SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 17, No. 4, pp. 547–561, 1998

Printed in the USA
0730-7268/98 $6.00 + .00

TOWARD A BETTER UNDERSTANDING OF THE BIOAVAILABILITY, PHYSIOLOGY, AND TOXICITY OF SILVER IN FISH: IMPLICATIONS FOR WATER QUALITY CRITERIA

CHRISTER HOGSTRAND*† and CHRIS M. WOOD‡
†T.H. Morgan School of Biological Sciences and Graduate Center for Toxicology, University of Kentucky, 101 Morgan Building, Lexington, Kentucky 40506-0225, USA
‡Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada

(*Received* 29 *May* 1997; *Accepted* 8 *September* 1997)

Abstract—In its ionic form, silver (Ag^+) is highly toxic to fish (96-h 50% lethal concentration [LC50]: low μg/L range). However, concentrations of Ag^+ in aquatic environments are extremely low and other more common forms of silver show only low to moderate toxicities (e.g., 96-h LC50: silver thiosulfate >100,000 μg Ag/L; silver chlorides >100 μg Ag/L). In bioassays with freshwater fish, acute toxicity appears to be derived exclusively from the Ag^+ ion concentration of the water. Some other forms of silver are bioavailable but do not show obvious contribution to acute toxicity. Complexation of Ag^+ by chloride, dissolved organic carbon, and sulfide are important in reducing silver toxicity. The protective action of hardness (i.e. calcium) is modest. When added as the readily dissociating silver nitrate salt, the toxicity of silver is considerably lower in seawater (96-h LC50 range: 330–2,700 μg Ag/L) than in freshwater (96-h LC50 range: 5–70 μg Ag/L). Acute silver toxicity to fish is caused by failure of the organism to maintain constant Na^+ and Cl^- concentrations in the blood plasma. In freshwater fish, Ag^+ exerts its toxic effects on the Na^+ and Cl^- transport across the gills, whereas the intestine has been indicated as the site of toxicity in seawater fish. Although there are still many gaps in our understanding of silver effects on fish, it can be concluded that present regulatory standards for silver can be much improved by taking into account the important geochemical modifiers of silver toxicity.

Keywords—Silver Fish Physiology Toxicology Review

INTRODUCTION

Impact of silver on aquatic environments is an area of research that investigators have been relatively late in exploring. While toxicities of more abundant and notorious metals, such as aluminum, cadmium, copper, lead, and mercury have been studied extensively, the literature on the effects of silver on aquatic animals and plants was until recently largely limited to the intermittent appearance of acute toxicity data. During the last several years, the situation has started to change and we are now beginning to understand some of the environmental factors that modulate silver bioavailability and toxicity. Furthermore, the mechanisms for acute silver toxicity have been investigated and, at least for freshwater fish, the key physiological and biochemical effects of acute exposure to the silver ion (Ag^+) have been characterized. In this review, which deals exclusively with effects on fish, we have attempted to evaluate critically the available information to illustrate what we do, and do not, know about silver toxicity. Current regulatory standards for silver are put into perspective with this emerging picture, and we have specifically pointed out areas where more research is needed to improve water quality criteria for silver. Comparable information for benthic organisms is provided by Hirsch [1,2] and Berry et al. [3]. The selection of data presented in this review reflects our personal opinion on experimental conditions and quality of data.

Water-breathing animals are typically more vulnerable to toxicants than animals that breathe air. The direct reason for this difference is not that fish are necessarily more sensitive to toxicants on a dose basis. Rather, many toxic chemicals, including ionic silver and other metal ions, are highly water soluble and get into direct contact with the very sensitive gills. The fish gill is a sophisticated, delicate organ that has multiple physiological functions. In addition to gas exchange, the gill is a central ionoregulatory organ, the major site of acid–base regulation, and the principal route for excretion of nitrogenous waste products. Disruption of any of these functions could potentially be fatal. At very high concentrations, waterborne toxicants produce a stereotyped structural damage to the gill epithelium, which leads to increased diffusion distances and impaired gas exchange [4]. The end result of such gross gill damage is a drop in the blood oxygen tension and suffocation. It is now well established that for most metals (aluminum excepted) the concentrations required to produce gross gill pathology are much higher than those encountered even in heavily contaminated waters. Unfortunately, this does not mean that natural fish populations are safe from the impact of metals because ionoregulatory functions of the gill have been found to be impaired fatally from exposure to quite low concentrations of many metals. Indeed different types of ionoregulatory disturbances are probably the most common causes of toxicity at environmentally realistic metal concentrations [5].

While the intestinal tract in most cases may be the dominating pathway for metal assimilation, it is usually branchial uptake of dissolved metal that is significant for short-term acute toxicity in freshwater fish. The bioavailability and toxicity of waterborne metals are very speciation dependent. Typically, it is the free metal ion that is the most toxic form, and

* To whom correspondence may be addressed (hogstra@pop.uky.edu).

metals complexed with dissolved organics and inorganic anions show lower degrees of bioavailability and toxicity [6]. This general rule is, however, not always valid. Notable exceptions are the very toxic methyl mercury and tributyl tin complexes. The situation is further complicated by the fact that the gill surface has high-affinity binding sites that compete with other ligands in the water for metal ions [7]. Moreover, the properties of these binding sites can change over time as a result of metal acclimation and other physiological processes.

GENERAL CONSIDERATIONS OF SILVER SPECIATION AND BIOAVAILABILITY

Silver is present in the environment primarily as silver sulfides and silver chloride ($AgCl_n^{1-n}$) complexes [8,9]. Dissolved organic carbon (DOC) and colloid complexes with silver are probably also prevalent [10–12]. While silver sulfide dominates under reducing conditions, the other silver species are more important in oxidizing waters where fish are likely to reside. The free silver ion (Ag^+), which is undoubtedly the most toxic species of silver (see below), is present at concentrations worth consideration only in freshwater, not in brackish water or seawater. In typical oxygenated freshwaters (e.g., from the Great Lakes Basin), the fraction of silver that is present as Ag^+ is <40% (often much less) of the total dissolved silver concentration, and most of the remaining silver is bound to DOC or forms an uncharged $AgCl_{(aq)}$ complex [8,13,14]. As the chloride concentration is increased, the activity of Ag^+ drops rapidly and becomes vanishingly low. Under oxidizing conditions in waters ranging from brackish to oceanic (0.8–36‰ salinity), $AgCl_2^-$ is the predominant dissolved silver species [8,14]. However, in laboratory toxicity tests there is often extensive formation of the insoluble $AgCl_{(s)}$ complex, cerargyrite, in most of the brackish water range (0.8–15‰) [13,14]. Cerargyrite is unlikely to form in natural waters because of the high silver concentrations needed for its formation [14]. Seawater also contains appreciable fractions of the larger $AgCl_3^{2-}$ and $AgCl_4^{3-}$ complexes, and the relative contributions from these compounds increase with increasing salinity. While chloride concentration is the major inorganic determinant of speciation of dissolved silver in oxidizing waters, water hardness and pH play very minor roles in the equilibrium equation [8]. The DOC binds silver with high affinity [10,11], and preliminary results from bioassays show that the DOC concentration of the water is a strong modifier of silver toxicity [15,16]. These results suggest that DOC is likely an important variable for silver speciation in natural waters.

The concentrations of silver in tissues of field-collected fish are generally low (Table 1). In most studies the highest levels of silver were found in the liver. This is consistent with results from toxicokinetic experiments, which indicate that the liver is a major silver accumulatory organ in fish [11,17,18]. Reported concentrations of silver in liver of feral fish range from 0.01 μg/L (wet weight) in blackfin icefish (*Chaenocephalus aceratus*) from Antarctica [19] to 0.8 μg/L (wet weight) in winter founder (*Pleuronectes americanus*) from the New York Bight [20]. We found the silver concentration in farmed rainbow trout (*Oncorhynchus mykiss*) to be very high in comparison with that described for fish species collected in the field. Farmed rainbow trout fingerlings, used as controls in various experiments, showed hepatic silver concentrations averaging 4 μg/g (wet weight) [21] and the silver level in the liver of larger rainbow trout (250–450 g) was approximately 10 μg/g (wet weight) [22,23]. The rainbow trout is known to have unusually high levels of copper in the liver (reviewed by Olsson et al. [24]). Silver and copper are chemically and toxicologically related (see below), which suggests that the relatively high hepatic silver concentrations could be secondary to the unusual copper accumulation of the rainbow trout.

Table 1. Concentrations of silver in tissues of field-collected fish[a]

Tissue	Concn.	No. species	Reference
Intestine	0.135	1	[95]
Gill	0.71	1	[95]
Kidney	0.38	1	[95]
Liver	0.01–0.8	8	[19,20,95,103–107]
Ovaries	0.035–0.06	2	[106,107]
Muscle	0.002–0.2	7	[19,20,103–106]
Scales	0.1–0.3 (dry wt.)	7	[108]

[a] The silver concentrations are shown as μg/g wet weight, except where indicated (scales). In a few cases, where the silver concentrations in the original research articles were expressed on a dry weight basis, a conversion factor of ×0.25 has been applied.

Because of the very high toxicity of Ag^+ in comparison with other silver species [21,25] and the general rule of free metal ions as the most bioavailable metal species, it might be assumed that only the Ag^+ is taken up by fish to any appreciable extent. On balance, the literature on silver accumulation in fish does not support this view, though admittedly, the available information is limited and difficult to compare because of differences in exposure concentrations and durations. In Table 2 we have compiled data from our laboratories on hepatic silver accumulation from short-term exposures of fish to waterborne silver. During such short-term experiments, silver elimination should be negligible [18] and accumulation of silver in the liver should reflect uptake. By normalizing for exposure duration and concentrations of dissolved silver in the different experiments, it is possible to make some general statements about the bioavailability of different silver species. Although large amounts of silver were accumulated in rainbow trout exposed to a very high concentration of silver thiosulfate ($Ag[S_2O_3]_n^{n-}$), the concentration-specific accumulation rate (CSAR), which indicates bioavailability, was 1,000 lower for $Ag(S_2O_3)_n^{n-}$ than for silver added as silver nitrate ($AgNO_3$) to freshwater. These data suggest that $Ag(S_2O_3)_n^{n-}$, which is the form of silver resulting from photographic processing [26], is not readily bioaccumulated by fish. The CSAR for negatively charged $AgCl_n^{1-n}$ to fish in seawater appears to be equally low, indicating that the negatively charged $AgCl_n^{1-n}$ complexes present in full-strength seawater accumulate very slowly in fish. In contrast, the uncharged $AgCl_{(aq)}$ complex may be highly bioavailable. If high concentrations of silver are added as $AgNO_3$ to brackish water, large quantities of insoluble cerargyrite will form and the remaining dissolved silver will largely be present as $AgCl_{(aq)}$ and $AgCl_2^-$ [14,21,27]. Under these conditions substantial amounts of silver enter the fish and the CSAR is quite high. Because little silver accumulates in the liver of fish exposed in seawater (where $AgCl_2^-$ is the dominant complex), it can be concluded that $AgCl_{(aq)}$ is the complex efficiently taken up in brackish water. Thus, while $AgCl_{(aq)}$ is substantially less toxic than the Ag^+, it may be almost as bioavailable as the latter. In contrast, negatively charged silver complexes, such as $AgCl_2^-$, $AgCl_3^{2-}$, $AgCl_4^{3-}$, and $Ag(S_2O_3)_n^{n-}$, probably have low bioavailabilities to fish.

Table 2. Relative bioavailability of different forms of dissolved silver, added as $AgNO_3$ or $Ag(S_2O_3)_n^{n-}$ [a]

Conditions	Dissolved Ag species	Fish species	Total dissolved Ag (μg/L)	AR (μg/[g × d])	CSAR (ng/[g × d × ppb])	Reference
$AgNO_3$ in 0.05 mM Cl^-	Ag^+ (90.1%) $AgCl_{(aq)}$ (9.8%) $AgCl_2^-$ (0.0%)	Rainbow trout	1.7 5.6	2.75 2.45	2,800 440	[13]
$AgNO_3$ in 0.22 mM Cl^-	Ag^+ (66.5%) $AgCl_{(aq)}$ (32.6%) $AgCl_2^-$ (0.0%)	Rainbow trout	1.7 7.5	2.42 2.72	2,400 360	[13]
$AgNO_3$ in 0.8 mM Cl^-	Ag^+ (34%) $AgCl_{(aq)}$ (60%) $AgCl_2^-$ (5.2%)	Rainbow trout	4.3 7.2 9.3	1.56 2.24 2.4	360 310 260	[21]
$AgNO_3$ in 50 mM Cl^-	Ag^+ (0.1%) $AgCl_{(aq)}$ (14%) $AgCl_2^-$ (76%)	Rainbow trout	120	30.0	250	[21]
$AgNO_3$ in 510 mM Cl^-	Ag^+ (0.0%) $AgCl_{(aq)}$ (1%) $AgCl_2^-$ (57%) $AgCl_3^{2-}$ (23%) $AgCl_4^{3-}$ (19%)	Starry flounder	250	0.084	0.34	[95]
$Ag(S_2O_3)_n^{n-}$ in 0.8 mM Cl^-	Ag^+ (0.00%) $AgS_2O_3^-$ (1%) $Ag(S_2O_3)_2^{2-}$ (99%)	Rainbow trout	98,000	37.0	0.38	[21]

[a] Accumulation rate (AR) refers to the increase in liver silver concentration per day. The concentration-specific accumulation rate (CSAR) is the AR normalized for exposure concentration. The speciation of silver at each exposure condition is given according to computer modeling by MINEQL$^+$ (version 3.01). The data suggest that the silver species, Ag^+ and $AgCl_{(aq)}$ are highly bioavailable whereas $Ag(S_2O_3)_n^{n-}$ and negatively charged $AgCl_n^{1-n}$ complexes have very low bioavailabilities.

EFFECTS OF SILVER ON FRESHWATER FISH

Silver toxicity in freshwater

Acute waterborne toxicity. There is now a large body of information on the acute toxicity of silver to freshwater fish. However, much of it is in the ''gray literature.'' The earlier studies have been summarized by Cooper and Jolly [28] and the U.S. Environmental Protection Agency (EPA) [29] and the more recent work by Eisler [30]. In the peer reviewed literature, representative measurements of 96-h 50% lethal concentration (LC50) values under well-controlled conditions have been reported for rainbow trout (*O. mykiss*) [13,21,31–35], flagfish (*Jordanella floridae*) [36], fathead minnow (*Pimephales promelas*) [25,33,34,36–38], channel catfish (*Ictalurus punctatus*) [37], bluegill (*Lepomis macrochirus*) [34,35,39], arctic grayling (*Thymallus arcticus*) [40], and coho salmon (*Oncorhynchus kisutch*) [40]. With very few exceptions, these studies have relied on the use of the highly soluble salt $AgNO_3$ as the source of silver, and there is general agreement that the 96-h LC50 for $AgNO_3$ lies in the range of 5 to 70 μg total Ag/L (0.05–0.65 μM; Fig. 1). This makes $AgNO_3$ one of the most toxic metal salts in freshwater laboratory tests, more potent than even copper or cadmium salts [41], a conclusion first recognized by Erichsen-Jones [42] in classic experiments on stickleback (*Gasterosteus aculeatus*). However, it is important to emphasize that tests with $AgNO_3$ are of questionable environmental relevance, because $AgNO_3$ dissociates completely (log *K* value = −0.3 [43]), to yield substantial proportions of free ionic Ag^+ in solution. In most natural waters, only a small portion of the total dissolved silver will exist as Ag^+, but mounting evidence (see below) suggests that the ionic Ag^+ fraction is the primary or sole source of acute toxicity to freshwater organisms in such tests. Both geochemical speciation programs (MINTEQA [44] MINEQL$^+$ [45]) and the few available measurements [46–48] tell us that the concentrations of ionic Ag^+, relative to these acute levels, are quite low (<0.2 μg/L), even in highly polluted natural waters. This is because Ag^+ binds avidly to a variety of anionic ligands, both organic and inorganic.

Of much greater environmental relevance is the potential toxicity of various forms of complexed silver. The available information is sparse but suggests strongly that complexed silver is far less toxic than ionic Ag^+. Ligands of importance

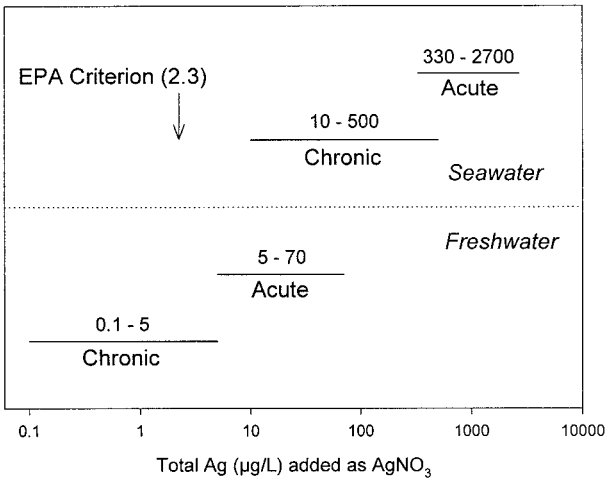

Fig. 1. Toxicity ranges of silver, added as $AgNO_3$, to fish in freshwater and seawater. The figure is based on literature cited in the text. Note that the range for chronic toxicity in seawater is based on very few studies and that information on silver toxicity to fish classes other than teleostei is not available. The EPA criterion is the current U.S. EPA acute water quality criterion for silver in seawater (2.3 μg/L [29]), which refers to total recoverable silver. For freshwater systems, the EPA criterion depends on water hardness according to the equation: maximum total recoverable Ag (μg/L) = $e^{(1.72[\ln \text{hardness}]-6.52)}$. There are no chronic criteria for silver in effect.

include thiosulfate, sulfide, chloride, and DOC. Silver thiosulfate is the major form of silver in the discharge of photographic industries prior to effluent processing [26], silver sulfide (Ag_2S) is the major form after reduction in sewage treatment [47], while Cl^- and DOC are readily available in most natural waters.

Dissolved organic carbon complexes silver with high affinity [10]; for example, Janes and Playle [11] estimated a log $K \sim 9.0$ for natural DOC collected from a marsh. To date, the only direct toxicity studies on Ag–DOC complexes are two preliminary reports (abstracts) by Brooke et al. [15] and Klaine et al. [16] on 30-d-old and newly hatched fathead minnow, respectively. In both investigations, raising DOC (reagent-grade humic acid) from 0 to 1 to 10 to 12 mg/L increased the 96-h LC50 for $AgNO_3$ by two- to fourfold. Anecdotally, Brooke et al. [15] noted that when fathead minnow and *Daphnia* $AgNO_3$ toxicity tests were performed in natural St. Louis River water, LC50 values were raised 10- to 60-fold relative to values in water of defined composition. They attributed this effect to the high DOC concentration (value not reported) of the St. Louis River. There is a clear need for more research, preferably using a range of DOCs collected from the field.

Early work by the photographic industry suggested that thiosulfate (log K = 8.8–14.2 for various complexes) and sulfide complexation (log K = 19.2) greatly reduced silver toxicity [49,50], but the first rigorous demonstration was provided by LeBlanc et al. [25]. In acute tests on fathead minnow, silver sulfide and $Ag(S_2O_3)_n^{n-}$ complexes, presented separately, exhibited 96-h LC50 values 15,000- to 20,000-fold greater (>240,000–280,000 μg/L) than values for $AgNO_3$ (16 μg/L). This relative lack of toxicity for $Ag(S_2O_3)_n^{n-}$ was confirmed by Hogstrand et al. [21] with rainbow trout. The 96-h LC50 value for silver to rainbow trout was 161,000 μg/L relative to 12 μg/L for $AgNO_3$, a 13,000-fold difference. In these trials, the $Ag(S_2O_3)_n^{n-}$ complex was highly soluble, being made from a 4:1 excess molar ratio of thiosulfate to silver, and remained fully dissolved up to these very high concentrations. The very insoluble silver sulfide was tested as a slurry [25].

A similar lack of solubility complicates tests with $AgCl_n^{1-n}$ complexes that are weaker in the dissolved form (log K = 3.3–5.5). The precipitated form, cerargyrite, has a log K of 10.15. When particulate slurries made from suspensions of AgCl salt were tested, no mortality was observed over 7 d in rainbow trout at the highest level tested, 1,000 μg/L [21]. An alternate way of testing $AgCl_n^{1-n}$ is to add $AgNO_3$ to waters containing a high background level of NaCl (e.g., 50 mM). When this approach was adopted, 96-h LC50 values were >4,600 μg/L (40% mortality only) for fathead minnow in flow-through tests [25] and >100,000 μg/L (0% mortality) for rainbow trout in static-renewal tests [21]. Speciation calculations are problematical because of the time-dependent precipitation of cerargyrite ($AgCl_{(s)}$) under these conditions, but Hogstrand et al. [21] estimated that about 120 μg/L of dissolved $AgCl_n^{1-n}$ was present at the highest level in their tests. Thus chloride complexation reduced $AgNO_3$ toxicity at least 10-fold, and in practice by several more orders of magnitude when precipitation was taken into account. In this regard, the ability of both native chloride and sulfide to greatly reduce toxicity by precipitating Ag^+ out of solution in natural waters should be recognized.

With this background in mind, it is clear that acute guidelines based on $AgNO_3$ toxicity are environmentally relevant only if the geochemistry of silver in the test waters and receiving waters is understood. Regulatory agencies such as the U.S. EPA have long recognized the importance of only one geochemical variable, water hardness, in modifying silver toxicity. Hardness largely reflects the concentration of calcium in most natural waters. Indeed, hardness is the only geochemical variable incorporated into the current regulatory approach for silver in the form of the ''hardness equation'' [29]:

$$\text{maximum total recoverable Ag } (\mu\text{g/L}) = e^{(1.72[\ln \text{ hardness}]-6.52)}$$

where hardness is expressed in units of mg/L of $CaCO_3$ equivalents. For example, at hardnesses of 50, 100, and 200 mg/L, the mandated maximum total recoverable silver level would be 1.2, 4.1, and 13 μg/L. While the goal of incorporating geochemistry into environmental regulation is laudable (e.g., see Bergman and Dorward-King [51] for a modern exposition of the geochemical approach), we question the validity, and therefore the usefulness, of this particular equation for the reasons laid out below.

Examination of the original document that derived this equation [29] indicates that the data set used was strongly dependent on a series of non-peer reviewed interlaboratory comparison tests (''round robin'') with $AgNO_3$ on fathead minnow, rainbow trout, and *Daphnia*, summarized by Lemke [52]. Hogstrand et al. [21] reanalyzed some of these data (for rainbow trout) and in fact found no correlation with water calcium concentration. However, there was a strong positive correlation of the 96-h LC50 values with water chloride concentrations, a protective effect. Galvez and Wood [13] reanalyzed the data for both fathead minnow and rainbow trout in greater detail using geochemical speciation analysis by MINEQL$^+$ [45]. They concluded that the observed toxicity was largely related to the concentration of the free Ag^+ ion in solution, for which the major determinant was water chloride. The original document [29] noted that other data were available at the time in both the gray and peer-reviewed literature (e.g., Davies et al. [32]) showing a much smaller or no protective effect of hardness, and a protective effect of chloride, but elected not to use this information in the derivation of the equation.

It is widely recognized that Ca^{2+} stabilizes biological epithelia and protects against the damaging effects of many metals by competition for binding sites at gill surfaces [53,54]. However, in the case of silver, the real degree of protection by hardness appears to be quite modest, in accord with recent findings on the mechanism of Ag^+ toxicity to freshwater fish (see below). For example, Davies et al. [32] reported that increasing water hardness from 26 to 350 mg/L elevated the 96-h LC50 of $AgNO_3$ for rainbow trout only 2-fold, whereas the ''hardness equation'' predicts a >80-fold elevation. Nebeker et al. [33] found no protective effect in *Daphnia* life-cycle tests between 60 and 180 mg/L (hardness equation prediction = 7-fold difference). With fathead minnow in the hardness range 50 to 250 mg/L (prediction = 16-fold difference), preliminary data (abstracts) indicate a 2.5-fold elevation of the 96-h LC50 in 30-d-old fish [15] and no protective effect at all in newly hatched larvae [16]. Overall, simple use of the hardness equation for the conditions described above would have greatly underprotected at high hardness and overprotected at low hardness.

Galvez and Wood [13] found that on an equimolar basis, water chloride had a 10-fold greater protective effect than water calcium in extending the survival time of rainbow trout in a lethal concentration of $AgNO_3$. More importantly, as il-

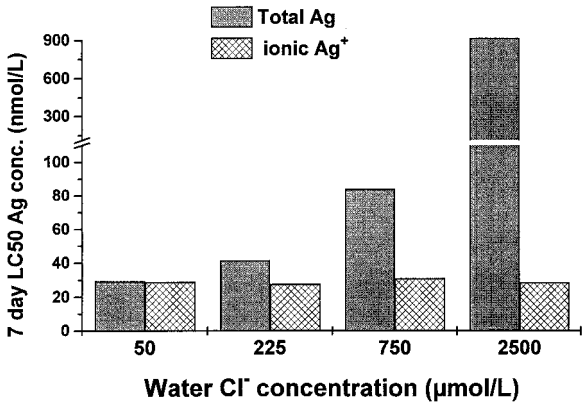

Fig. 2. The contribution of the free silver ion, Ag^+, to acute toxicity in rainbow trout in freshwater. The figure shows the 168-h LC50 of silver expressed as total silver (added as $AgNO_3$ to the water) and the concentration of the Ag^+ in the water (calculated by MINEQL$^+$, version 3.01). The dissolved silver species present were almost entirely Ag^+, $AgCl_{(aq)}$, and $AgCl_2^-$. Precipitation of cerargyrite, $AgCl_{(s)}$, occurred only at the highest Cl^- concentration (2,500 μM). Silver toxicity seems to be determined exclusively by the Ag^+ concentration of the water, and the 168-h LC50 value for Ag^+ is approximately 30 nM (3.2 μg Ag^+/L). Data for the plot were obtained from Hogstrand et al. [21] and Galvez and Wood [13].

lustrated in Figure 2, 168-h acute toxicity tests carried out in concert with experimental manipulations of water chloride, over widely differing ranges of total $AgNO_3$ and hardness, demonstrated the critical importance of ionic Ag^+. These bioassays, which were performed by manipulation of the amounts of dissolved $AgCl_n^{1-n}$ and precipitated cerargyrite, revealed an almost constant LC50 value when silver was expressed as the concentration of the free ion Ag^+ (Fig. 2). Regardless of the concentration of total silver, the 168-h LC50 for free Ag^+ was ~30 nmol/L = 3.2 μg/L [13,21].

In view of the small protective influence of hardness, the large variation in chloride in natural fresh waters and its effect on the proportion of silver existing as Ag^+, we believe that there is a pressing need to rework the hardness equation for silver. Ideally, the equation should include other geochemical factors, most particularly chloride, DOC, and perhaps sulfide. Research currently in progress [15,16,55] may help meet this goal.

Chronic waterborne toxicity. In freshwater fish, the data base for chronic toxicity is only a small fraction of that for acute toxicity, but it appears clear that chronic thresholds are much lower than acute thresholds for tests based on $AgNO_3$ (Fig. 1). The most detailed and lengthy study is that of Davies et al. [32] who exposed rainbow trout (starting with eyed eggs) for 18 months to very low levels of silver as $AgNO_3$ (concentrations were nominal) in flowing water of low hardness (26 mg/L, $[Cl^-]$ not reported). Based on chronic mortality alone, the maximum acceptable toxicant concentration (MATC) was between 0.09 and 0.17 μg Ag/L, while premature hatching and reduced growth rates were seen at slightly higher levels (<0.5 μg Ag/L). In this same study, the 96-h LC50 was 6.5 μg Ag/L, suggesting an extremely high acute-to-chronic ratio (ACR ~ 54) that is rather unusual for metals (e.g., for Cu, the ACR is about 3 [56]). Support for this high ratio is provided by Nebeker et al. [33] who conducted similar exposures of rainbow trout (steelhead) for 60 d only (from fertilized eggs to postswimup stage) in water of low hardness (36 mg/L) and low chloride (7.2 mg/L = 200 μmol/L). Based on growth inhibition, the MATC was <0.1 μg/L, whereas the 96-h LC50 was 9.2 μg/L, yielding an ACR of ~100. A 32-d embryo–larval exposure of fathead minnows in water of low hardness (45 mg/L, $[Cl^-]$ not reported) again yielded a relatively low MATC (0.37–0.65 μg/L, based on survival) and high ACR (~28) [37]. Norberg-King [38], using the same water quality for a 7-d subchronic test with fathead minnows, reported an MATC of about 1 μg/L, and an ACR of ~8, but recognized that the subchronic test was less sensitive than longer chronic procedures (e.g., early life-stage tests).

In a 28-d physiological study with juvenile rainbow trout in moderately hard water (hardness = 140 mg/L, chloride = 25 mg/L or 700 μmol/L), Galvez et al. [27] found no effect of $AgNO_3$ on growth at 0.5 μg Ag/L, but a small increase in food consumption and a slight disturbance of ionoregulation, supporting the idea that the MATC is below this level. At the other end of the spectrum are reports such as those of Diamond et al. [35], who used natural river water (hardness = 35 mg/L, chloride not reported) to conduct both acute and 7-d larval survival and growth tests with rainbow trout and found an ACR >1.0. Here the presence of unmeasured natural anionic ligands (e.g., DOC) may have served as an ameliorating influence. Similarly Coleman and Cearley [57] found that bluegill tolerated 70 μg Ag/L as $AgNO_3$ for 6 months without significant effects on growth, but water chloride levels were exceptionally high (193 mg/L = 5,500 μmol/L), which likely left little Ag^+ in solution.

Based on the one available study [25], the astonishing ameliorating effect of sulfide and thiosulfate complexation seen for acute toxicity also holds true for chronic toxicity. In 30-d embryo–larval tests with fathead minnows, the MATC for silver was >11,000 μg/L using silver sulfide (the highest concentration of the slurry that could be achieved) and 16,000 to 35,000 μg/L (growth inhibition) when $Ag(S_2O_3)_n^{n-}$ was employed. Here the ACRs were approximately 20 for $Ag(S_2O_3)_n^{n-}$ and could not be calculated for silver sulfide, but the key point is that relative to MATCs reported for $AgNO_3$ in other studies cited above, sulfide and thiosulfate offered greater than five orders of magnitude of protection against chronic toxicity. There is a need for more studies on the chronic toxicity of waterborne silver compounds, especially $AgCl_n^{1-n}$ and Ag–DOC complexes.

Food route toxicity. This is a potentially important area about which almost nothing is known in freshwater fish. The final fate of silver from the water column is precipitation as insoluble silver sulfide (or perhaps chloride complexes when very high silver levels are present) or adsorption onto particulate organic matter, all of which may be ingested by benthic invertebrates or absorbed by algae and plants, and thereby enter the food chain. Furthermore, waterborne silver in various forms readily enters aquatic organisms and builds up in the tissues (see below for fish examples), providing another route of trophic transfer. Eisler [30] has reviewed tissue burdens in aquatic plants and animals collected from the wild. Reported concentrations may exceed 100 mg total Ag/kg (dry weight) in some mollusks living in contaminated sites. However, in a simple laboratory ecosystem (algae, *Daphnia*, fathead minnows) contaminated with 5,000 μg Ag/L as $Ag(S_2O_3)_n^{n-}$, the *Daphnia* took up silver to higher levels than found in the water, but the fish did not biomagnify silver from their diet of contaminated *Daphnia*, and no toxic effects were observed over 10 weeks of exposure [50]. In a radiotracer experiment, juvenile carp fingerlings contaminated by 3 d exposure to 4.3

μg/L of ^{110m}Ag as AgCN were fed to fingerling brown trout (*Salmo trutta*) [58] at a daily ration of about 5%. The brown trout grew at normal rates and steadily accumulated ^{110m}Ag radioactivity, reaching a concentration of about 12% of that in the prey by the end of the experiment at 34 d; about 63% of the ^{110m}Ag was found in the liver. By extrapolation, the authors predicted that biomagnification might occur by 500 d but noted that in an earlier unpublished study where ^{110m}Ag-contaminated gammarids were fed to carp (*Cyprinus carpio*), uptake was very low and there was no evidence of biomagnification. Whether or not biomagnification occurs, it must be emphasized that an internal buildup of silver in itself does not imply an impairment of function.

There is an obvious need for more controlled feeding studies, preferably using diets in which the silver has been biologically incorporated, rather than simply added as a silver salt. In this regard, preliminary data from a 4-month feeding study of juvenile rainbow trout with a diet enriched 30-fold above control levels with biologically incorporated silver has indicated slow but steady silver uptake and no impairment of physiology [59].

Silver bioavailability and reactivity in freshwater

At the outset, a caveat is required. In terms of the mechanism of acute toxicity, silver appears to be a surface-active toxicant, doing its damage on or in the gills (see below). Silver also enters freshwater fish across the gills [17], but entry into the body (bioaccumulation) bears little or no relation to acute toxicity. It is therefore essential to distinguish those aspects of bioavailability, which relate to acute toxicity from those that relate to entry.

By analogy to earlier work on copper and cadmium where gill metal burden related directly to toxicity [60], Janes et al. [61] developed a geochemical modeling approach based on the geochemical program MINEQL$^+$ [45] to understand silver binding to trout gills. Empirical data were generated for gill silver burden using 2- to 3-h exposures of live fish to 0 to 0.22 μmol/L of $AgNO_3$ (0–24 μg/L) in dilute water; times and concentration range were chosen to ensure saturation of only high affinity (presumably high specificity) binding sites. In support of the concept that silver binding under these conditions was directly causative of toxicity, they demonstrated a linear relationship between gill silver burden and the net rate of sodium loss to the water, which is thought to be the key event in toxicity (see below). Additions of various concentrations of competitive cations (Ca^{2+}, Na^+, and H^+) and complexing anionic ligands for Ag^+ (Cl^-, thiosulfate, DOC) were used to determine the conditional equilibrium binding constants for the gills (log *K* values and binding site density). The affinity of the gill for Ag^+ proved to be remarkably high (log $K \sim 10$.), two to three orders of magnitude greater than for Cu^{2+}. Interestingly, based on the affinity of these toxicity-related Ag^+ binding sites for other cations, they appear to be very similar to those previously characterized for Cu^{2+} and very different from those for Cd^{2+} [62]. The Ca^{2+} (i.e., hardness) interactions at these sites appear to be particularly weak, which may explain the limited protection offered by water hardness. When the gill binding constants were added into MINEQL$^+$ [45], the model successfully predicted the observed gill silver burdens of fish tested for 2 to 3 h in a range of natural waters. However, the prediction broke down in very hard, alkaline water and during prolonged exposures (145 h), which may indicate the kinetic constraints of an equilibrium model.

Recently, this type of geochemical modeling has been endorsed as a potential regulatory tool for predicting metal toxicity in receiving waters [51]. The approach goes one step beyond simply speciating silver in the water column, because the gill itself is considered as a negatively charged ligand. To illustrate in simple terms, even if there is no free Ag^+ in the water, the high affinity gill ligands may be able to ''strip'' Ag^+ off lower affinity ligands. The general qualitative conclusion is that any cation that competes with Ag^+ for binding sites on the gill, or any anion that competes with the gill sites for Ag^+ binding, will tend to reduce toxicity. The greater the ligand log *K* values and the greater the concentrations, the greater should be the protective effects.

An innate assumption of this approach is that the species that binds to the gill sites and does the acute damage is Ag^+. We believe the summated toxicological evidence (above) and physiological data (below) strongly support this concept. However, as discussed earlier, it is far from clear that Ag^+ is necessarily the species that enters the fish and bioaccumulates, and it is also far from clear that when one measures gill silver burden under real-world conditions, one is measuring only Ag^+ bound to silver toxicity sites. Silver exposure may induce production of the metal binding protein metallothionein in the gills, liver, and other tissues, which will immobilize the metal in a nontoxic form [21,23]. To cite an extreme example, when adult rainbow trout were exposed for 6 d to an extremely high level (30,000 μg/L) of $Ag(S_2O_3)_n^{n-}$ where there was essentially no free Ag^+ (<0.003 μg/L), silver accumulated on the gills (to 3.2 μg/g, wet weight), in the plasma (0.88 μg/ml), and most notably in the liver (73 μg/g, wet weight), yet there was negligible physiological evidence of toxicity [23]. In all three tissues, these absolute levels were two- to threefold greater than seen in trout after 6 d exposure to 10.9 μg Ag/L (as $AgNO_3$) where free Ag^+ was 3 to 4 μg/L [22]. The latter exposure was right at the 168-h LC50 level for Ag^+ (Fig. 2), and of course, there was substantial physiological evidence of toxicity despite the much lower tissue accumulations. Similarly, when juvenile rainbow trout were exposed to very high levels of AgCl (no free Ag^+, dissolved $AgCl_n^{1-n} \sim 120$ μg/L) for 7 d, there was no toxicity, but gill and liver total silver burdens were 10- to 40-fold greater than in moribound trout at the 168-h LC50 level for Ag^+ [21].

From these observations, it is clear that silver derived from complexed forms as well as from the free ion may build up on/in the gills and may enter fish, though we do not yet know either the actual species that enter or the route of entry. It is also clear that internal silver accumulation is unrelated to acute toxicity, though we do not know if the same is true for chronic toxicity. In juvenile trout, Galvez et al. [27] reported a 40-fold greater liver build-up of silver (to ~25 μg/g, wet weight) in a 28-d chronic exposure (2 μg Ag/L added as $AgNO_3$) where growth inhibition occurred, than in a lower exposure (0.5 μg Ag/L added as $AgNO_3$) where growth inhibition did not occur. We also know very little about turnover and excretion of internal silver residues; two radiotracer studies reported negligible depuration over approximately 1 month for ^{110m}Ag accumulated from either the diet [58] or the water [63] in brown trout. There is an obvious need for more research in all of these areas.

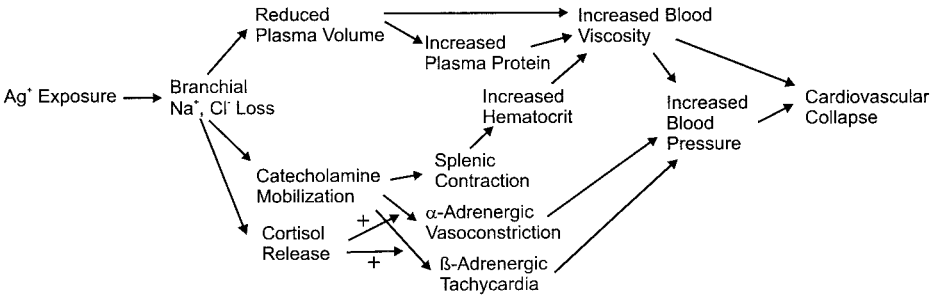

Fig. 3. Suggested etiology of acute silver toxicity in freshwater fish. Exposure to the free silver ion, Ag^+, results in a net loss of Na^+ and Cl^- from the blood plasma. This osmolyte loss causes a sequence of events that eventually leads to a fatally increased blood viscosity and arterial blood pressure. Cardiovascular collapse is likely to be the final cause of death.

Physiological effects of silver in freshwater

Acute responses to waterborne silver. Until very recently, nothing was known about the toxic mechanism of any form of silver, except that at very high, environmentally irrelevant concentrations, $AgNO_3$ caused death by suffocation [28]. However this response, reflecting gross gill damage, is common to virtually all metals at such industrial levels [4]. In the past several years, work from our laboratories has focused on the physiological responses of adult rainbow trout during exposure to approximately 10 μg/L of silver as $AgNO_3$ in moderately hard water (hardness = 140 mg/L, chloride = 25 mg/L or 700 μM) where free Ag^+ was at the 168-h LC50 level (3–4 μg/L; Fig. 2). With the exception of silver entry (see above), the whole suite of responses observed are attributed to the action of the Ag^+ component because none of them occurred when Ag^+ was removed by thiosulfate complexation [23], and they were reduced in a dose-dependent fashion when increasing amounts of Ag^+ were removed (at constant total silver) by chloride complexation [55].

At these more realistic levels of silver, toxicity has nothing to do with suffocation: blood O_2 levels remain high and lactate levels low [22]. Instead, the primary mechanism of toxicity is an almost total inhibition of active Na^+ and Cl^- uptake at the gills that starts immediately upon exposure and is complete by 8 h [64]. The specificity of the effect is shown by the fact that diffusive effluxes of Na^+ and Cl^- are not substantially affected [64], nor is active Ca^{2+} uptake inhibited [22]. With time, effluxes are gradually reduced below control levels, but there is no recovery of influxes during continued exposure, so Na^+ and Cl^- balances remain negative [65]. By 6 d, plasma levels of Na^+ and Cl^- have fallen by about 30%, and the fish are close to death from ionoregulatory failure [22,65].

In vivo Michaelis–Menten kinetic analysis on trout exposed to a lower level of $AgNO_3$ for 48 h demonstrated that Ag^+ does not affect the affinity of the gill transport system for Na^+ but rather reduces the maximal transport capacity of the system [64]. An important cause, though not necessarily the only cause, of this decreased transport capacity is a marked reduction in gill Na^+, K^+ ATPase activity (85% by 48 h at 10 μg Ag/L added as $AgNO_3$) [64]. This enzyme is located in the basolateral membranes of the branchial ion transport cells and is thought to play a key role in powering active Na^+ and Cl^- uptake processes (see Wood [5] and Perry [66] for current uptake models). An earlier in vitro study on mammalian Na^+, K^+ ATPase demonstrated the potency of $AgNO_3$ and silver acetate in inhibiting activity [67], and this was confirmed for the trout gill enzyme by adding $AgNO_3$ to assay homogenates at total silver concentrations similar to those measured in the gills of $AgNO_3$-exposed trout [64]. Comparable $NaNO_3$ additions had no effect. Preliminary work [68] has pinpointed the mechanism of action of silver as competitive inhibition at the Mg^{2+} binding site (cytoplasmic side) on the α-subunit of the enzyme. This will prevent ATP binding and hydrolysis, thereby reducing the transport capacity of the system, and manifest as a noncompetitive inhibition of ion transport. In another recent in vitro study, Cu^{2+} was indicated to act in a similar manner by preventing Mg^{2+} binding to the Na^+, K^+ ATPase [69]. However, the Cu^{2+} inhibition at the Mg^{2+} binding site was mixed, whereas the inhibition by Ag^+ was purely competitive. These effects at the molecular level are very similar to those of copper at the gills (reviewed by Wood [5] and Taylor et al. [70]), which is interesting inasmuch as Janes and Playle [11] found that the cationic affinities of the silver toxicity sites of trout gills were very similar to those of the copper toxicity sites.

When $AgNO_3$ is removed from the dilution water, recovery of active Na^+ influx in vivo occurs just as rapidly as the initial blockade [64]. This observation suggests that the enzymatic inhibition is fully reversible, and that the rate of silver elimination from the gills is rapid, considering that the enzyme is on the basolateral, rather than the apical surface of the transport cells. This is in accord with the fact that during continued exposure, silver accumulation in the blood can be detected after only 24 h and continues linearly thereafter [65]. At present, we know nothing about how silver reaches the basolateral surface or about whether Ag^+ also exerts effects on apical processes. Studies with specific cation-channel or ion-exchange blockers (e.g., amiloride) may prove informative in this regard.

The etiology of Ag^+-induced mortality, i.e., the sequence of events connecting net gill ion losses to death, appears to be very similar to that worked out earlier for low pH stress [71], apart from a few small differences. Interestingly, the same sequence of events also seems to explain the toxic sequence for copper [72], again emphasizing the similarity between silver and copper effects. Figure 3 presents a model for silver effects, based on data from Wood et al. [22], Morgan et al. [64], and Webb and Wood [65]. The net loss of Na^+ and Cl^- from the plasma is due exclusively to the complete inhibition of branchial Na^+ and Cl^- influx by Ag^+, whereas during low pH and copper stress there is usually only partial inhibition of influx but simultaneous elevation of diffusive efflux. Regardless, the branchial loss sets up an osmotic imbalance between plasma and tissues. This causes a net shift of water from the extracellular to intracellular compartment (principally white muscle) within the fish, and by day 6, plasma volume

has fallen almost 40%, coincident with the loss of electrolytes. At the same time, the blood becomes more viscous because of the greater concentrations of protein and red blood cells left behind. An additional important cause of greater red cell concentration is a discharge of stored erythrocytes from the spleen, probably a result of stress-induced activation of the sympatho-chromaffin cell axis (i.e., catecholamine mobilization). Interestingly, red cell swelling, which is yet another factor contributing to the elevation of hematocrit during low pH and copper exposure, does not occur during Ag^+ exposure. Perhaps silver in the blood plasma in some way interferes with the β-adrenergically stimulated Na^+/H^+ exchange on the erythrocyte membrane, which is thought to be responsible. We hypothesize that death finally results from circulatory failure caused by critically low blood volume, high viscosity, and high blood pressure. The latter would be due to both the viscosity increase and the stimulatory effects of catecholamines on cardiac output and vascular resistance, probably augmented by the sensitization of adrenergic responsiveness caused by cortisol mobilization. We emphasize that certain elements of this sequence (catecholamine mobilization, high blood pressure) remain conjectural at present, but the similarity to H^+ and copper toxic syndromes is striking.

Additional phenomena occur, some of which may exacerbate toxicity (e.g., blood acidosis, ammonia build-up) while others (e.g., progressive elevations of glucose and cortisol) are universal stress responses. In clinical terms (see Wood et al. [22]), the blood acid–base response is a progressive metabolic acidosis (fixed acid build-up reflected in decreased plasma HCO_3^-) with partial respiratory compensation (decrease of the partial pressure of CO_2, Pa_{CO_2}, in the arterial blood). Later in the sequence, arterial pH drops moderately, while Pa_{CO_2} and $[HCO_3^-]$ are severely depressed. The respiratory compensation is due to a visible hyperventilation by the fish which ''blows off'' CO_2, thereby lowering Pa_{CO_2} and actually raising Pa_{O_2}. If this compensation did not occur, the fall in pH_a would be more severe. Lactic acid production does not occur; rather, the metabolic acidosis is explained by a steady net uptake of acidic equivalents from the environmental water during Ag^+ exposure [65]. Indeed the cumulative net uptake of acidic equivalents from the water is far greater than needed to explain the extracellular acidosis; this factor also probably causes intracellular metabolic acidosis, signified by a steady loss of K^+ to the environment in the face of unchanged plasma $[K^+]$ [65].

The exact mechanism responsible for the uptake of acidic equivalents is unclear, but the same phenomenon is seen in trout exposed to low pH in the same water quality [71]. Because ion uptake at the gills is coupled to acid–base regulation (i.e., Na^+/H^+ and Cl^-/HCO_3^- exchange mechanisms [5]), it is likely related to the disturbance of ion exchange. Branchial carbonic anhydrase, the enzyme catalyzing the production of the acidic (H^+) and basic equivalents (HCO_3^-) that serve as the counterions for these exchanges, was significantly inhibited in trout exposed to Ag^+ in vivo [64]. The inhibition could be duplicated in vitro by the addition of $AgNO_3$ to gill homogenates. In vitro inhibition of erythrocytic carbonic anhydrase by $AgNO_3$ has also been reported in the white sucker, *Catostomus commersoni* [73].

With copper toxicity, recent interest has focussed on the build-up of ammonia in the blood plasma, which is thought to be caused by an inhibition of ammonia excretion at the gills (reviewed by Taylor et al. [70]). While plasma ammonia progressively increases during Ag^+ exposure, a careful time-course study has shown that inhibition of ammonia excretion never occurs in freshwater, but rather a progressive rise, reaching about fourfold control levels by 6 d [65]. Thus plasma ammonia retention is in fact a product of an increased metabolic production rate rather than a reduced excretion rate, and the parallel rise in plasma cortisol levels is likely the cause. The proteolytic effects of cortisol in fish are well known [74]. In light of the general similarity of silver and copper responses, these observations suggest that the cause of ammonia retention in copper-exposed fish should be re-assessed. Conversely, swimming performance should be examined in silver-exposed fish, because emerging evidence indicates that high plasma ammonia is responsible for the reduced exercise ability of copper-exposed trout [70]. If exercise performance is similarly reduced during chronic silver exposure, there could be detrimental consequences for fitness, though whether Ag^+ concentrations in nature ever reach high enough levels is unknown.

Chronic responses to waterborne silver. The impaired uptake of Na^+ and Cl^- that characterizes acute Ag^+ toxicity is clearly demonstrable at acutely sublethal Ag^+ concentrations. In rainbow trout exposed to silver at 20% of the 144-h LC50 (i.e., 2 μg Ag/L added as $AgNO_3$ in the same water quality), the branchial influx of Na^+ and Cl^- were markedly depressed within a few hours after onset of exposure [64]. Even at 5% of the 144-h LC50 concentration (0.5 μg /L), a small ionoregulatory effect was evident [27]. These data suggest that the toxic mechanism does not change at sublethal levels. Interestingly, during prolonged exposure (28 d) at these low levels, there was some indication of recovery of plasma ion concentrations. At 0.5 μg Ag/L, the trout ate more, suggesting that they were using dietary salts to ''eat their way out of trouble,'' a phenomenon recently documented in trout subjected to chronic sublethal low pH exposure [75]. At 2 μg Ag/L, both appetite and growth rate were significantly inhibited [27]. The mechanism responsible was not identified, but notably the latter fish accumulated 40-fold greater total silver levels in the liver, and there was a small induction of hepatic metallothionein. Another possible chronic mechanism is interference with the metabolism of zinc, an essential element. In both smallmouth bass and bluegills chronically exposed to sublethal silver, Coleman and Cearley [57] demonstrated an apparent inverse relationship between whole-body silver accumulation and whole-body zinc levels, suggesting that silver was displacing zinc from proteins. Whole-body copper levels were unaffected. Silver has a known ability to displace zinc from sulfhydryl groups in proteins, including metallothionein itself [76].

Metallothionein is a low molecular weight, cysteine-rich protein now known to play critical roles in the normal metabolism of essential metals (copper, zinc) and in the immobilization and detoxification of both essential and nonessential metals (mercury, cadmium; reviewed by Kägi and Schäffer [76] and Olsson et al. [24]). Recently, it has been found that silver (a nonessential metal) is also an effective inducer of metallothionein synthesis in both mammals [77] and fish [21,23,78,79]. Silver binds extremely avidly to metallothionein and displaces other metals, such as copper and zinc from this protein [76,80]. At present it is not known whether silver directly interacts with the transcriptional machinery to induce metallothionein synthesis or acts indirectly by mobilizing zinc from pre-existing metallothionein or other zinc stores. Relative to the potency of other metals, both the speed of induction (<7 d), the extent of induction (up to 10-fold background

Table 3. Acute toxicity of silver to seawater teleost fish

Species	Life state	Salinity (‰)	96-h LC50 (μg/L)	168-h LC50 (μg/L)	Reference
Mummichog (*Fundulus heteroclitus*)	Adult	24	2,700	—	[82]
Sheepshead minnow (*Cyprinodon variegatus*)	Juvenile	28	1,170	—	[109]
Shiner perch (*Cymatogaster aggregata*)	Adult	29–30	356	—	[110]
English sole (*Parophrys vetulus*)	Adult	29–30	800	—	[110]
Tidepool sculpin (*Oligocottus maculosus*)	Juvenile	25	330	119	[81]
	Juvenile	32	657	472	[81]
Coho salmon (*Oncorhynchus mykiss*)	Smolt	29–30	487	—	[110]
Rainbow trout (*O. mykiss*)	Smolt	25	401	—	[14]

levels seen in various waterborne exposure regimes), and the widespread nature of the response (e.g., liver, gills, kidney) to silver are remarkable [21,23]. Furthermore, preliminary data suggest that binding of silver to metallothionein totally abolishes the inhibition of the Na^+, K^+ ATPase by silver [68]. This may explain the apparent absence of toxicity associated with large internal accumulations of this metal. The importance of metallothionein in detoxifying silver depends of course upon whether metallothionein actually binds silver to a significant extent in vivo. Studies on the subcellular distribution of silver in tissues and mechanisms of metallothionein gene activation by silver should, therefore, be helpful in elucidating the role of metallothionein in silver detoxification.

EFFECTS OF SILVER ON SEAWATER FISH

Silver toxicity in seawater

Acute waterborne toxicity. Background information on acute silver toxicity to fish in seawater is considerably more limited than that available for freshwater fish. While there is a notable variability in LC50 values among different marine species, most studies are in agreement that silver is much less toxic in seawater than it is in freshwater (Fig. 1). Table 3 summarizes acute toxicity data for silver on fish in seawater. In the gray literature, there are additional scattered reports on acute silver toxicity to several other teleost species. These reports were recently included in a comprehensive overview by Eisler [30] and will not be discussed here.

The 96-h LC50 values for silver (tested via additions of $AgNO_3$) to juvenile and adult fish in seawater range from 330 μg Ag/L (3.06 μM) for the tidepool sculpin (*Oligocottus maculosus*) [81] to 2,700 μg Ag/L (25 μM) for mummichog (*Fundulus heteroclitus*) [82]. These values are two orders of magnitude above the current U.S. EPA [29] seawater acute limit of 2.3 μg Ag/L. The lowest 96-h LC50 value for silver to juvenile or adult seawater fish is more than four times higher than the highest value obtained with freshwater fish (Fig. 1). The variability in silver toxicity to marine fish is probably partially species related, but there is also an influence of salinity. By increasing the salinity from 25 to 32‰ the 96- and 168-h LC50 values to the tidepool sculpin increased by 100 and 300%, respectively [81]. A diametrically opposite relationship between salinity and mortality during silver exposure was observed for seawater-adapted rainbow trout. In an experiment where the silver concentration was kept constant at the 96-h LC50 value (400 μg Ag/L) determined in 25‰ salinity, it was found that mortality went up if the salinity was increased to 30‰, whereas no mortalities were observed in 15 or 20‰ salinities [14]. At 15‰ salinity it may, in fact, be practically impossible to induce 96-h acute silver toxicity to rainbow trout. The reason is that even a modest increase in the amount of silver added on top of the nonlethal 400 μg Ag/L (a concentration >100,000 times higher than that found in marine environments) tested would initiate cerargyrite precipitation and effectively prevent further increase in the concentration of dissolved silver [14]. Because of the mechanism of acute silver toxicity in seawater (see Physiology section below), there are reasons to believe that the increase in mortality while going from 25 to 30‰ salinity was due to a constraint in the ability of the rainbow trout to hypoosmoregulate (osmoregulate in seawater). In contrast to the fully marine tidepool sculpin, the rainbow trout is an anadromous teleost and the parr–smolt conversion, associated with seaward migration, presents a formidable physiological challenge to these fish. Thus, the increased sensitivity of salmonids to silver with increased salinity (in the seawater range, 17–36‰) is likely due to the combined stress of silver and salinity. We believe that salmonids and other anadromous species may be an exception, and that for most marine fish the toxicity of silver is reduced as salinity goes from 25‰ to oceanic seawater (32–36‰; Fig. 4). Given that concentrations of dissolved silver in open oceans are extremely low (0.1–0.2 ng/L) [83], silver toxicity, if any, would be expected only in coastal environments

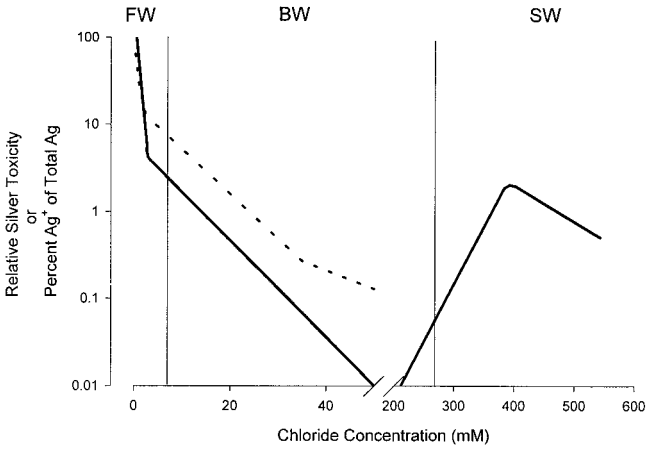

Fig. 4. Relative acute toxicity of silver to teleost fish (solid line) over the full range of chloride concentrations from dilute freshwaters to oceanic seawater (note the logarithmic scale of the y-axis). FW = freshwater; BW = brackish water; SW = seawater. Toxicity in dilute freshwater is arbitrarily set to 100, and toxicity at all other chloride concentrations is expressed relative to this value. The graph was generated from actual acute toxicity data, with different fish species reviewed in the text, but should be viewed as a generalized model. The percentage of silver present as Ag^+, at a total dissolved silver concentration of 10 μg/L, is indicated by the dotted line (assuming no S^{2-} or DOC present). In SW, $[Ag^+]$ is less than 0.01% of the total dissolved silver. A more extensive model of silver speciation as functions of silver concentration and salinity is presented by Ferguson and Hogstrand [14].

close to anthropogenic inputs. There is a demand for further toxicity studies in salinity ranges typical of estuarine environments (15–30‰) where silver concentrations can be somewhat higher (0.3–2.0 ng/L) [83], and the influence of salinity may be considerable.

Another factor that has been shown to modulate silver toxicity in marine fish is the ammonia concentration of the water [81]. Silver exposure in itself causes a rise in plasma ammonia levels. This effect has been observed both in freshwater and seawater fish during silver exposure [65,84]. Excretion of ammonia is largely governed by the laws of simple diffusion, and increased water ammonia concentrations will therefore result in impaired excretion and build-up of plasma ammonia [85]. When the tidepool sculpin was exposed to a range of silver concentrations in combination with different ammonia concentrations, it was found that ammonia in the water amplified and accelerated silver toxicity (i.e., both LC50 and LT50 values were increased) [81]. The importance of this observation lies in the fact that ammonia is an ubiquitous pollutant arising from industrial, domestic, and agricultural discharges, nonpoint-source runoff, and biogenic production; one might well expect silver contamination and ammonia build-up to occur in the same water bodies. Ammonia versus silver interactions warrant further investigation in both seawater and freshwater systems at environmentally realistic water ammonia concentrations.

Chronic waterborne toxicity. We were unable to obtain information on standardized chronic toxicity endpoints (i.e., full life-cycle tests, partial life-cycle tests, and extended early life-stage tests) for silver on seawater fish. Voyer et al. [86] and Klein-MacPhee et al. [87] each described short early life-stage tests to derive chronic values for silver on the winter flounder. The earlier study [86] aimed to investigate the influence of dissolved silver on cadmium toxicity at three different salinities, 10, 21, and 32‰. Embryos were exposed, from 24 h postfertilization to hatch, to different concentrations of silver, cadmium, or a combination of both. Silver ($AgCl_n^{1-n}$) alone had no adverse effect on the survival and successful hatching of embryos at concentrations up to 174 μg Ag/L. When tested in combination with cadmium, silver actually reduced the toxicity of the former in a dose-dependent manner. For example, at a cadmium concentration of 1,066 μg/L and a salinity of 21‰, the number of viable hatches was reduced to 36%, but at the same concentration of cadmium in combination with 167 μg/L of silver, hatching viability was essentially no different from the control (90%). In the subsequent study by Klein-MacPhee et al. [87], a silver concentration somewhere between 180 and 386 μg/L was required to reduce hatching in winter flounder, but a more extended exposure resulted in effects at much lower silver concentrations. Thus, embryos and larvae were exposed to waterborne silver during an 18-d period from stages of early cleavage to yolk sac reabsorption. Through this protocol, silver was found to cause effects including reduced hatching frequency, time to hatch and larvae size, and increased larvae mortality and malformations. These are all typical effects on fish that have been observed from similar studies on numerous inorganic and organic chemicals [88,89]. Winter flounder larvae mortality at 54, 92, and 180 μg Ag/L was 3, 31, and 97%, respectively, with a control mortality of 6% [87]. The lowest concentration of silver that displayed any effect was 92 μg/L. The highest tested concentration with no effect was 54 μg/L and the 18-d chronic effect level was calculated to be 70 μg/L. Thus, the MATC for winter flounder was 35,000 higher than the highest silver levels found in coastal waters of North America.

Silver concentrations higher than 500 μg/L were reported as lethal to cunners (*Tautogolabrus adspersus*) in 24‰ salinity seawater [90]. Gould and MacInnes [91] exposed cunners to 500 μg/L of silver for 96 h in 24‰ seawater and found reduced oxygen consumption by gill tissue and altered enzyme activities in liver and muscle. During a 96-h exposure to 120 μg Ag/L in the same salinity, all fish survived but the gill tissue showed a depressed oxygen consumption rate [90]. Exposure of winter flounder to 10 μg Ag/L for 60 d caused no effects on gill tissue oxygen consumption, but the liver did display reduced activity of aminotransferase, a family of enzymes involved in nitrogen metabolism [92]. Jackim et al. [93] measured the effects of a 96-h exposure to 30–40 μg/L of silver on liver enzymes in mummichog (*F. heteroclitus*) and found that four out of the five enzymes assayed displayed reduced activities. The most pronounced effects observed were for catalase (32% reduction) and xanthine oxidase (27% reduction).

From the limited data available on subchronic silver effects on marine fish it can be concluded that the threshold for disturbances of embryo–larvae life stages in winter flounder is approximately 70 μg/L of dissolved silver. Effect on aminotransferase in the same species was observed during chronic exposure to only 10 μg/L of dissolved silver. All observed chronic effects occurred at concentrations of dissolved silver that were considerably higher than the current acute water quality criterion for total recoverable silver in seawater, 2.3 μg/L [94]. There is clearly a need to broaden the database to define better both acute and chronic criteria for silver in seawater.

Physiological effects of silver in seawater

Physiology of acute silver toxicity. Through recent work, the etiology of acute toxicity to freshwater fish has now been relatively well delineated from the level of systems physiology to the molecular mechanisms of action (see above) [22,23,64,65,68]. In contrast, our knowledge of the key events that lead to acute silver toxicity in seawater fish extends no further than some preliminary data from our laboratories [84,95]. Experiments were conducted on the starry flounder (*Platichtys stellatus*) analogous to those previously employed to determine the events leading to acute silver toxicity in freshwater rainbow trout [22,65]. Because of the modest toxicity of silver to marine fish, the exposure levels were set to 250 and 1,000 μg/L as compared with the 10 μg/L used in the freshwater studies on rainbow trout. It should be noted that these exposure levels are four orders of magnitude higher than those found in coastal waters around the U.S.; undoubtedly they would not be found in nature. Exposure to such environmentally unrealistic silver concentrations were necessary to induce the physiological effects that lead to acute silver toxicity to starry flounder at 32‰ salinity and, in fact, only two out of eight fish in the highest exposure concentration (1,000 μg Ag/L) died during the 144-h experiment [84].

As in freshwater fish, the physiological mechanism of acute silver toxicity to the starry flounder seems to be an osmoregulatory failure [84]. However, because starry flounder like other marine teleost fish are hypoosmotic in relation to the surrounding water, the result of osmoregulatory failure is an increase rather than a decrease in the Na^+ and Cl^- concentrations of the blood. Thus, the effect on osmoregulation is quite opposite to that in freshwater fish where silver causes a net loss

of Na^+ and Cl^- to the environment (see above) [22]. Again, the effect of silver closely resembles that of copper. In freshwater fish, exposure to waterborne copper results in reduced plasma Na^+ and Cl^- levels, whereas in seawater fish the plasma Na^+ and Cl^- levels increase during exposure [72,96,97]. In silver-exposed starry flounder (1,000 μg/L), rapidly rising plasma Na^+ and Cl^- concentrations preceded mortality [84]. Moribound fish displayed plasma Cl^- concentrations exceeding 200 mM, compared with an average of 143 mM before the start of the exposure. This osmotic stress was manifested by a markedly elevated plasma glucose concentration.

The effects of silver exposure were considerably milder at the lower test concentration (250 μg/L) than at the higher lethal level of exposure (1,000 μg/L). There was no mortality, and the plasma Cl^- concentration was only slightly elevated during the 144 h of exposure. However, a different and rather surprising effect was observed. During the early part of the exposure to either of the silver concentrations, there was a substantial increase in concentration of plasma ammonia [84]. Later in the experiment, the ammonia levels subsided. A similar increase in plasma ammonia has been observed in silver-exposed freshwater rainbow trout [65], which suggests that this is a general effect of silver exposure. In this regard, it may be significant that winter flounder showed a nitrogen metabolism disturbance, in the form of reduced aminotransferase activity in the liver, at a silver concentration of only 10 μg/L [92]. It is, however, unclear if this enzyme inhibition has any connection to the elevated plasma ammonia levels observed in starry flounders at the much higher silver concentrations of 250 and 1,000 μg/L. Silver and copper are not only chemically related but clearly display many similar toxicological properties. Such similarities also apply to the effects on ammonia metabolism. Wilson and Taylor [72,97] were first to show an effect of metals on plasma ammonia levels in fish. During exposure of rainbow trout to copper in both freshwater [72] and seawater [97], they found markedly increased plasma ammonia concentrations. In contrast to the situation in starry flounders, the plasma ammonia level remained elevated in the rainbow trout throughout the exposure. This difference may, however, be related to the fish species rather than to the toxicants because the plasma ammonia level stayed elevated during exposure of rainbow trout to silver in freshwater [65]. A lack of concentration dependency of the effect, together with the decreasing plasma ammonia level later in the experiment when flounder from the 1,000 μg Ag/L group started to die, strongly suggest that ammonia toxicity was not the primary cause of death. A similar conclusion was made by Wilson and Taylor [97] concerning hyperammoniaemia during copper exposure in seawater trout. It remains possible that ammonia could contribute to the toxicity of silver during conditions that impair ammonia excretion [81].

The site of silver uptake and toxic action may be another important difference between responses in freshwater and seawater. Silver toxicity to freshwater fish is probably caused by the binding of Ag^+ to sites on or inside the gill cells (see above) [11,22,64,68]. There is virtually no Ag^+ present in seawater; more importantly, we found no evidence of silver accumulation in gill tissue from starry flounders exposed to 250 μg Ag/L. Instead, there was an 80-fold increase in the silver concentration of intestinal tissue as compared with the control. These data suggest that the intestine was the primary uptake route for silver in the starry flounder. It is interesting to note that accumulation of waterborne cadmium follows the same pattern. In mummichog, exposed to cadmium at salinities ranging from freshwater to 25‰ seawater, the accumulation of cadmium switched from gill to intestinal tissue as the salinity was increased [98]. Similarly, the intestinal mucosa of cadmium exposed Atlantic cod (*Gadus morhua*) showed three times higher levels of cadmium than any other tissue examined including gills (C. Hogstrand, unpublished observations). Thus, it is possible that the intestinal tract rather than the gills is the primary uptake site for several waterborne metals.

The intestine appears to be the site of silver toxicity as well as uptake in marine fish. Branchial Na^+, K^+ ATPase has been indicated as a major target for toxicity during exposure of fish to Ag^+ in freshwater [64,68]. However in starry flounder exposed to a silver concentration of 250 μg/L in seawater, there was no inhibition of Na^+, K^+ ATPase activity in either the gills or the intestine (C. Hogstrand, E.A. Ferguson, and C.M. Wood, unpublished observations). In fact, the Na^+, K^+ ATPase activity in the gills was increased as a result of silver exposure. This increase was most likely a compensation for an impaired water uptake in the intestine because the drinking rate was markedly reduced in the exposed fish. Marine fish normally drink considerable quantities of water to prevent dehydration by the hyperosmotic environment. Water is pulled osmotically from the intestinal lumen by the active pumping of Na^+ and Cl^- into the body [99]. The excess of Na^+ and Cl^- is then extruded from the body across the gills. A reduced water uptake would tend to result in a more concentrated blood plasma, but at the level of exposure used in our experiment (250 μg/L), the fish were apparently able to compensate by an upregulation of the branchial Na^+, K^+ ATPase. To date no measurements on Na^+, K^+ ATPase activities have been performed in fish exposed to lethal levels of dissolved silver in seawater.

Thus, we propose that acute silver toxicity in seawater is caused by an osmoregulatory malfunction at the level of the intestine. This disturbance leads to a progressive increase in plasma Na^+ and Cl^- concentrations, which is the ultimate cause of acute toxicity. Present results suggest that the gills are not the site of toxicity to silver in marine fish and there are indications that this would apply to cadmium and perhaps other metals as well. As discussed in earlier sections, a geochemical gill-binding model has recently been suggested as a regulatory tool to determine acute metal toxicity mathematically in defined water qualities [11,51,62]. While this approach shows great promise in freshwater systems, it may not be directly applicable to the marine environment if the gills are not a target of metal toxicity.

Silver uptake and elimination in seawater fish. As discussed above, silver is considerably less bioavailable in seawater than in freshwater and the uptake route of dissolved silver in fish may fundamentally differ between these two extremes; freshwater fish take up silver across the gills, whereas the intestine appears to be the uptake route of importance for marine teleosts. There is evidence that silver accumulation from seawater is strongly salinity dependent. In a recent study, tidepool sculpins accumulated high levels of silver from 25‰ seawater, but no detectable silver accumulation occurred at a salinity of 32‰ [81]. Interestingly, silver accumulation was probably not related to short-term acute toxicity because even at lethal exposure concentrations of silver there was no evidence for silver accumulation above that of the control. While the results were very clear-cut, the explanation for this strongly salinity-dependent silver accumulation is not so obvious. It can be argued that silver is more readily available at lower

salinities because more of the bioavailable $AgCl_{(aq)}$ species is present. However, speciation calculations indicated that while there indeed was a shift toward the $AgCl_{(aq)}$ complex with decreasing salinity, the increase in $AgCl_{(aq)}$ activity while going from 32 to 25‰ salinity is probably too modest to account for the vast difference in observed silver accumulation [14,81]. Another possibility is that the physiology of the fish changes as the salinity is decreased so that more silver is taken up or excreted. Currently we are unaware of any physiological mechanism that would have this effect, but a search for such mechanisms should be an important topic for future studies.

In one of his series of classic papers on metal accumulation, Pentreath [18] examined the uptake of ^{110m}Ag from seawater and food in a marine teleost, the plaice (*Pleuronectes platessa*), and an elasmobranch, the thornback ray (*Raja clavata*). Both species accumulated ^{110m}Ag slowly, but both uptake rate and concentration factor of the isotope were greater in the thornback ray than in the plaice [18]. This is very interesting because the plaice should drink, whereas the ray should not. Furthermore, the ion transport mechanism in an elasmobranch gill may be set up like a freshwater teleost gill [100]. Retention rate of silver from ^{110m}Ag-labeled food organisms (*Nereis* sp.) was also much faster in the ray than in the plaice. In both the plaice and the ray, the liver was a major accumulatory organ of ^{100m}Ag. However, the concentration of ^{110m}Ag to the liver was much more pronounced in the ray. Elimination rates of silver were surprisingly fast. The biological half-time ($t_{1/2}$) for ^{110m}Ag in plaice was no more than 12 to 31 d. As a comparison, the $t_{1/2}$ for ^{65}Zn in plaice is 100 to 300 d, depending on the source of the metal [101,102]. Elimination rates of ^{110m}Ag seem to be much slower in the thornback ray; the $t_{1/2}$ of ^{110m}Ag accumulated from water was 277 d and that for ^{110m}Ag of dietary origin was 300 to 1,400 d [18]. For both species it was calculated that under natural conditions accumulation of silver from the water should be insignificant in comparison with the uptake from the diet [18]. However, the situation might have been different if the experiments were conducted at a lower salinity (25‰) where the uptake rate of silver from water seems to be substantially higher. A major conclusion from this comparative study is that the elasmobranch accumulated and retained much more silver than the teleost. The few studies to date that have been conducted on the physiological and toxicological effects of silver on marine fish have been performed only on teleosts; it is important to carry out similar investigations on elasmobranchs.

CONCLUSIONS

It is now evident that there is a lot we do know and even more we do not know about the toxicity and bioavailability of silver to fish. In freshwater, acute toxicity of waterborne silver is critically dependent on speciation and is caused only by the free Ag^+ ion. The free Ag^+ ion is a surface-active toxicant that does its damage by binding to specific sites on or inside the gills. A key toxic effect is inhibition of branchial Na^+, K^+ ATPase activity, which leads to blockade of active Na^+ and Cl^- uptake across the gills. The resulting net loss of ions leads to a complex stress response in which the fish may eventually die from circulatory failure. These actions are very similar but not identical to the actions of Cu^{2+} and H^+. Increased metabolic ammonia production and internal build-up occur as part of this acute stress response. While this stimulated ammoniogenesis is not the direct cause of toxicity, there is a need for research to evaluate whether it leads to secondary sublethal effects such as decreased swimming ability.

Laboratory tests with $AgNO_3$ almost invariably overestimate acute silver toxicity in the field because of the abundance of natural ligands (Cl^-, DOC, colloid organics, S^{2-}), which avidly bind Ag^+ and markedly reduce its toxicity. Indeed, it is doubtful that silver discharges in the freshwater environment ever result in high enough Ag^+ levels to cause acute toxicity. Relative to Cl^-, DOC, and S^{2-}, which exert important protective effects against toxicity from dissolved silver, the modifying action of hardness (Ca^{2+}) on Ag^+ toxicity is slight. The U.S. EPA hardness equation currently used for regulating acute toxicity is faulty, and research is urgently needed to replace it with a relationship that includes these other more important geochemical modifying factors.

Waterborne silver readily enters freshwater fish, perhaps as Ag^+ and/or $AgCl_{(aq)}$, but there is no evidence that internal silver accumulation plays any role in acute toxicity. Silver may also be taken up from the diet, but to date there is no evidence that dietary silver causes toxicity. Further research is needed on both topics. Available data on chronic toxicity in freshwater are sparse but suggest that silver is toxic at very low levels. There is an urgent need for more research in this area in which the absolute exposure levels and geochemical speciation of silver are carefully monitored. The potential for acclimation should also be assessed.

Acute toxicity of waterborne silver (added as $AgNO_3$) falls greatly as the Cl^- concentration in the water increases, reaching negligible toxicity in brackish water (Fig. 4). Toxicity increases again at higher salinity but in full-strength seawater remains less than 25% (in most cases 2–10%) of typical freshwater values. As essentially no free Ag^+ is present, toxicity must originate from one or more of the dissolved silver chloride complexes that predominate in seawater. Similar to the conclusion for freshwater, but based on much more limited data, it seems unlikely that silver discharges into the marine environment ever result in high enough Ag levels to cause acute toxicity to fish. Current acute standards for Ag in seawater set by U.S. EPA are two orders of magnitude lower than available LC50 data. Clearly there is a need for acute toxicity studies on a wider range of marine fish species, and at a wider range of salinities, to confirm these conclusions.

In seawater, the key mechanism of acute toxicity again appears to involve osmoregulatory failure. However, in contrast to the situation in freshwater, plasma Na^+ and Cl^- levels rise rather than fall, and the fish may die from the elevated Na^+ and Cl^- concentrations combined with dehydration. The intestine rather than the gills appears to be the site of toxic impact and silver build-up, resulting in an inhibition of drinking, which is obligatory to sustain water balance in marine fish. As in freshwater fish, internal ammonia accumulation accompanies silver toxicity but is not the direct cause of death. A potential for enhanced silver toxicity by ammonia exists in situations where environmental ammonia levels are elevated. These conclusions remain tentative, and more work is needed to substantiate them. Almost nothing is known about chronic silver toxicity in seawater fish. Less silver seems to enter marine than freshwater teleosts, a difference that may be due to the different routes of entry and/or different silver speciation in the water column. Nothing is known about the toxic potential of accumulated silver in marine fish. These are all important areas for future research.

Acknowledgement—We thank Joe Gorsuch for the invitation to write this review and for constructive comments on its content. Jim McGeer, Nic Bury, Ian Morgan, Fernando Galvez, Joseph Shaw, Elizabeth Ferguson, and Nathan Webb all provided very useful input for which we are grateful. Original research reported here was funded by grants to C. Hogstrand from the Silver Council and to C.M. Wood from the National Association of Photographic Manufacturers, Kodak Canada, and the NSERC Canada Industrially Oriented Research Program.

REFERENCES

1. Hirsch MP. 1998. Toxicity of silver-spiked sediments to the freshwater amphipod (*Hyalella azteca). Environ Toxicol Chem* 17:601–604.
2. Hirsch MP. 1998. Bioaccumulation of silver from laboratory-spiked sediments by the oligochaete (*Lumbricus variegatus*). *Environ Toxicol Chem* 17:605–609.
3. Berry W, Cantwell M, Edwards P, Serbst J, Hansen D. 1998. Predicting the toxicity of silver spiked sediments using interstitial water metal and acid volatile sulfide normalizaitons. *Proceedings,* 5th International Conference, Transport, Fate and Effects of Silver in the Environment, Hamilton, ON, Canada, September 28 to October 1 (in press).
4. Mallatt J. 1985. Fish gill structural changes induced by toxicants and other irritants: A statistical review. *Can J Fish Aquat Sci* 42:630–648.
5. Wood CM. 1992. Flux measurements as indices of H^+ and metal effects on freshwater fish. *Aquat Toxicol* 22:239–264.
6. Kramer JR, et al. 1997. Chemical speciation and metal toxicity in surface freshwaters. In Bergman HL, Dorward-King EJ, eds, *Reassessment of Metals Criteria for Aquatic Life Protection,* Technical Publication Series. SETAC, Pensacola, FL, USA, pp 57–70.
7. Playle RC. 1997. Physiological and toxicological effects of metals at gills of freshwater fish. In Bergman HL, Dorward-King EJ, eds, *Reassessment of Metals Criteria for Aquatic Life Protection,* Technical Publication Series. SETAC, Pensacola, FL, USA, pp 101–105.
8. Cowan CE, Jenne EA, Crecelius EA. 1985. Silver speciation in seawater: The importance of sulfide and organic complexation. In Sigleo AC, Hattori A, eds, *Marine and Estuarine Geochemistry,* Lewis, Chelsea, MI, pp 135–303.
9. Kramer JR. 1995. Aqueous silver in the environment. *Proceedings,* 3rd International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, August 6–9, pp 19–30.
10. Sujari ANA, Bowen HJM. 1986. Interactions of silver with humates and other species in natural waters. *J Radioanal Nucl Chem Lett* 106:213–221.
11. Janes N, Playle RC. 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 14:1847–1858.
12. Wen LS, Santschi PH, Gill GA, Peternostro CL, Lehman RL. 1997. Colloidal and particulate silver in rivers and estuarine waters of Texas. *Environ Sci Technol* 31:723–731.
13. Galvez F, Wood CM. 1997. The relative importance of water hardness (Ca) and chloride levels in modifying the acute toxicity of silver to rainbow trout. *Environ Toxicol Chem* 16:2363–2368.
14. Ferguson EA, Hogstrand C. 1998. Acute silver toxicity to seawater-acclimated rainbow trout: Influence of salinity on toxicity and silver speciation. *Environ Toxicol Chem* 17:589–593.
15. Brooke L, Erickson R, Kahl M, Vande Venter F, Harting S, Markee T, Stephan C, Spehar R. 1994. Effects of laboratory test conditions on the toxicity of silver to aquatic organisms. *Proceedings,* 2nd International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, September 11–14, pp 119–122.
16. Klaine SJ, et al. 1995. Influence of water quality parameters on silver toxicity: Preliminary results. *Proceedings,* 3rd International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, August 6–9, pp 65–78.
17. Hibiya T, Oguri M. 1961. Gill absorption and tissue distribution of some radionucleotides (Cr-51, Hg-203, Zn-65, and Ag-110m. 110) in fish. *Bull Jpn Soc Sci Fish* 27:996–1000.
18. Pentreath RJ. 1977. The accumulation of ^{110m}Ag by the plaice, *Pleuronectes platessa,* and the thornback ray, *Raja clavata* L. *J Exp Mar Biol Ecol* 29:315–325.
19. Szefer P, Pempkowiak J, Skwarzek B, Bojanowski R, Holm E. 1993. Concentrations of selected metals in penguins and other representative fauna of the Antarctica. *Sci Total Environ* 138: 281–288.
20. Greig RA, Wenzloff DR. 1977. Trace metals in finfish from the New York Bight and Long Island Sound. *Mar Pollut Bull* 6: 395–409.
21. Hogstrand C, Galvez F, Wood CM. 1996. Toxicity, silver accumulation and metallothionein induction in freshwater rainbow trout during exposure to different silver salts. *Environ Contam Toxicol* 15:1102–1108.
22. Wood CM, Hogstrand C, Galvez F, Munger RS. 1996. The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*). 1. The effects of ionic Ag^+. *Aquat Toxicol* 35:93–109.
23. Wood CM, Hogstrand C, Galvez F, Munger RS. 1996. The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*). 2. The effects of $Ag(S_2O_3)_n$. *Aquat Toxicol* 35:111–125.
24. Olsson P-E, Kling P, Hogstrand C. 1998. Mechansims of heavy metal accumulation and toxicity in fish. In Langston H, Bebianne C, eds, *Metal Metabolism in Aquatic Environments,* Chapman and Hall, London, UK (in press).
25. LeBlanc GA, Mastone JD, Paradice AP, Wilson B. 1984. The influence of speciation on the toxicity of silver to fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 3:37–46.
26. Bard C, Murphy JJ, Stone DL, Terhaar CJ. 1976. Silver in photoprocessing effluents. *J Water Pollut Control Fed* 48:389–394.
27. Galvez F, Hogstrand C, Wood CM. 1998. Physiological responses of juvenile rainbow trout to chronic low level exposures to waterborne silver. *Comp Biochem Physiol C* (in press).
28. Cooper CF, Jolly WC. 1970. Ecological effects of silver iodide and other weather modification agents: A review. *Water Resour Res* 6:88–98.
29. U.S. Environmental Protection Agency. 1980. Ambient water quality criteria for silver. EPA-440/5-80-071. Office of water regulations and standards, Criteria and Standards Division, Washington, DC.
30. Eisler R. 1997. Silver hazards to fish, wildlife and invertebrates: A synoptic review. U.S. Fish and Wildlife Service, Biology Report 32. Toxicity of metal mining wastes. *Bull Environ Contam Toxicol* 17:66–73.
31. Hale JG. 1977. Toxicity of metal mining wastes. *Bull Environ Contam Toxicol* 17:66–73
32. Davies PH, Goettl JP Jr, Sinley JR. 1978. Toxicity of silver to rainbow trout (*Salmo gairdneri*). *Water Res* 12:113–117.
33. Nebeker AV, McAuliffe CK, Mshar R, Stevens DG. 1983. Toxicity of silver to steelhead and rainbow trout, fathead minnows and *Daphnia magna. Environ Toxicol Chem* 2:95–104.
34. Holcombe GW, Phipps GL, Suliman AH, Hoffman AD. 1987. Simultaneous multiple species testing: Acute toxicity of 13 chemicals to 12 diverse freshwater amphibian, fish, and invertebrate families. *Arch Environ Contam Toxicol* 16:697–710.
35. Diamond JM, Mackler DG, Collins M, Gruber D. 1990. Derivation of a freshwater silver criteria for the New River, Virginia, using representative species. *Environ Toxicol Chem* 9:1425–1434.
36. Lima HB, Curtis C, Hammermeister DE, Call J, Felhaber TA. 1982. Acute toxicity of silver to selected fish and invertebrates. *Bull Environ Contam Toxicol* 29:184–189.
37. Holcombe GW, Phipps GL, Fiandt JT. 1983. Toxicity of selected priority pollutants to various aquatic organisms. *Ecotoxicol Environ Saf* 7:400–409.
38. Norberg-King TJ. 1989. An evaluation of the fathead minnow seven-day subchronic test for estimating chronic toxicity. *Environ Toxicol Chem* 8:1075–1089.
39. Buccafusco RJ, Ellis SJ, LeBlanc GA. 1981. Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus*). *Bull Environ Contam Toxicol* 26:446–452.
40. Buhl KJ, Hamilton SJ. 1991. Relative sensitivity of early life stages of Arctic grayling, coho salmon, and rainbow trout to nine inorganics. *Ecotoxicol Environ Saf* 22:184–197.
41. McDonald DG, Reader JP, Dalziel TRK. 1989. The combined effects of pH and trace metals on fish ionoregulation. In Morris R, Taylor EW, Brown DJA, Brown JA, eds, *Acid Toxicity and Aquatic Animals,* Society of Experimental Biology Seminar Series, Cambridge University Press, Cambridge, UK, pp 221–242.
42. Erichsen-Jones JR. 1939. The relation between the electrolytic

solution pressures of the metals and their toxicities to the stickleback (*Gasterosteus aculeatus* L.). *J Exp Biol* 16:425–437.
43. Morel FMM, Hering JG. 1993. *Principles and Applications of Aquatic Chemistry*. John Wiley and Sons, New York, NY, USA.
44. U.S. Environmental Protection Agency. 1991. MINTEQA2/PRODEFA2. A geochemical assessment model for environmental systems: Version 3.0 user's manual. EPA/600/3-91/021. Center for Exposure Assessment Modeling, Athens, GA.
45. Schecher WD, McAvoy DC. 1994. *MINEQL⁺ Users Manual, Version 3.01*. Hallowell, ME, USA.
46. Chudd JM. 1983. Measurement of pAg in natural water samples. *Environ Toxicol Chem* 2:315–323.
47. Lytle PE. 1984. Fate and speciation of silver in publicity owned treatment works. *Environ Toxicol Chem* 3:21–30.
48. Cooley AC, Dagon TJ, Jenkins PW, Robillard KA. 1988. Silver and the environment. *J Imaging Technol* 14:183–189.
49. Terhaar CJ, Ewell WS, Dziuba SP, Fassett DW. 1972. Toxicity of photoprocessing chemicals to fish. *Photogr Sci Eng* 16:370–377.
50. Terhaar CJ, Ewell WS, Dziuba SP, White WW, Murphy PJ. 1977. A laboratory model for evaluating the behavior of heavy metals in an aquatic environment. *Water Res* 11:101–110.
51. Bergman HL, Dorward-King EJ. 1997. *Reassessment of Metals Criteria for Aquatic Life Protection*. SETAC, Pensacola, FL, USA.
52. Lemke AE. 1981. Interlaboratory comparison: Acute testing set. EPA 600/3-81-005. Office of pesticides and toxic substances, U.S. Environmental Protection Agency, Washington, DC.
53. Pagenkopf GK. 1983. Gill surface interaction model for trace metal toxicity to fishes: Role of complexation, pH and water hardness. *Environ Sci Technol* 17:342–347.
54. Evans DH. 1987. The fish gill: Site of action and model for toxic effects of environmental pollutants. *Environ Health Perspect* 71:47–58.
55. McGeer JC, Galvez F, Wood CM. 1997. Effects of water chloride concentration on physiological response to silver exposure. *Proceedings*, Annual Meeting, Society for Experimental Biology, Canterbury, UK, April 7–9, p 25.
56. U.S. Environmental Protection Agency. 1985. Ambient water quality criteria for copper—1984. EPA 440/5-84-031. Office of Water Regulations and Standards, Washington, DC.
57. Coleman RL, Cearley JE. 1994. Silver toxicity and accumulation in largemouth bass and bluegill. *Bull Environ Contam Toxicol* 12:53–61.
58. Garnier J, Baudin JP. 1990. Retention of ingested ^{110m}Ag by a freshwater fish, *Salmo trutta*. *Water Air Soil Pollut* 50:409–421.
59. Galvez F, Hogstrand C, Wood CM. 1996. The physiological effects of dietary silver exposure in juvenile rainbow trout (*Oncorhynchus mykiss*). *Proceedings*, 4th International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, August 25–28, pp 165–174.
60. Playle RC, Dixon DG, Burnison K. 1993. Copper and cadmium binding to fish gills: Modification by dissolved organic carbon and synthetic ligands. *Can J Fish Aquat Sci* 50:2667–2677.
61. Janes N, Richards J, Playle RC. 1995. Influence of temperature and thiosulfate on active and passive uptake of silver by rainbow trout. *Proceedings*, 3rd International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, August 6–9, pp 299–302.
62. Playle RC, Dixon DG, Burnison K. 1993. Copper and cadmium binding to fish gills: Estimates of metal–gill stability constants and modeling of metal accumulation. *Can J Fish Aquat Sci* 50: 2678–2687.
63. Garnier J, Baudin JP, Foulquier L. 1990. Accumulation from water and depuration of ^{110m}Ag by a freshwater fish, *Salmo trutta* L. *Water Res* 24:1407–1414.
64. Morgan IJ, Henry RP, Wood CM. 1997. The mechanism of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) is inhibition of gill Na^+ and Cl^- transport. *Aquat Toxicol* 38:145–163.
65. Webb NA, Wood CM. 1998. Physiological analysis of the stress response associated with acute silver nitrate exposure in freshwater rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 17:579–588.
66. Perry SF. 1997. The chloride cell: Structure and function in the gills of freshwater fishes. *Annu Rev Physiol* 59:325–347.
67. Nechay BR, Saunders JP. 1984. Inhibition of adenosine triphosphatases in vitro by silver nitrate and silver sulfadiazine. *J Am Coll Toxicol* 3:37–42.
68. Ferguson EA, Leach DA, Hogstrand C. 1996. Metallothionein protects against silver blockage of the Na^+/K^+-ATPase. *Proceedings*, 4th International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, August 25–28, pp 191–200.
69. Li J, Lock RAC, Klaren PHM, Swarts HGP, Schuurmans Stekhoven FMAH, Wendelaar Bonga SE, Flik G. 1996. Kinetics of Cu^{2+} inhibition of Na^+/K^+-ATPase. *Toxicol Lett* 87:31–38.
70. Taylor EW, Beaumont MW, Butler PJ, Mair J, Muallid MSI. 1996. Lethal and sub-lethal effects of copper upon fish: A role for ammonia toxicity. In Taylor EW, ed, *Toxicology of Aquatic Pollution: Physiological, Molecular and Cellular Approaches*, Vol 57, Society for Experimental Biology Seminar Series, Cambridge University Press, Cambridge, UK, pp 85–113.
71. Wood CM. 1989. The physiological problems of fish in acid waters. In Morris R, Taylor EW, Brown DJA, Brown JA, eds, *Acid Toxicity and Aquatic Animals*, Society of Experimental Biology Seminar Series, Cambridge University Press, Cambridge, UK, pp 125–152.
72. Wilson RW, Taylor EW. 1993. The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. *J Comp Physiol B* 163:38–47.
73. Christensen GM, Tucker JH. 1976. Effects of selected water toxicants on the in vitro activity of fish carbonic anhydrase. *Chem–Biol Interact* 13:181–192.
74. van der Boon J, van den Thillart GEEJM, Addink ADF. 1997. The effects of cortisol on intermediary metabolism in teleost fish. *Comp Biochem Physiol A* 100:47–53.
75. Dockray JJ, Reid SD, Wood CM. 1996. Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss*) on unlimited ration. *Can J Fish Aquat Sci* 25:2752–2763.
76. Kägi JHR, Schäffer A. 1988. Biochemistry of metallothionein. *Biochemistry* 27:8509–8515.
77. Palmiter RD. 1994. Regulation of metallothionein genes by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription factor, MTF-1. *Proc Natl Acad Sci USA* 91:1219–1223.
78. Cosson RP. 1994. Heavy metal intracellular balance and relationship with metallothionein induction in the liver of carp after contamination by silver, cadmium and mercury following pretreatment by zinc or not. *Biometals* 7:9–19.
79. Cosson RP. 1994. Heavy metal intracellular balance and relationship with metallothionein induction in the gills of carp: After contamination by Ag, Cd, and Hg following pretreatment with Zn or not. *Biol Trace Element Res* 46:229–245.
80. Sheuhammer AM, Cherian G. 1986. Quantification of metallothioneins by a silver saturation method. *Toxicol Appl Pharmacol* 82:417–425.
81. Shaw JR, Wood CM, Birge WJ, Hogstrand C. 1998. Toxicity of silver to the marine teleost (*Oligocottus maculosus*): Effects of salinity and ammonia. *Environ Toxicol Chem* 17:594–600.
82. Dorfman D. 1977. Tolerance of *Fundulus heteroclitus* to different metals in salt waters. *Bull NJ Acad Sci* 22:21–23.
83. Schafer MM. 1995. Sampling and analytical techniques for silver in natural waters. *Proceedings*, 3rd International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, August 6–9, pp 99–112.
84. Hogstrand C, Wood CM. 1996. The toxicity of silver to marine fish. *Proceedings*, 4th International Conference, Transport, Fate and Effects on Silver in the Environment, Madison, WI, USA, August 25–28, pp 109–112.
85. Wood CM. 1993. Ammonia and urea metabolism. In Evans DH, ed, *The Physiology of Fishes*, CRC, Boca Raton, FL, USA, pp 379–425.
86. Voyer RA, Cardin JA, Heltshe JF, Hoffman GL. 1982. Viability of embryos of the winter flounder *Pseudopleuronectes americanus* exposed to mixtures of cadmium and silver in combination with selected fixed salinities. *Aquat Toxicol* 2:223–233.
87. Klein-MacPhee G, Cardin JA, Berry WJ. 1984. Effects of silver on eggs and larvae of the winter flounder. *Trans Am Fish Soc* 113:247–251.
88. Birge WJ, Black JA, Ramey BA. 1981. The reproductive tox-

icology of aquatic contaminants. *Hazard Assess Chem* 1:59–115.
89. Heath AG. 1995. *Water Pollution and Fish Physiology*. CRC, Boca Raton, FL, USA.
90. Thurberg FP, Collier RS. 1977. Respiratory response of cunners to silver. *Mar Pollut Bull* 8:40–41.
91. Gould E, MacInnes JR. 1978. Short-term effects of two silver salts on tissue respiration and enzyme activity in the cunner (*Tautogolabrus adspersus*). *Bull Environ Contam Toxicol* 18: 401–408.
92. Calabrese A, Thurberg FP, Gould E. 1977. Effects of cadmium, mercury, and silver on marine animals. *Mar Fish Rev* 39:5–11.
93. Jackim E, Hamlin JM, Sonis S. 1970. Effects of metal poisoning on five liver enzymes in the killifish (*Fundulus heteroclitus*). *J Fish Res Bd Can* 27:383–390.
94. U.S. Environmental Protection Agency. 1986. Quality criteria for water 1986. EPA-440/5-86-001. Office of Water Regulations and Standards Division, U.S. Environmental Protection Board, Washington, DC.
95. Hogstrand C, Shaw JR, Ferguson EA, Birge WJ, Wood CM. 1995. Physiological effects of silver on marine fish. *Proceedings,* 3rd International Conference, Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, August 6–9, pp 143–149.
96. Stagg RM, Shuttleworth TJ. 1982. The accumulation of copper in *Platichthys flesus* L. and its effects on plasma electrolyte concentrations. *J Fish Biol* 20:491–500.
97. Wilson RW, Taylor EW. 1993. Differential responses to copper in rainbow trout (*Oncorhynchus mykiss*) acclimated to sea water and brackish water. *J Comp Physiol B* 163:239–246.
98. Kuroshima R. 1992. Cadmium accumulation in the mummichog, *Fundulus heteroclitus,* adapted to various salinities. *Bull Environ Contam Toxicol* 49:680–685.
99. Schmidt-Nielsen K. 1997. *Animal Physiology: Adaptations and Environment.* Cambridge University Press, New York, NY, USA.
100. Evans DH. 1993. Osmotic and ionic regulation. In Evans DH, ed, *The Physiology of Fishes,* CRC, Boca Raton, FL, USA, pp 315–341.
101. Pentreath RJ. 1973. The accumulation and retention of ^{65}Zn and ^{54}Mn by the plaice, *Pleuronectes platessa. J Mar Biol Ecol* 12: 1–18.
102. Pentreath RJ. 1976. Some further studies on the accumulation and retention of ^{65}Zn and ^{54}Mn by the plaice, *Pleuronectes platessa. J Mar Biol Ecol* 21:179–189.
103. Heit M. 1979. Variability of the concentrations of seventeen trace elements in the muscle and liver of a single striped bass, *Morone saxatilis. Bull Environ Contam Toxicol* 23:1–5.
104. Greig RA, Wenzloff DR. 1977. Final report on heavy metals in small pelagic finfish, euphausid crustaceans and apex predators, including sharks, as well as on heavy metals and hydrocarbons (C_{15+}) in sediments collected at stations in and near deepwater dumpsite 106. III. Contaminant inputs and chemical characteristics. Baseline report of the environmental conditions at dumpsite 106. U.S. Department of Commerce, NOAA, Rockville, MD.
105. Greig RA, Wenzloff DR, Nelson B, Shelpuk C. 1977. Trace metals in organisms from ocean disposal sites of the middle eastern United States. *Arch Environ Contam Toxicol* 6:395–409.
106. Hellou J, Warren WG, Payne JF, Belkhode S, Lobel P. 1992. Heavy metals and other elements in three tissues of cod *Gadus morhua* from the northwest Atlantic. *Mar Pollut Bull* 24:452–458.
107. McDermott DJ, Alexander GV, Young DR, Mearns AJ. 1976. Metal contamination of flatfish around a large submarine outfall. *J Water Pollut Fed* 48:1913–1918.
108. Papadopoulu C, Kassimati EM. 1977. Stable elements in skeletal formations of fish species from Greek waters. *Thalassia Jugosl* 13:187–192.
109. U.S. Environmental Protection Agency. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027F. Cincinnati, OH.
110. Dinnel PA, Stober Q, Link JM, Letourneau MW, Roberts WE, Felton SP, Nakatani RE. 1983. Methodology and validation of a sperm cell toxicity test for testing toxic substances in marine waters. Final Report Grant R/TOX-1. University of Washington, Seattle, WA, USA.