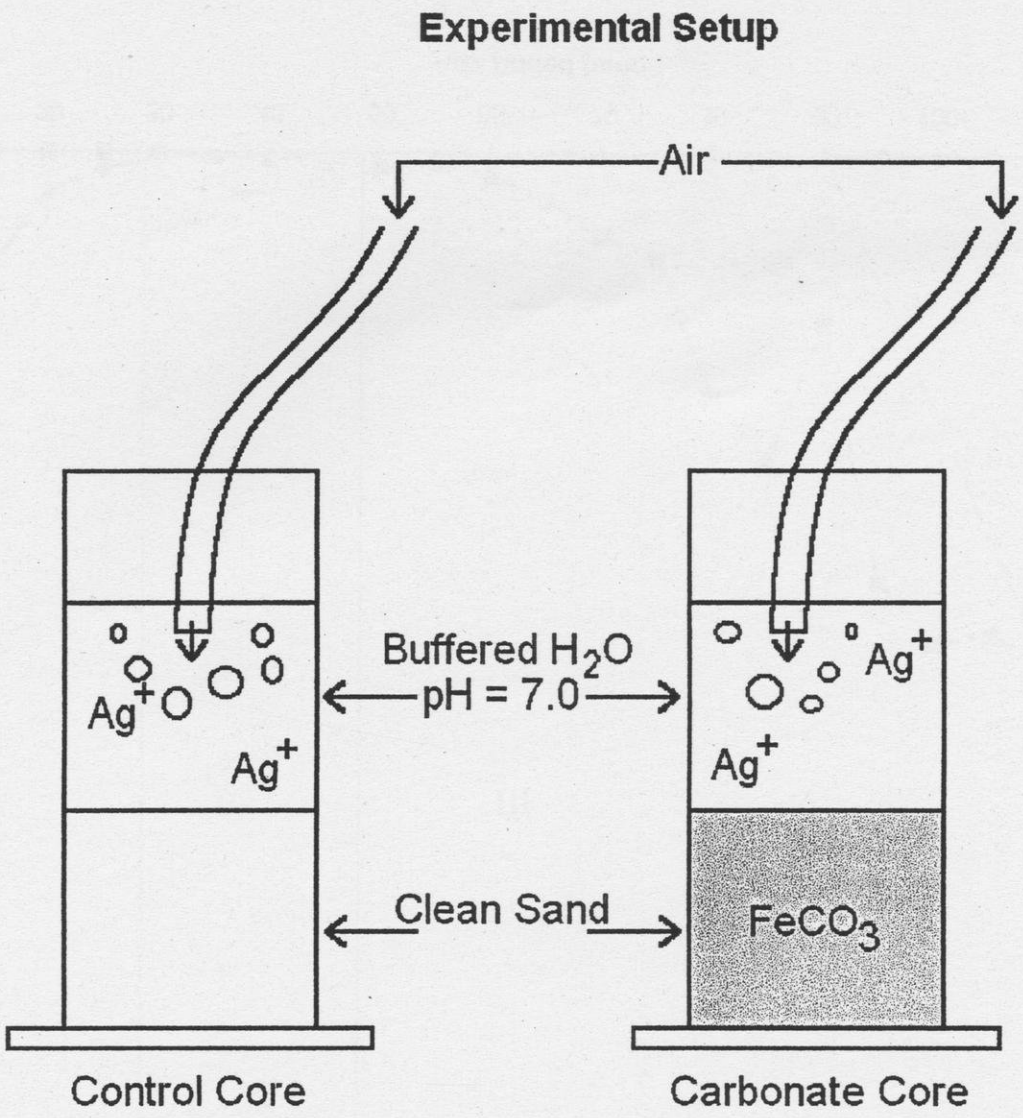
Experimental Setup
Air
Buffered H_2O
pH = 7.0
Ag^+
Ag^+
Ag^+
Ag^+
Clean Sand
$FeCO_3$
Control Core
Carbonate Core

Figure 2

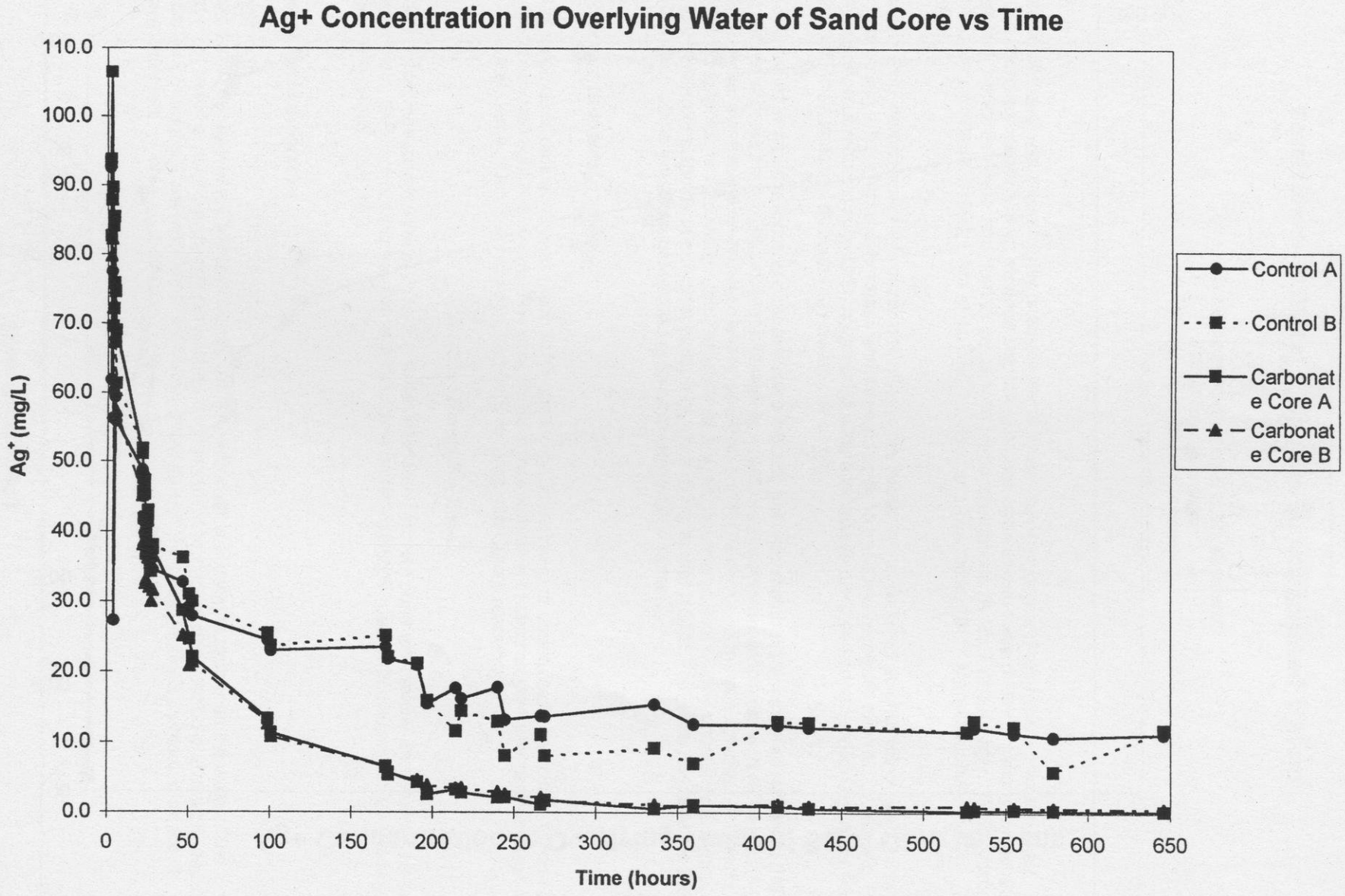
Ag+ Concentration in Overlying Water of Sand Core vs Time
Ag+ (mg/L)
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
50
100
150
200
250
300
350
400
450
500
550
600
650
Time (hours)
Control A
Control B
Carbonate Core A
Carbonate Core B

Figure 3

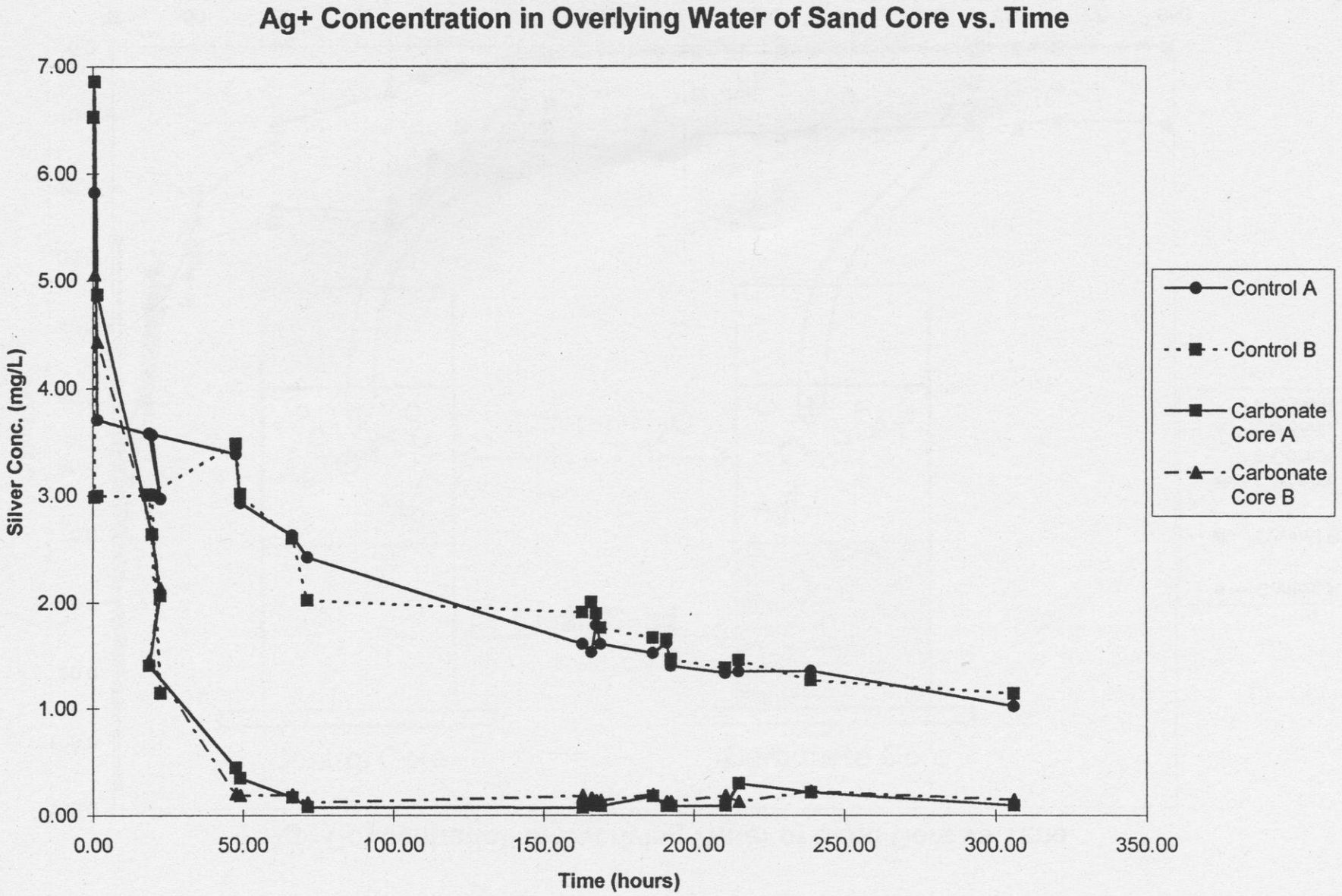
Ag+ Concentration in Overlying Water of Sand Core vs. Time
Silver Conc. (mg/L)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.00
50.00
100.00
150.00
200.00
250.00
300.00
350.00
Time (hours)
Control A
Control B
Carbonate Core A
Carbonate Core B

Figure 4

Persistence of Metal Sulphides in Oxygenated Waters

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Soluble sulphides, e.g., Na_2S, are highly unstable in the presence of oxygen and are rapidly oxidized in air. Despite this phenomenon, however, reduced sulphur (SII-) has been shown to be present in many oxygenated surface waters, both freshwater and marine, at *nano*molar concentrations (Adams and Kramer, 1999; Kramer *et al.*, 1999; Rozan and Benoit, 1999; Elliot *et al.*, 1987; Luther and Tsamakis, 1989; Manolopoulos, Ph.D. Dissertation).

The relevance and importance of this occurrence lies in the interaction of reduced sulphur with metals. Sulphide (SII-) exhibits a high affinity for type-B metals and strongly binds to trace heavy metals such as Zn, Cu, Ag, Hg. Thus, the presence of reduced sulphur in oxygenated waters increases dissolved concentrations of trace heavy metals and aids their transport through the environment.

Possible sources of reduced sulphur to natural waters include, bacterial metabolism, hindered sulfhydryl (HS^-) groups in natural organic matter (NOM), metal thiolates, mixed metal sulphide complexes with NOM [i.e., NOM - metal - S(II)], metal sulphides alone (particulate or colloidal) and NOM occluded metal sulphides. The method by which dissolved sulphide is stabilized in the presence of oxygen, however, is not clearly understood. Hypotheses implicate the binding of sulphide to certain metals, e.g., Cu and Zn, and/or NOM. This work attempted to enhance our understanding of the presence of reduced sulphur in oxygenated waters.

Cline's Reactive Sulphide

Measurable sulphide is defined as that which is reactive with Cline's reagent under acidic conditions to form a complex which absorbs light at 670 nm. The concentration of sulphide is measured colorimetrically against a series of Na_2S standards. Only sulphide can form H_2S to react with Cline's reagent:

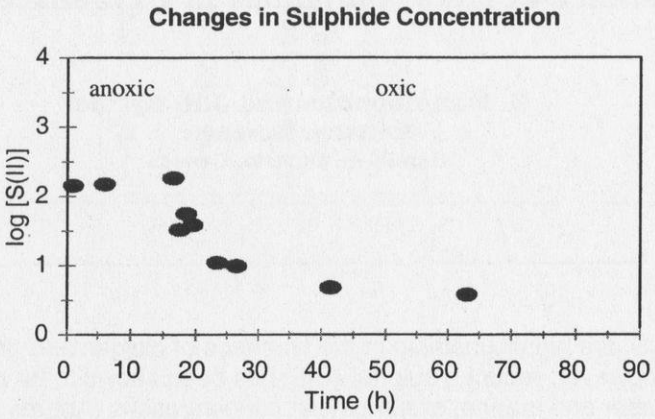
$$S^{2-} + 2\ H_3O^+ \rightleftharpoons H_2S + 2\ H_2O$$

This method of sulphide determination does not suffer interferences from common cations and anions. However, mercaptans (thiols, S-R) are not Cline reactive while some metal sulphides react with the reagent very slowly or only partially, e.g., Ag_2S, CuS, NiS.

Oxidation of Metal Sulphides

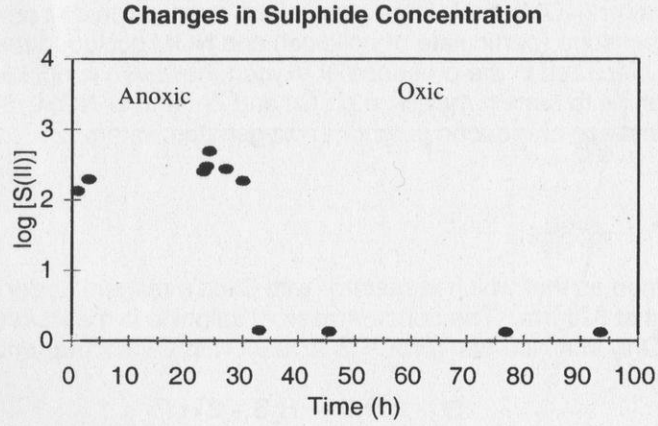
Oxidation of FeS

FeS suspended in deoxygenated water is rapidly oxidized when air is allowed to penetrate the water. The concentration of reactive sulphide quickly diminishes during the first few hours of oxidation and steadily declines to nondetectable (< 5 nmol/L) concentrations. However, even after 46.5 h of oxidation, 3.8 μmol/L of reactive sulphide are detected.



Oxidation of FeS + 2% Zn^{2+}

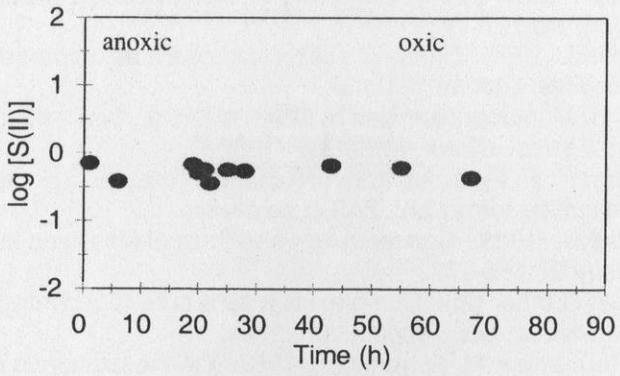
The addition of Zn^{2+} to FeS suspended in deoxygenated water slightly suppresses the rate of FeS oxidation upon exposure to air. After the first few hours of oxidation the concentration of reactive sulphide levels off at 1.3 - 1.4 μmol/L and persists after 70 h of oxidation.



Oxidation of CuS

Unlike FeS which oxidizes in a matter of a few hours, CuS suspended in oxygenated water undergoes very slow oxidation on the order of days or even months. Over a period of 48 h of oxidation, the concentration of reactive sulphide remained constant at 0.4 - 0.6 μmol/L.

Changes in Sulphide Concentration



Reactive Sulphide in DOC

Several samples of NOM taken from a variety of locations were analyzed for dissolved organic carbon and reactive sulphide concentration upon dissolution of the samples in water. Nanomolar levels of sulphide were detected in the DOC. No correlation between sulphide and DOC concentrations, however, was established to help predict the origin of sulphide in natural samples.

Sample Location	S^{2-} (nmol/L)	DOC (mgC/L)	S^{2-}/DOC
Aurevann, Norway	10	3.67	2.72
Gjerstad (limed), Norway	30	5.18	5.79
Gjerstad (unlimed), Norway	50	6.10	8.20
Hellerudmyra (May-96), Norway	10	6.03	1.66
Hellerudmyra (Oct-96), Norway	10	7.05	1.42
Humex B, Norway	50	7.14	7.00
Maridalsvann, Norway	ND*	2.31	0
Trehorningen, Norway	10	3.80	2.63
Swanee River	ND*		
Luther Marsh	49	3.80	12.89

*ND - not detectable

Conclusions

- Cline reactive sulphide has been measured at *nano*molar concentrations in oxic waters as well as in dissolved organic matter.
- Oxidation of metal sulphides exhibits a wide range of rates. FeS oxidizes extremely rapidly in a matter of hours while CuS may persist in oxygenated waters for days or even months.
- Metal sulphides are proposed as a source of sulphide in oxygenated waters. Cu and Zn in particular appear to stabilize sulphide allowing its persistence for relatively long periods of time in oxygenated waters. This hypothesis is further supported by the findings of Theberge *et al.* (1997) and Luther *et al.* (1999).
- Metal sulphides are likely associated with NOM. Metals may complex sulphide sites in NOM or may be occluded in the NOM.

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Silver in Sediments of the Elbe River and of Lake Constance

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Introduction

If compared with other heavy metals (p. ex. Cd, Hg, Pb, Cu, Zn, Ni, Cr), the study of silver in the geosphere has been somewhat neglected in Europe. The main reason lies in the fact that silver is not listed at all in the "*Klarschlammverordnung*" (Germany) or in the "*Leidraad Bodemsanering*" (Netherlands) or in comparable lists of many other European countries. As a consequence, very few analyses exist for silver in soils, sediments and sewage sludge.

In this report, Ag concentrations in sediments are derived from the analysis of the < 20 μm grain fraction (in few cases, if indicated, < 63 μm). This fraction with its high specific surface area is assumed to represent the primarily fine-grained *suspended load* of a water body, resulting in *mud* after deposition, in contrast to the coarser fraction, > 20 μm, the *bed load*, resulting in *sand* (with a very low specific surface area) when deposited.

The < 20 μm analytical results of recent unpolluted sediments are comparable with the "average shale" concentrations (Turekian & Wedepohl, 1961) of ancient ("fossil") fine-gained sediments and sedimentary rocks (mudstones, shale), considered as geogenic background values.

For Lake Constance muddy sediments no fractionation was required: more than 95% of the sediments composition lie in the < 20 μm grain size range.

The Elbe River (Fig. 1)

After the German reunification in 1989, the sediments of the Elbe River (*Czech* Labe), draining about 90% of the Czech Republic and large parts of eastern Germany, known for their extreme concentrations of cadmium and mercury, underwent a thorough investigation (Mueller & Furrer, 1998) leading to the point sources of heavy metals in the catchment area. Additional analyses for silver led to the surprising result that this metal occurs in concentrations up to 500 times higher than in the "average shale" (0.07 mg/kg).

Main tributaries of the Elbe from the left side are the Moldau (*Czech* Vitava) in the Czech Republic, Mulde and Saale in Germany draining the Erzgebirge, and from the right side Schwarze Elster and Havel in Germany.

The limnic part of the Elbe reaches until Geesthacht. The city of Hamburg with its large harbour system is already part of the estuary, strongly influenced by the North Sea.

Fig. 2 depicts the Ag concentration of the Elbe sediments in its total length during two sampling campaigns (Prange et al., 1997). It is clearly to be seen that between October 1992 and October 1995 a considerable decrease of the Ag concentration took place. The sharpest decrease was found in the Czech part of the Elbe near Hradec Kralove and in the mouth of the Moldau River. In both campaigns, the Mulde River, although with a strongly reduced Ag concentration, remained the main polluter of the Elbe River.

Our campaign carried out one year later in October 1996, resulted in a similar picture as in 1995, the Mulde River with about 11 mg/kg Ag representing still the highest Ag source.

In 1992, the "Synthesia" industrial center in the lower Elbe near Hradec Kralove with the center of photographic industry in the Czech Republic, and the mouth of the Moldau (Vitava) River draining the Czech capital Prague, contributed the highest Ag concentrations. Fig. 3 depicts the concentrations of Hg, Cd and Ag in the sediments of the Czech part of the Elbe between Hradec Kralove and the Czech-German border after Borovec (1995). The role of a silver point source between Hradec Kralove and Pardubice is clearly to be seen.

Fig. 4 shows the Ag content of the sediments of the Vitava/Moldau River and its tributaries in two campaigns in 1995 and 1996 (Schindler et al., 1997). A general decrease of the Ag content is to be observed in 1996. Still very high concentrations, however, occur after the river has entered the city limit of Prague, causing a high Ag import into the Elbe River. The reason for this strong increase of the Ag concentration is not yet clear. Prague is well known as the city of gold and silver jewelry; photographic industry and film consumption might also play an important role.

In a dated sediment core "Jessipek" (near Hradec Kralove, Czech Rep.) the maximum silver concentration was found in 1992 with 45 mg/kg (Fig. 5); at "Bucher Brack" (near Tangermunde), 129 km downstream from the mouth of the Mulde River, the maximum was dated to 1981 with 25 mg/kg (Prange et al., 1997). Both concentration curves are related to photographic industry: Core "Jessipek" is situated below the largest Czech producer of photographic materials. Core "Bucher Eck" contains at least part of the silver freight from the largest producer of photographic materials at Wolfen in the lower course of the Mulde in the former "German Democratic Republic," which was closed only in 1989.

As a result of all measures applied during the past decade to minimize pollution in the Elbe River, the Ag concentration of the sediment at Geesthacht, lowermost limnic station of the Elbe, between 1989 and 1996 was reduced continuously from 11.6 to 3.3 mg/kg (Fig. 6, Ackermann, 1999).

The present still high Ag content of suspended material transported from the Mulde into the Elbe River (between Feb. 1998 and Jan. 1999, a mean Ag concentration of 5.6 mg/kg was measured, Mueller, in prepn.) – is believed to be related to the former large mining district in the hinterland of the Mulde River – the "Silber-Erzgebirge" ("Silver-Ore Mountains") and no longer to the producer of photographic material at Wolfen.

In the tidal area of the Elbe a strong dilution of all heavy metal contents in the sediments takes place by mixing with relatively "clean" marine sediments (Mueller & Forstner, 1975). Within the North Sea still a clear zonation with decreasing heavy metal concentrations (except for Pb) from the mouths of the Elbe and Rhine Rivers into the open sea is to be observed (Irion & Mueller, 1988). Analyses of silver carried out from identical samples by the German Federal Geological Survey not included in this publication are now presented for the first time in Fig. 7. Ag concentrations decrease seawards from the mouth of the Elbe with around 1 mg/kg to < 0.4 mg/kg.

Sequential extractions with a six-step technique slightly modified after Kersten & Forstner (1987) carried out on sediments (size fraction < 63 μm) of the Czech part of the Elbe sediments by Borovec (1996) led to a result: "The largest amount of Ag (82%) is bonded to non or partially crystalline manganese oxide or hydrous oxide phases" (step 3 of the extraction). Only about 18% are extracted in the organics/oxidizable sulfides step 5.

Our own measurements (in prepn.) generally result in a much higher percentage of step 5 eluates depending on the S-concentration of the sediments.

Lake Constance

Lake Constance, surrounded by Germany, Austria and Switzerland is the largest and most important drinking water reservoir of Central Europe.

Sedimentological-geochemical investigations of continuously deposited, fine-grained, dated sediments from the central part of the lake, water depth 254 m (Fig. 8), represent the chronological history of heavy metals, organic contaminants and nutrients during the past 100 years.

The results of our investigations concerning silver can be summarized as follows (Mueller 1997, 1999):

Between 1890 and 1960/70 a strong increase of the heavy metals Pb, Zn, Cd, and Ag, accompanied by a parallel increase of polycyclic aromatic hydrocarbons (PAH) can be observed.

The Ag concentration increased during this period from 0.17 mg/kg to 1.08 mg/kg (Enrichment factor 6.3). Hereafter a steady decrease followed to a new minimum of 0.12 mg/kg in 1995.

The co-occurrence of heavy metals and polycyclic aromatic hydrocarbons and their parallel evolution already described in 1977 by Mueller, et. al., leads to the conclusion that both groups of contaminants are predominantly products of the incomplete, pyrolytic, combustion of fossil fuels, especially of coal. After the 60s, the improved techniques in coal combustion and the partial replacement of coal by oil and atomic energy led to a strong reduction of both PAH and heavy metals.

In addition, as a result of an international agreement between Germany, Switzerland and Austria, the communities in the total catchment area of the lake had to be provided with sewage treatment plants, including the "third step," phosphate precipitation.

We assume that silver in Lake Constance sediments mostly originates from atmospheric wet and dry deposition. More than 90% of the silver in the anoxic sediments is bound to S. According to K. Plessow (1999 in prepn.) the mean Ag concentration of 80 dust samples collected in 15 German cities between 1990 and 1998, was 5.6 mg/kg.

In a study carried out by Heinrichs (1993), the highest enrichment factors (related to Al) were found for Ag, Cd and Se in urban dust, spider webs and humic soils.

In a more recent compilation, Heinrichs and Brumsack (1997) came to the conclusion that the composition of urban dusts from Germany is nearly identical with urban dusts collected worldwide.

The same authors in their 1997 study state that Ag in urban dusts at a ratio of about 1:1 stems from two sources: traffic and high-temperature processes (combustion of coal, oil and gas, waste incineration, steel production, etc.). The portion of Ag derived from natural sources can be neglected (Fig. 9).

In the September 1 issue of *Environmental Science and Technology*, 1999, 33, 2953-2957, C. GOBEIL reports on "Silver in Sediments from the St. Lawrence River and Estuary and the Saguenay Fjord."

The Ag content of the vertical sediment profile in the St. Lawrence River closest to Lake Ontario (Station 865), exposing the highest Ag concentrations, varied from 0.05-1.1 mg/kg (Lake Constance for comparison: 0.12-1.1 mg/kg).

The contamination in four dated sedimentary cores increased markedly after 1930, reached a maximum during the 1980s, and has been diminishing since, but is still far above the pre-1930 concentrations. For comparison: maximum Ag concentrations in Lake Constance were measured already between 1960-1975 and decreased to background concentrations in 1995 as of 1890.

In contrast to our explanation of a preferential *atmospheric* pathway and deposition of elevated Ag concentrations found in Lake Constance sediments, GOBEIL concludes: *"The direct discharge of wastewaters is likely the most important pathway for the introduction of anthropogenic Ag in these environments, but the input from the Great Lakes can also be important as suggested by very high Ag concentrations in a core collected in the river near Lake Ontario."*

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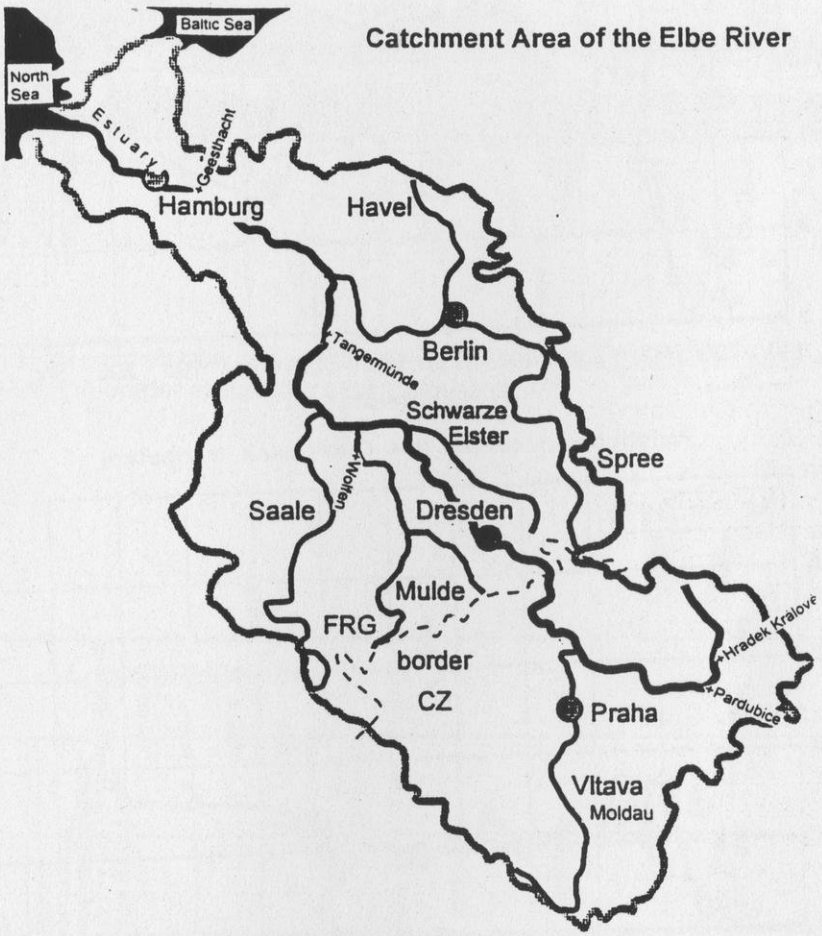

Fig. 1 Catchment area of the Elbe (*Czech* Labe) River.
Area 148,268 km^2, Length 1103 km, Inhabitants ca. 25 Mio, Discharge 880 m^3/s.

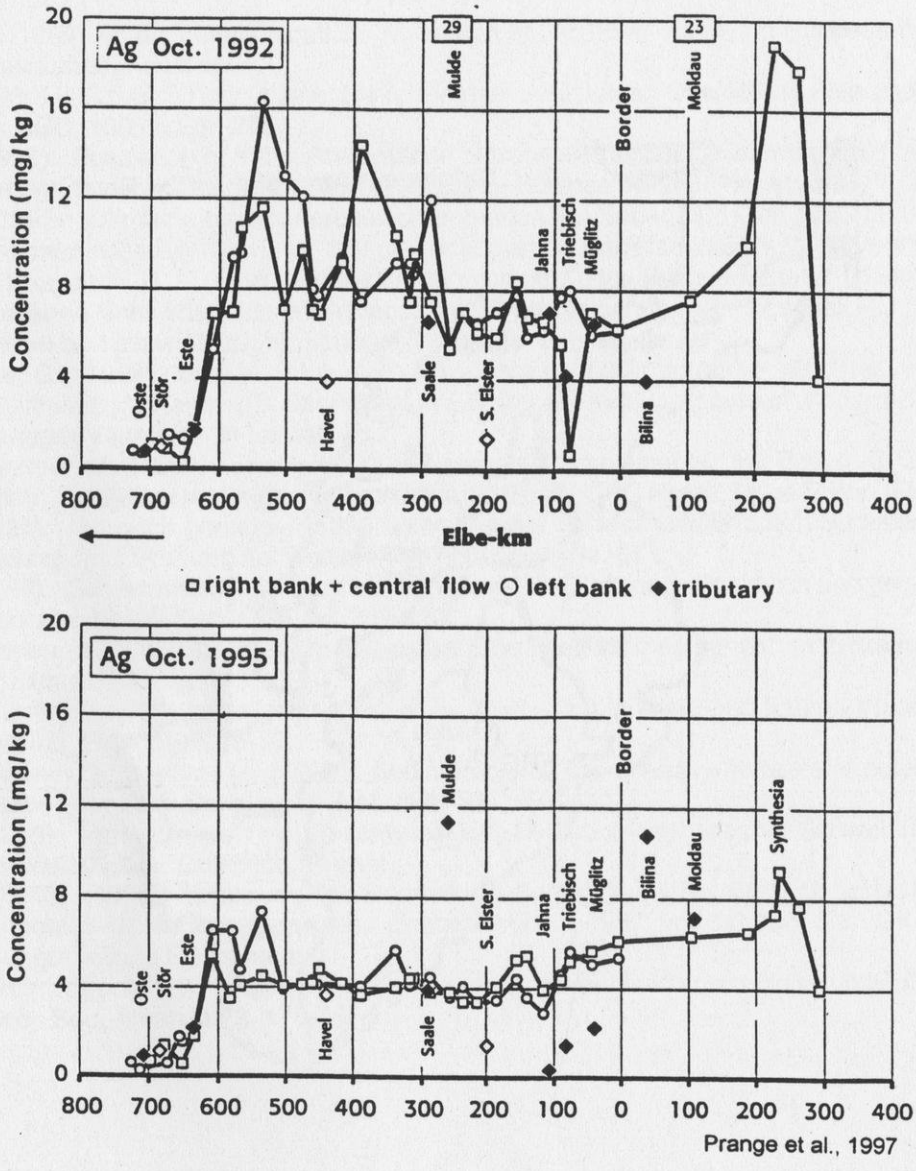

Fig. 2 Results of sediment analyses (fraction < 20 μm) from two sampling campaigns in 1992 and 1995 (after Prange, et al., 1997). Km counting starts at the Czech/German border (km 0) in two directions: upstream (Czech Republic), downstream (Germany).

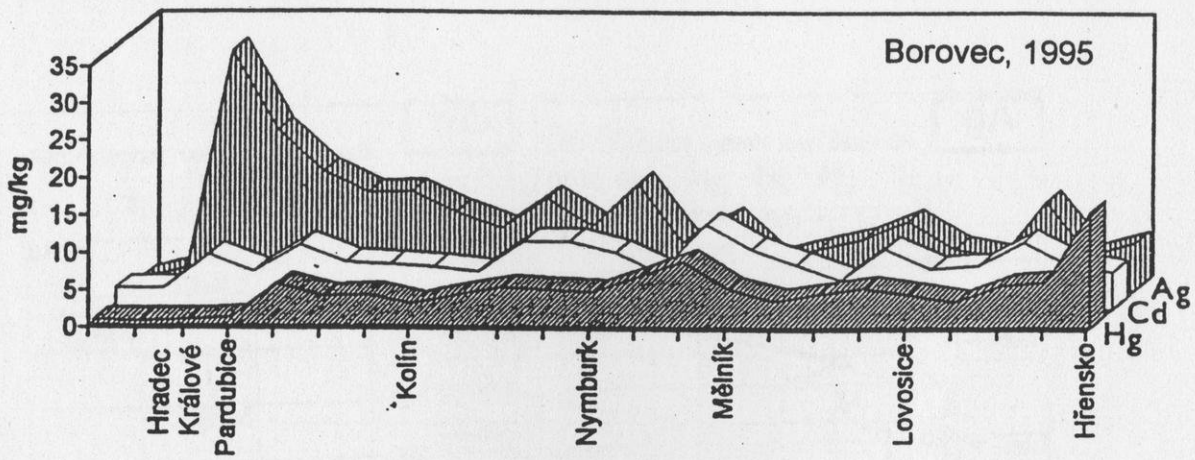

Fig. 3 Ag-, Cd- and Hg- concentrations in sediments (< 63 μm) of the Elbe River between Hradec Kralove and the Czech/German border at Hrensko after Borovec (1995).

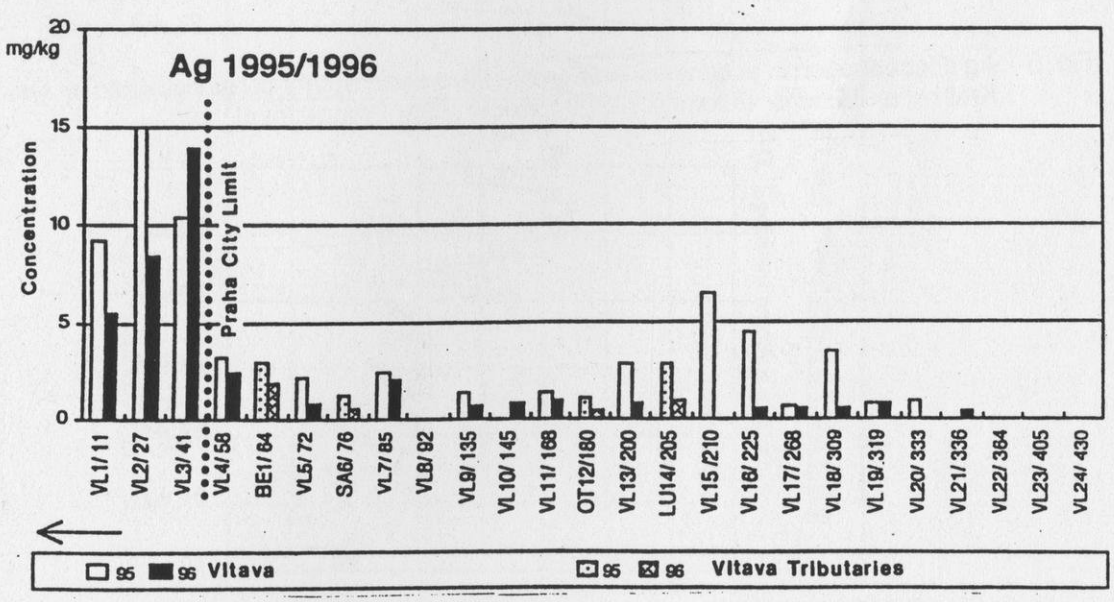

Fig. 4 Ag concentration in sediments (< 20 μm) of the Moldau/Vitava River and its major tributaries during sampling campaigns 1995 and 1996 (after Schindler, et al., 1997).

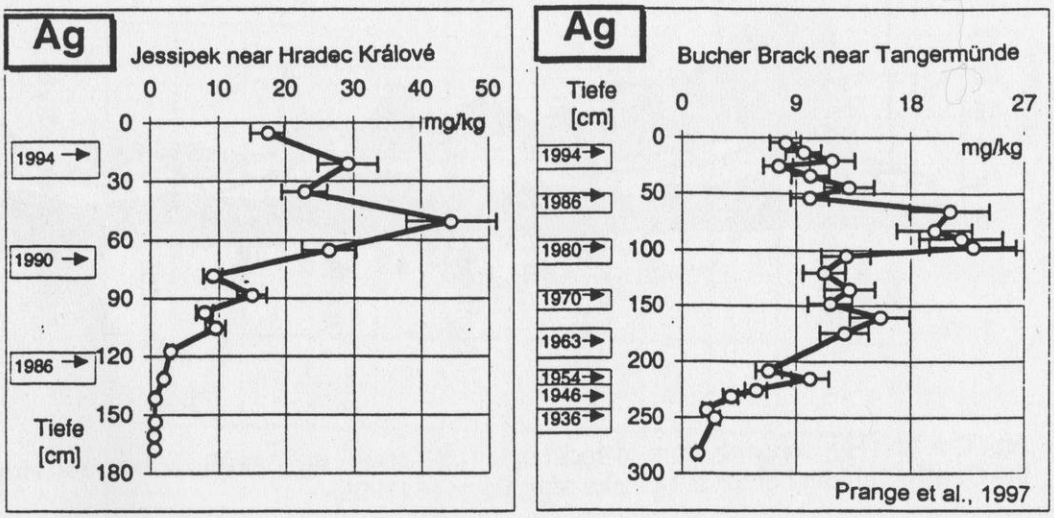

Fig. 5 Ag concentration in sediments (< 20 μm) of two sedimentary cores collected in Jessipek near Hradec Kralove and Bucher Eck near Tangermunde. After Prange, et al., 1997.

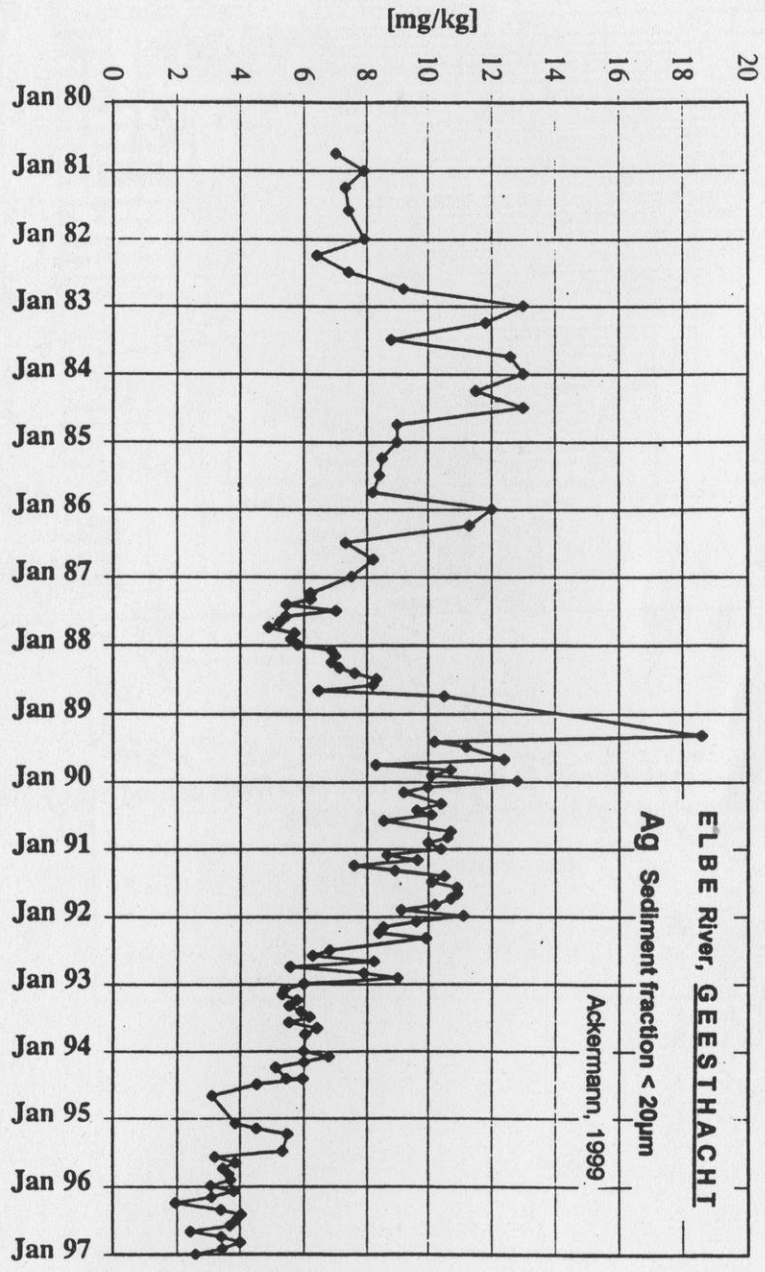

Fig. 6 Ag concentration in sediments (< 20 μm) at Geesthacht, lowermost limnic section of the Elbe River, between January 1980 and January 1997 (after Ackermann, 1999).

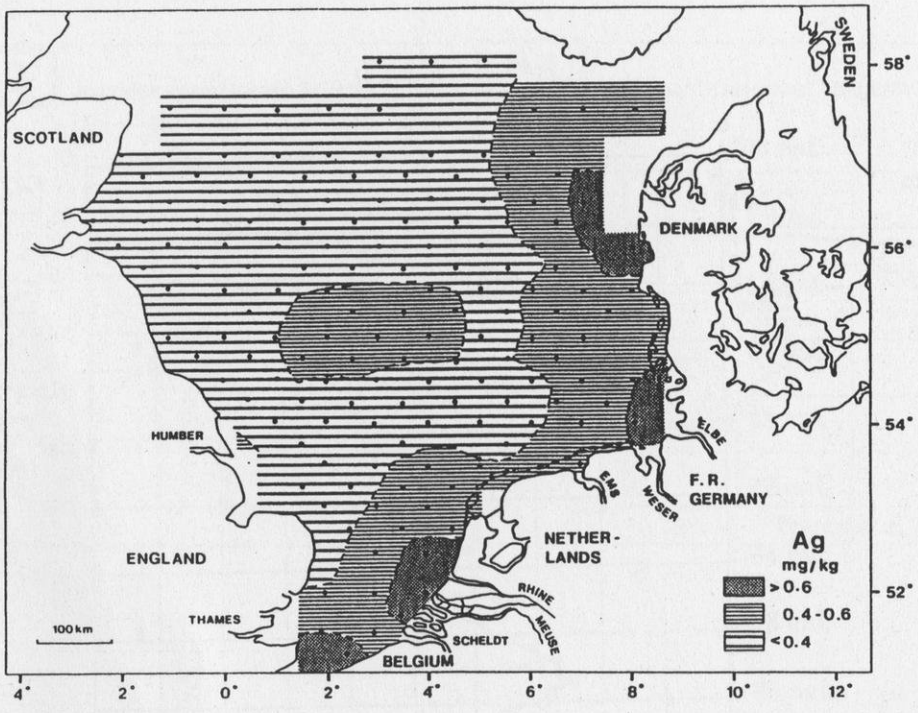

Fig. 7 Ag concentration in sediments of the North Sea. Analyses carried out by the German Federal Geological Survey on material studied by Irion & Mueller (1988).

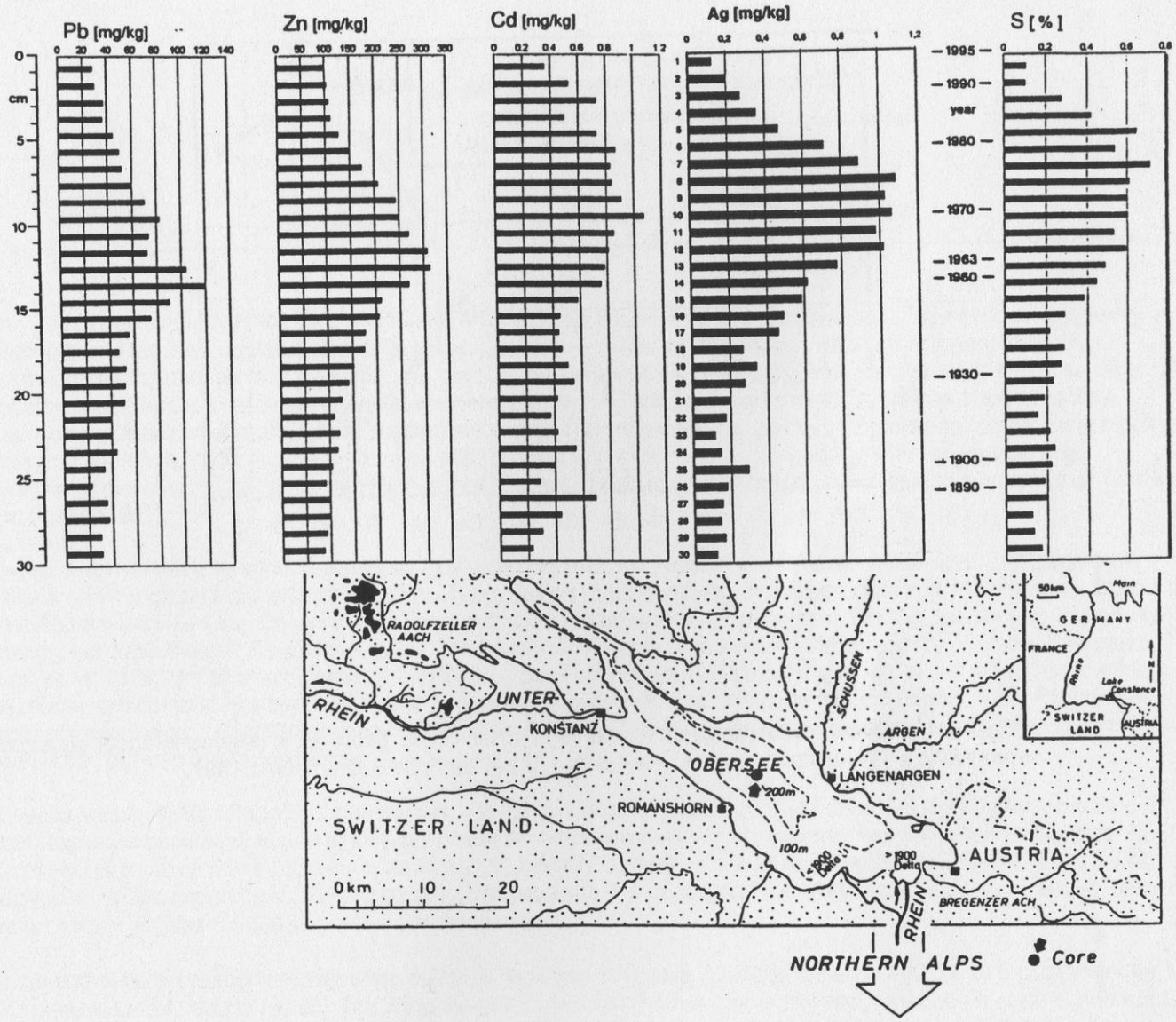

Fig. 8 Ag, Pb, Zn, Cd and S concentration in a dated sediment core from the central part of Lake Constance. After Mueller 1997, 1999.

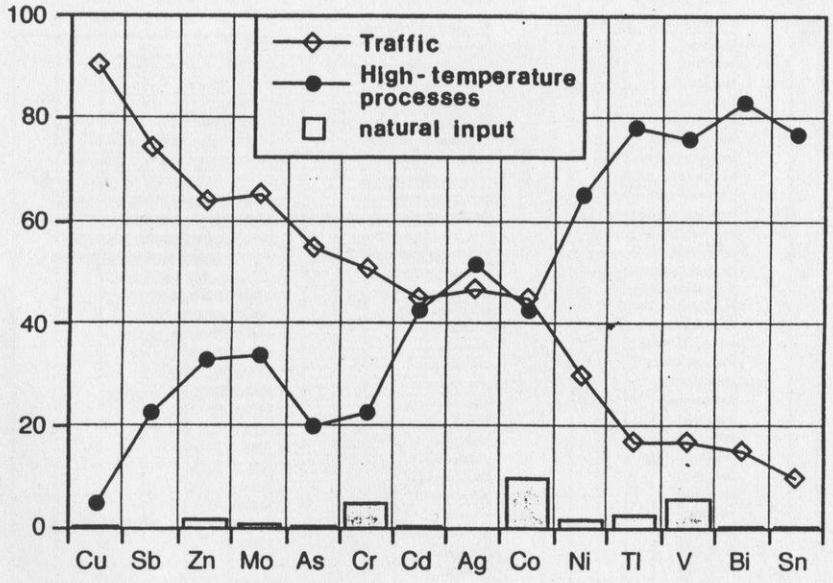

Fig. 9 Origin of environmentally important elements in urban dusts. Note Ag is derived in about equal portions from traffic and high-temperature processes. After Heinrichs & Brumsack, 1997.

Adding Magnesium to the Silver-Gill Binding Model

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The gills of freshwater fish are sensitive membranes that are the primary site of active ion uptake and, consequently, are susceptible to the ionoregulatory interference of waterborne metals (Morgan *et al.*, 1997). It has generally been accepted that the toxic form of a metal is the metal cation because it is readily available to interact at the gill surface. Located on the surface of the gill are negatively charged binding sites that serve as the initial site of metal toxicity. Cations (e.g., calcium or magnesium) present in the water can reduce metal toxicity by competing for the same binding sites and, therefore, reducing the amount of metal binding to the gill surface. Negatively charged ligands (e.g., thiosulfate) dissolved in the water can form complexes with the metal and render it unavailable to bind to the gill (Janes and Playle, 1995).

The silver-gill model (Figure 1) includes various water chemistry parameters to illustrate interactions of Ag and other cations with the gill surface, and interactions of complexing ligands with Ag^+ in the water column. The model is based on conditional equilibrium stability constants (log *K* values) which describe the strength of binding between metals and other ligands, including the gill. Conditional equilibrium stability constants can be inserted into aquatic chemistry programs, such as MINEQL$^+$, for testing different scenarios of water chemistry and the ensuing toxicity of a given metal. So far, the gill modeling technique has been applied to freshwater only (Playle *et al.*, 1993; Janes and Playle, 1995; Playle, 1998; Richards and Playle, 1998; Wood *et al.*, 1999). With the inclusion of magnesium, the model will be applicable not only to freshwater, but also to brackish water.

Juvenile rainbow trout (*Oncorhynchus mykiss*, 2 to 12 g), were exposed to 0.05 μM silver (as $AgNO_3$) for three to four h. Each exposure bucket contained eight L of reverse osmosis water and was supplemented with either calcium, as $CaSO_4$ (0 mM to 8.6 mM), or with magnesium, as $MgSO_4$ (0 mM to 210 mM) or with thiosulfate, as $Na_2S_2O_3$ (0 μM to 2.0 μM), depending on the experiment. Control experiments were run where no additions were made to the reverse osmosis water. Water pH was 6.5-7.0 and water temperature was ~15°C.

At the end of an experiment, all fish were sampled, their gills extracted, and then rinsed in 100 mL of E-pure water for 10 s to remove loosely bound metal. Gills were then digested in 5x their weight of 1 N HNO_3 for three h at 80°C and further diluted 10x in E-pure water. Analysis for metal content in the gills was done by graphite furnace atomic absorption spectrophotometry (AAS). Water samples were taken from each bucket at one h and three h and acidified with 16 N HNO_3. Water metal concentrations were measured by graphite furnace AAS and water ion levels (Mg, Ca, Na) were measured by flame furnace AAS.

Fish exposed to 0.04 μM Ag accumulated significant amounts of Ag on their gills in the presence of up to 8.6 mM $CaSO_4$ (Figure 2). The lack of protection against Ag accumulation in concentrations of Ca similar to those found in seawater is supported by other studies done in our lab (Janes and Playle, 1995). Since there was no decrease in Ag accumulation at the highest concentration of Ca in the water, Ca does not easily out compete Ag for gill surface binding sites. Similar studies have resulted in the same findings (Galvez and Wood, 1997). An investigation into what concentration of Ca will be successful in keeping Ag off the gills is not possible, since we cannot physically get levels of Ca >10 mM to stay in solution. Also, we cannot run the experiments with much lower concentrations of Ag or we would not be able to measure Ag accumulation on the gills.

Significant gill Ag accumulation is seen with or without Mg added to the water, up to a $MgSO_4$ concentration of 210 mM (Figure 3). No protective effect of Mg against Ag accumulation indicates that Mg is not successful in out competing Ag for gill binding sites. A concentration of 210 mM Mg is likely not found in natural water (seawater Mg concentration is ~50 mM) therefore neither Ca nor Mg, the 'hardness' cations, will be able to prevent fast accumulation of high concentrations Ag on trout gills. Over chronic exposures, these cations may provide protection against the ionoregulatory failure caused by low concentrations of Ag through their ability to reduce the diffusive permeability of the gill (McDonald, 1983). Also, Mg in high concentrations may be able to compete for its own binding site on the Na^+, K^+ ATPase enzyme (see Figure 1; Wood *et al.*, 1999).

Since neither Ca nor Mg were able to out compete Ag for gill binding sites, a thiosulfate experiment was run to prove that not only can Ag accumulate on the gills, it also can be kept off. Significant gill Ag accumulation was seen in concentrations of thiosulfate up to 1.0 μM. At 2.0 μM thiosulfate, gill Ag was reduced to levels similar to control fish, ~0.4 nmol Ag/g wet tissue (Figure 4). These results are similar to those of Janes and Playle (1995). The protective effect of thiosulfate is due to complexation between the $S_2O_3^{2-}$ anion and the Ag^+ cation. The formation of this complex prevents Ag from interacting at the gill surface.

Since there was no decrease in gill Ag concentration with up to 209 mM $MgSO_4$ in the water, the Mg-gill Ag binding constant must be much lower than the log $K_{Ag\text{-}gillAg}$ value of 10. The concentration of Mg in the water was ~2 million times higher than the concentration of Ag, so initial modeling attempts were made with a log K of 3.8. Modeling with this value resulted in predicted gill Ag concentrations that were lower than what were actually seen experimentally. Lowering the log K value to 0.8 yielded predicted gill Ag concentrations that were close to what were actually seen. Because Mg binds at the gill so weakly compared with Ag, any protective effect against Ag binding due to Mg competition is highly unlikely.

Magnesium was studied to evaluate its ability to protect against waterborne Ag from accumulating on gills of rainbow trout. No protection was seen by either Ca or Mg against Ag accumulation, however, we were able to keep Ag off the gills using 2.0 μM thiosulfate. Modeling with the aquatic chemistry program, MINEQL$^+$, resulted in a log $K_{Mg\text{-}gillAg}$=0.8 (Figure 5), suggesting that protection from Ag accumulation by Mg competition is unlikely.

Acknowledgements

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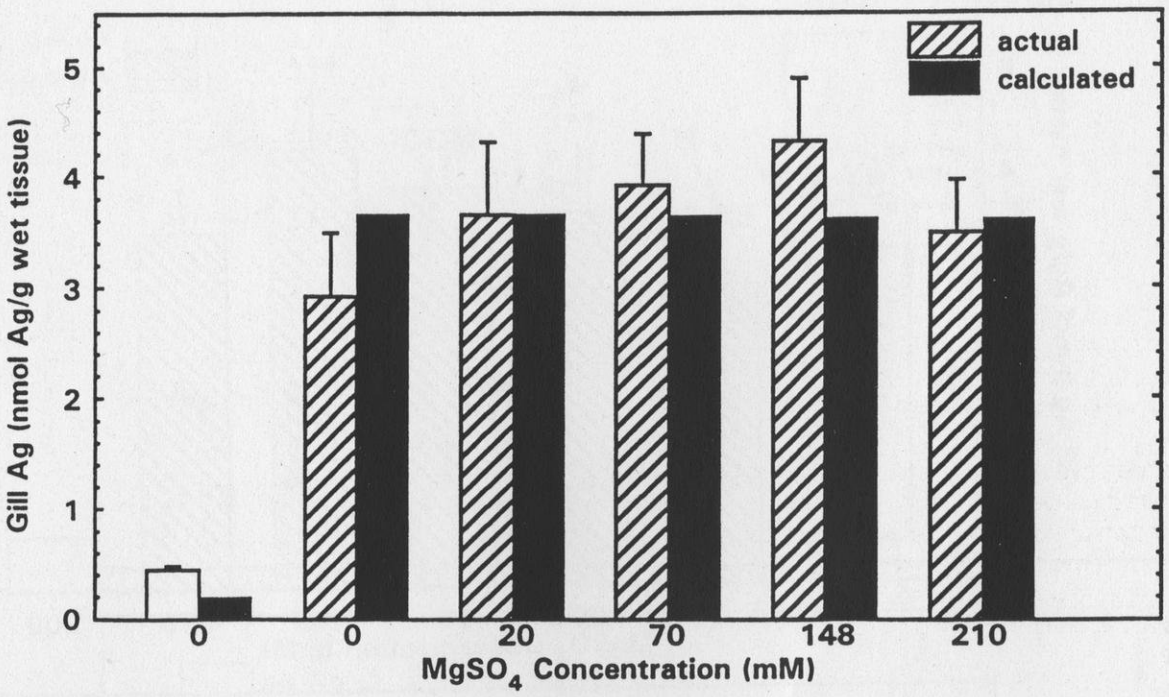


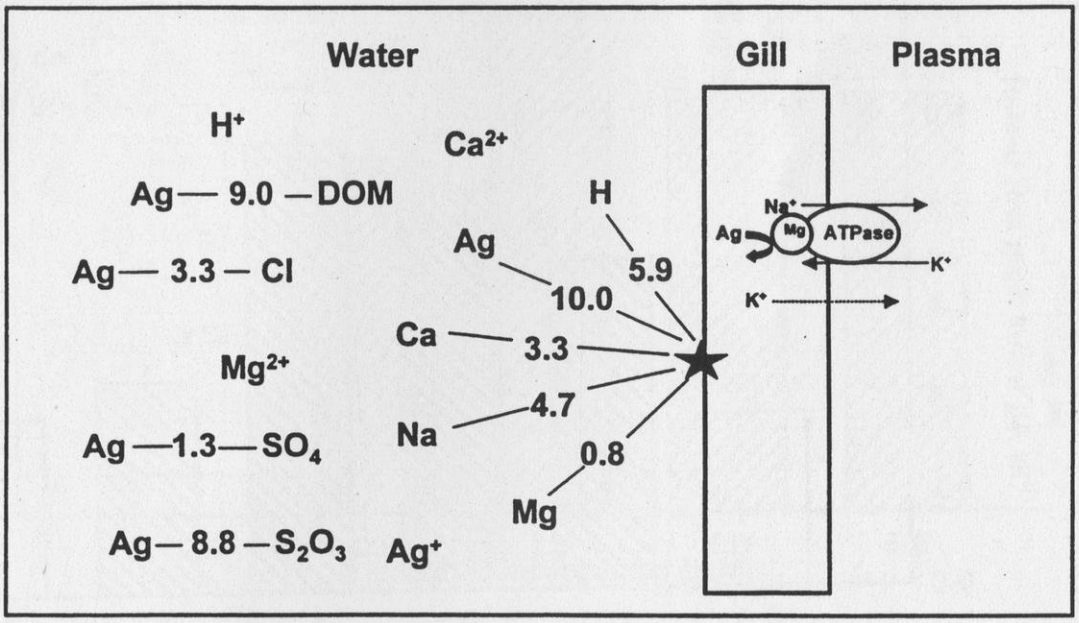
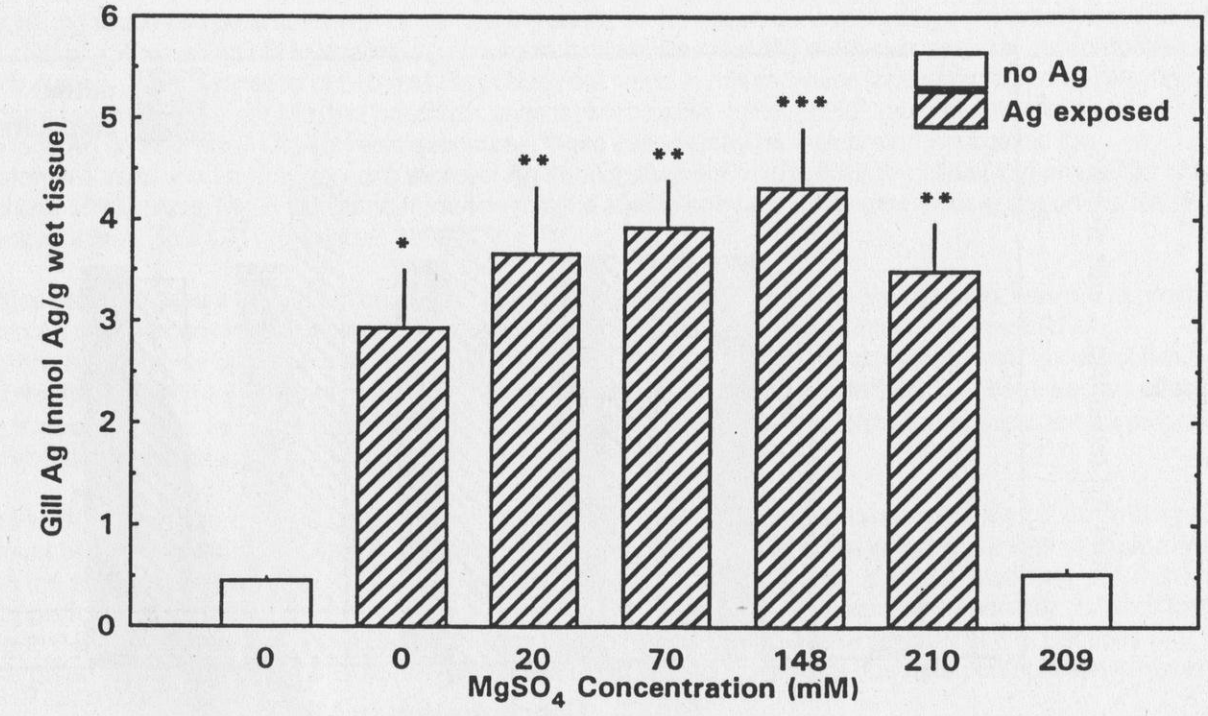


Figure 5 (top). Actual and predicted gill Ag accumulation in the presence of 0 to 210 mM $MgSO_4$ with 0.09 μM Ag in the water. Predicted gill Ag concentrations were calculated by MINEQL+ using a log $K_{Mg\text{-}gillAg}$=0.8.

Figure 6 (bottom). The silver-gill model of water ion interactions at fish gills with the addition of a log $K_{Mg\text{-}gillAg}$ value of 0.8. The binding strength of Mg to the gill is weak compared to other cations, indicating the poor protective effect of Mg against Ag binding.



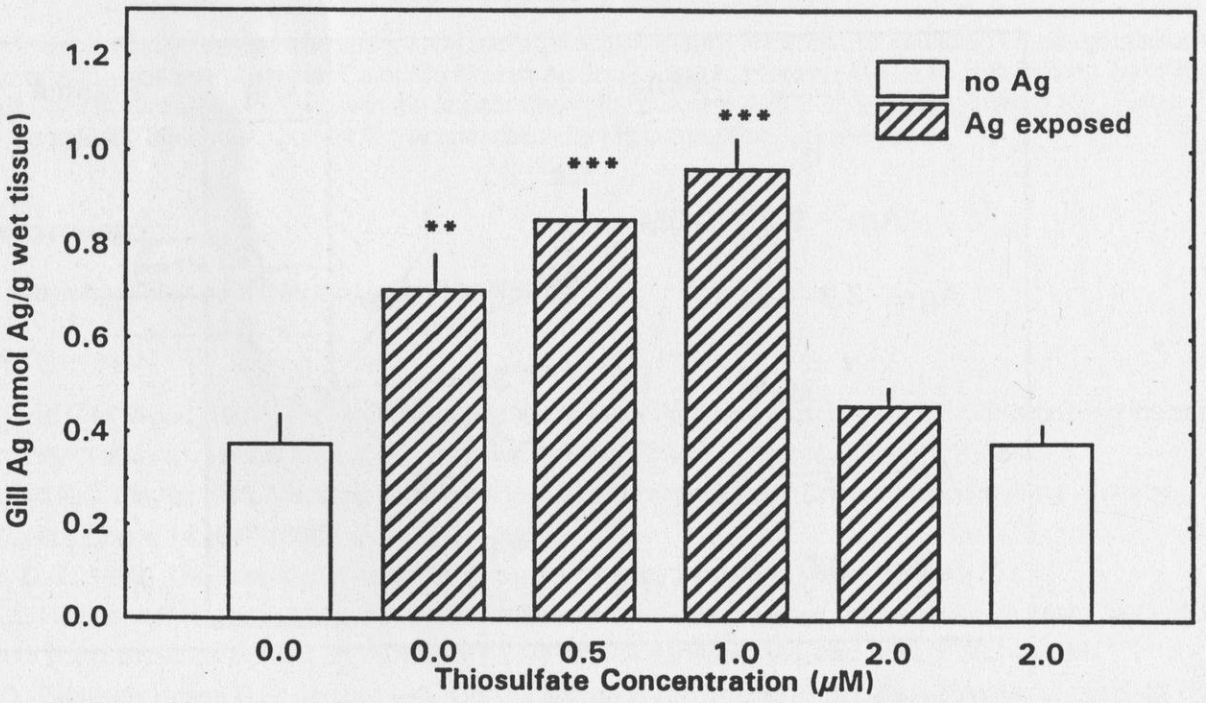
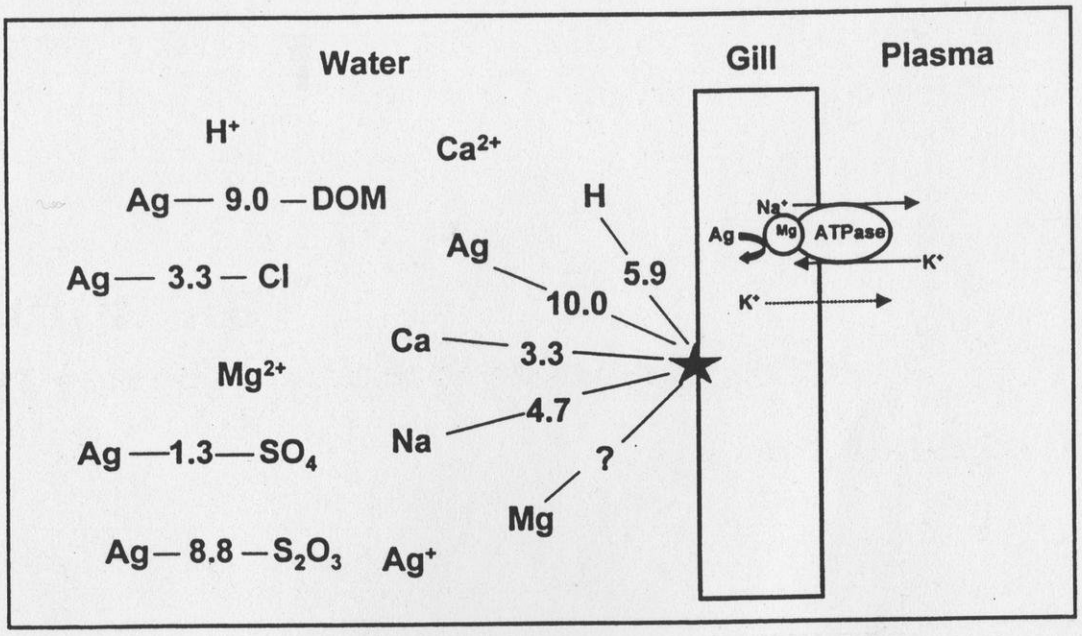


Figure 3 (top). Silver concentrations in gills of 5 to 10 g rainbow trout exposed to 0.09±0.02 μM $AgNO_3$ in the presence of $MgSO_4$. No decrease in Ag accumulation was seen in fish exposed to Ag with $MgSO_4$ present up to 210 mM.

Figure 4 (bottom). Silver concentrations in gills of 5 to 12 g rainbow trout exposed to 0.05±0.01 μM $AgNO_3$ in the presence of sodium thiosulfate. Significant concentrations of Ag accumulated on the gills with thiosulfate present up to a concentration of 1.0 μM. At 2.0 mM thiosulfate, Ag accumulation at the gills was prevented.



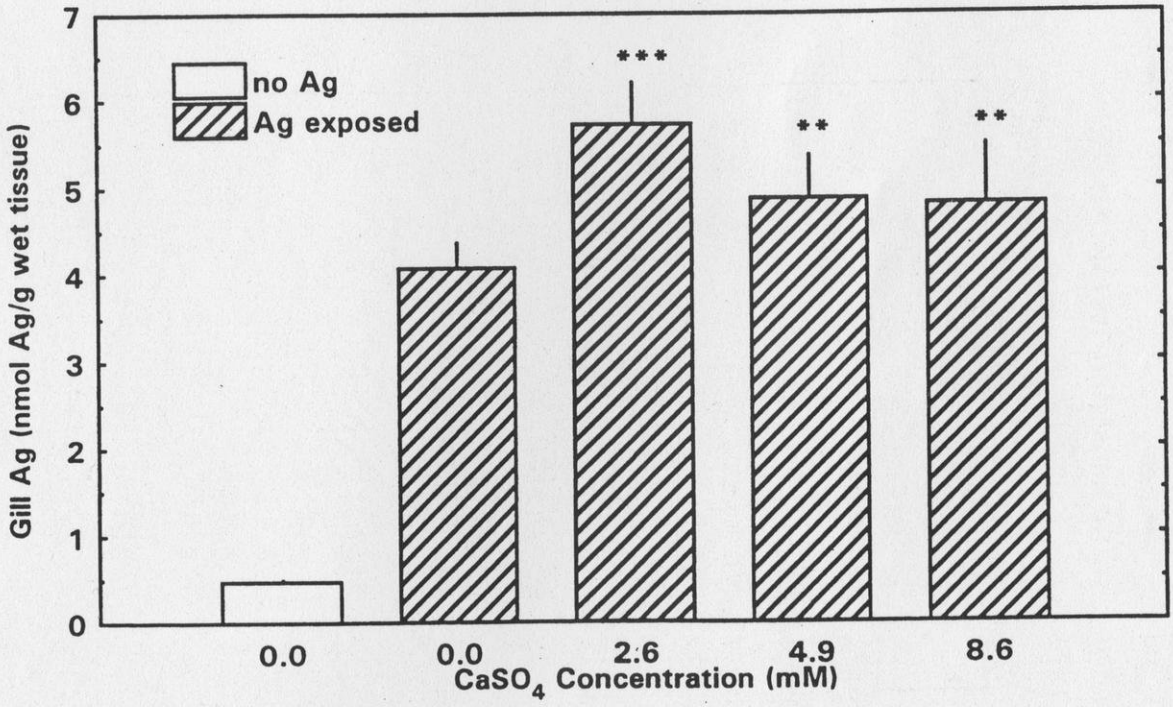


Figure 1 (top). The silver-gill model of water ion interactions at the surface of fish gills. Silver accumulation at the gill surface is reduced by the presence of competing cations in the water (Ca^{2+} or Mg^{2+}), or by the presence of complexing ligands ($S_2O_3^-$ or Cl^-). Individual log *K* values are found on the figure although the log $K_{Mg\text{-}gillAg}$ is not known. A single gill Ag binding site on the gill surface has been represented by a star.

Figure 2 (bottom). Silver concentrations in gills of 2 to 9 g rainbow trout exposed to 0.04±0.01 μM $AgNO_3$ in the presence of $CaSO_4$. No decrease in Ag accumulation occurred in any group exposed to Ag with Ca present up to a concentration of 8.6 mM.

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Transport, Fate and Effects of Silver in the Environment

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