ELSEVIER

Aquatic Toxicology 73 (2005) 406–417

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

# Accumulation and elimination of silver in *Daphnia magna* and the effect of natural organic matter

Chris N. Glover *, Chris M. Wood

*Department of Biology, McMaster University, Hamilton, Ont., Canada*

Received 4 November 2004; received in revised form 30 April 2005; accepted 3 May 2005

**Abstract**

Body burden is often used as an indicator of the toxic impact of metals such as silver. Natural organic matter (NOM) is reported to reduce silver toxicity to the highly sensitive freshwater crustacean *Daphnia magna*. However, the effect of NOM on silver burden in these organisms has not been investigated, and literature reports from other aquatic animals suggest that NOM can actually promote silver accumulation. In 24 h accumulation trials NOM exhibited a general trend of reducing whole body silver accumulation. Differences in accumulation profiles between NOM samples were attributed to chloride content stimulating uptake by the formation of diffusible silver chloride complexes. Silver accumulation assayed over 1 h exhibited considerable heterogeneity. Subsequent experiments conducted with varying light conditions during exposure and utilising gut dissection, suggested that these differences were in part due to variable gut silver accumulation. In addition to a general reduction in silver accumulation, NOM also facilitated enhanced elimination of silver from the animals. Rapid elimination of silver from *Daphnia*, coupled with speciation-, body compartment- and time of day-dependent accumulation suggests that silver body burden may be a poor indicator of silver toxicity in natural environments.


*Keywords:* Bioaccumulation; Biotic ligand model; Dissolved organic matter; Invertebrate; Metal; Uptake

## 1. Introduction

Knowledge of mechanisms underlying the toxicity of environmental contaminants is the key to predicting the harmful effects of such pollutants, and is thus integral to the development of realistic environmental protection legislation. For silver in the aquatic environment the mechanism of toxicity is well understood. Ionic silver ($Ag^+$) is a physicochemical mimic of the essential ion $Na^+$, and consequently appears to enter the ion transporting cells of uptake epithelia via apical sodium channels (Bury and Wood, 1999). Inside the cell silver disrupts carbonic anhydrase and $Na^+$, $K^+$-ATPase, the ionoregulatory enzymes that drive epithelial sodium transport (Morgan et al., 1997, 2004). Freshwater organisms rely on this pathway to replace sodium lost by passive diffusion from the concentrated

* Corresponding author at: National Institute for Nutrition and Seafood Research, Postboks 2029 Nordnes, 5817 Bergen, Norway. Tel.: +47 55 90 5155; fax: +47 55 90 5299.
*E-mail address:* chris.glover@nifes.no (C.N. Glover).

0166-445X/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2005.05.002

body to the dilute environment. Consequently, the inhibition of this pathway by silver can be fatal (Wood et al., 1996). This toxic mechanism has been elucidated primarily from experiments in freshwater rainbow trout, but a similar mechanism of action has been discerned in studied freshwater invertebrates (Grosell et al., 2002; Bianchini and Wood, 2003).

The environmental threat posed by silver in the aquatic environment is ameliorated by the presence of natural organic matter (NOM), a ubiquitous component of natural waters. In both laboratory (e.g. Karen et al., 999; Bury et al., 1999a, 2002) and natural waters (e.g. Erickson et al., 1998), NOM and other silver-binding ligands such as reduced sulfides (Bianchini et al., 2002a) have been shown to be effective in decreasing silver toxicity to aquatic organisms. This ameliorative action protects against acute silver toxicity to the extent that levels of silver in the environment only rarely exceed the capacity of these ligands to protect (Shafer et al., 1998). This protective ability of NOM is attributed to its strong binding affinity for silver ions (log *K* of 9.0–9.2, Janes and Playle, 1995; 7.5, Bury et al., 2002). In fish this results in a reduced capacity for silver to interact with the site of toxic action (branchial sodium uptake pathway) and is consistent with the observed decrease in gill silver burden (Janes and Playle, 1995; Bury et al., 1999b).

This well-defined interaction between the toxic metal and the sensitive site provides the basis of the biotic ligand model (BLM) approach for predicting environmental metal toxicity (see Di Toro et al., 2001). The BLM integrates knowledge of water chemistry with physiological mechanisms of toxicity to generate a site-specific assessment of the toxicity of a given metal to the biota therein. For example, as only silver ion is toxic, the complexation of silver by NOM will reduce the free silver ion activity, limiting the ability of silver to cross the uptake epithelium, and thus lessening toxicity. A core tenet of this approach is that the silver burden be indicative of toxicity, a relationship that has now been established for rainbow trout (Morgan and Wood, 2004).

Cladocerans are of particular interest from a silver toxicity perspective owing to their high sensitivity to waterborne silver (48 h $LC_{50} < 1\ \mu g\ L^{-1}$; Wood et al., 2002b). NOM appears to protect against silver toxicity to *Daphnia* (Karen et al., 1999; Bury et al., 2002; Glover et al., in review), but the effect of NOM on *Daphnia* silver burden is largely unknown. The investigation of NOM effect upon daphnid silver accumulation is particularly warranted given reports that NOM can actually enhance the silver body burden in another sensitive invertebrate species (zebra mussel; Roditi et al., 2000; Zimmermann et al., 2002). Such an effect in *Daphnia* could reduce the usefulness of silver body burden as an environmental indicator of silver toxicity in the aquatic environment.

## 2. Materials and methods

### 2.1. Daphnia maintenance

For all experimental manipulations described, adult (>7 days) *Daphnia magna* (ARO strain, Aquatic Research Organisms, Hampton, N.H.) were collected from an established laboratory culture. This culture was maintained under constant light (16:8 light:dark cycle), temperature (20–22 °C), and culture medium conditions. The culture medium was moderately hard laboratory water reconstituted from reverse osmosis deionised water with a composition based on that of Lake Ontario (1 mM $CaCO_3$, 0.15 mM $MgSO_4 \cdot 7H_2O$, 0.6 mM NaCl, pH 8.0). This synthetic Lake Ontario water (SLOW) also served as the basic experimental medium. Daphnids were fed daily with a slurry of yeast, alfalfa and digested trout chow (∼6 ml/L of culture water).

### 2.2. Natural organic matter (NOM)

A number of different commercially-available NOM stocks were used for the experiments described. Suwannee River NOM (SRN) and Nordic Reservoir NOM (NRN) were obtained as reference grade freeze-dried samples from the International Humic Substances Society (St. Paul, MN). Aldrich humic acid (AHA) was obtained from Sigma–Aldrich, also as a freeze-dried powder. Powdered samples were made up as liquid stocks of $\sim 2\ g\ L^{-1}$ using deionised water (>17.5 MΩ cm; Barnstead Nanopure II). All NOM concentrations reported are nominal (as total organic carbon), but based on measurement of NOM concentration in stock solutions according to the method described by Glover et al. (2005). Briefly, stock solutions were diluted and identical duplicate samples were

analysed both for total organic carbon content (Shimadzu 5050A) and absorbance at 300 nm (LKB Ultrospec 4054). Standard curves were constructed that exhibited a tight correlation ($r^2 > 0.75$) between absorbance and organic carbon content, permitting organic carbon content to be determined spectrophotometrically.

### 2.3. Silver accumulation protocol—24 h exposures

Accumulation assays were performed in acid-washed 250 ml glass beakers (Pyrex) containing 200 ml of SLOW, with an appropriate concentration of NOM (see figure legends). Silver was added as radiolabelled $^{110m}AgNO_3$ (RISØ National Laboratory, Roskilde, Denmark), to achieve an experimental concentration of 1 $\mu g\,L^{-1}$. To maximise radiolabel incorporation unlabelled silver was not used. Silver concentration in radiolabelled stock solutions was determined by graphite furnace atomic absorption spectrometry (GFAAS; Varian AA-1275 with GTA-9 atomizer). Silver and NOM (if any) were added together immediately prior to daphnid addition (i.e. no equilibration time). Consequently the experimental conditions resembled those close to point sources, where complete equilibration between silver and potential binding ligands has not occurred. Under such conditions silver toxicity is known to be greater than when a longer equilibration time is employed (Glover et al., in review). The experimental conditions thus likely represent a worst case scenario of environmental exposure.

Following 24 h in the radiolabelled solution steps were taken to minimise the contribution of adsorbed or trapped radiolabel that could interfere with silver accumulation measurement. Initially daphnids were placed in a high "cold" silver rinse solution (~1 $mg\,L^{-1}$ of unlabelled $AgNO_3$) for 15 s to eliminate radiolabelled silver loosely associated with the carapace. Animals were then transferred to clean SLOW (~1 min) to permit further exchange of radiolabelled water trapped in the carapace with unlabelled water. Daphnids were then blotted dry, weighed (UMT2, Mettler Toledo) and counted for γ-radioactivity (Canberra-Packard, Minaxi Auto-gamma 5000). Accumulated radioactivity was converted to actual silver accumulation by applying the appropriate specific activity conversion.

### 2.4. Silver accumulation protocol—1 h exposures

A series of 1 h silver accumulation experiments were performed in an identical manner to that described above. Shorter assays are often favoured in studies investigating uptake as they represent accumulation under "initial rate" conditions, thus avoiding the complication of regulatory processes that may occur at equilibrium. Initially, daphnids were exposed to increasing concentrations of Suwannee River NOM (SRN; 0, 1, 3 or 5 $mg\,C\,L^{-1}$), with the experiment performed twice, once in the morning and once in the afternoon. In another series daphnids were transferred into experimental solutions that contained AHA (8 $mg\,C\,L^{-1}$) or that were AHA-free, at time intervals 2 h apart (starting at 8 a.m. with the final assay starting at 6 p.m). All solutions were made fresh immediately prior to daphnid introduction and all daphnids were added to the experimental beaker from stock cultures, via a 30 s rinse in SLOW. After 1 h (in the dark) animals were removed and assayed for silver accumulation as above.

In a further series, adult daphnids were exposed to silver under identical conditions, with the exception that silver exposure was conducted either in the light or dark. At the time of transfer the stock culture was in the light phase of the daily cycle. Following the 1 h accumulation period, animals were removed from exposure beakers, rinsed in silver displacement and exchange solutions, and counted immediately for γ-activity. Animals were counted dry, eliminating the possibility for depuration to water during counting. Immediately following counting (<7 min following rinsing) animals were placed under a dissecting microscope and the guts were carefully dissected from the carapace and other tissues. If tearing or squeezing of gut tissues resulting in loss of gut contents (~10% of dissections) the animal was discarded from analysis. Dissected guts were then counted for γ-activity.

### 2.5. Silver elimination

In a 2 L acid-washed glass beaker a total of 20 daphnids were exposed to silver (1 $\mu g\,L^{-1}$, as $^{110m}AgNO_3$) for 24 h prior to time 0 measurement. Animals were not fed during this exposure period. Daphnids were removed, allowed to exchange trapped radiolabelled exposure water for unlabelled SLOW by subsequent

placement in two SLOW wash solutions (~1 min total), before being transferred into 2 ml microcentrifuge tubes containing either AHA (8 mg C $L^{-1}$) or AHA-free SLOW. Individual animals were counted for γ-activity immediately then transferred to a holding beaker containing 50 ml of water identical in composition to that of the counting tube (i.e. AHA or AHA-free). At times 2, 4, 6, 12, 24 and 72 h following removal from the exposure, this procedure was repeated. Before each measurement animals were briefly rinsed, transferred to a clean microcentrifuge tube containing water of identical composition, counted, and then transferred into a clean holding beaker containing 50 ml of fresh media. Animals spent no longer than 20 min in microcentrifuge tubes at each count. Feeding (~0.5 ml yeast, alfalfa, digested trout chow slurry) occurred immediately following the 24 h count, and subsequently after the 48 h measurement. This experiment was conducted twice, and because of similar results, data were combined. Owing to individual variation and a slightly higher starting silver content in the group assigned to the AHA solution, data have been expressed as the proportion of silver remaining at time 0, and were arcsine transformed prior to analysis.

### 2.6. Statistical analyses and elimination modelling

One-way or two-way analysis of variance was used to determine differences between treatment groups in silver accumulation trials. Silver elimination was modelled using Sigmaplot ver. 8.0.2 (SPSS Inc.; see Section 3 for details), with this software also used for area under the curve determination. Statistical differences between elimination curves were determined by Mann–Whitney *U*-test.

## 3. Results

Natural organic matter had a significant action on silver accumulation in *D. magna*. The nature and magnitude of this effect was dependent upon both the NOM used, and the accumulation assay conditions. Qualitative differences in silver uptake were observed between AHA and NRN following a 24 h accumulation period (Fig. 1). While AHA elicited a concentration-dependent decrease in silver accumulation with increasing AHA concentration, NRN engendered a biphasic accumulation response. A significant increase in silver accumulation at low NRN

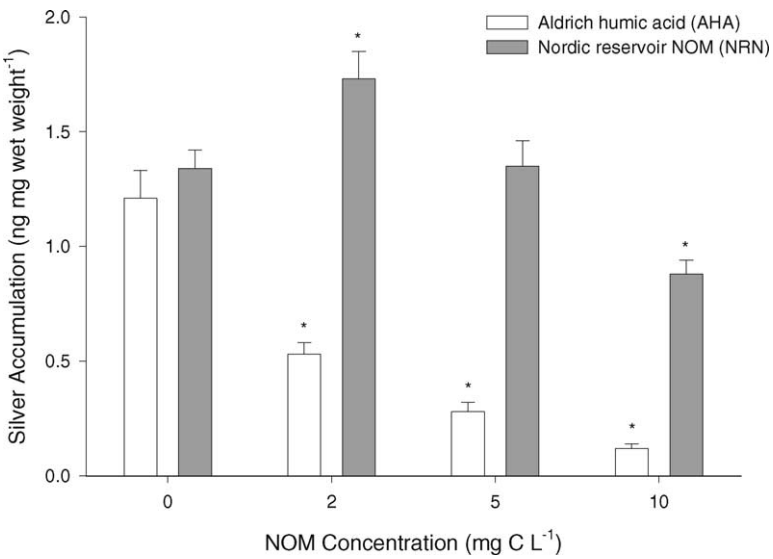


Fig. 1. Silver accumulation (ng mg wet $weight^{-1}$) in adult *Daphnia magna* following 24 h exposure to silver (1 μg $L^{-1}$) in the absence or presence of natural organic matter (0, 2, 5 or 10 mg C $L^{-1}$). Plotted values represent means (±S.E.M.) of five to six individuals. Significant differences (*) from controls (NOM-free) were tested at the $\alpha = 0.05$ level by two-way ANOVA with post-hoc L.S.D. analysis. Results of this analysis also determined a significant effect of NOM source upon silver accumulation (not shown).

levels (2 mg C $L^{-1}$) was followed by a significant decrease in silver accumulation at the highest NRN concentration (10 mg C $L^{-1}$).

One hour silver accumulation assays yielded highly variable results (Figs. 2 and 3). In Fig. 2 duplicate trials were conducted on animals from the same holding chamber, held under identical conditions, on the same day, with one trial conducted in the morning, the other in the afternoon. In the morning trial, the highest SRN level resulted in a significantly increased silver accumulation rate. In the afternoon trial, the control (SRN-free) silver accumulation rate was significantly elevated compared to the same condition in the morning trial. Furthermore, the highest SRN concentration resulted in a silver accumulation that was both significantly lower than the control condition and also significantly lower than the same concentration of the morning trial. This heterogeneity associated with time of day was discerned for other NOM samples assayed under similar conditions (not shown), and thus was not a pattern restricted to SRN.

To further investigate this variability daphnids were exposed to silver in the absence or presence of NOM at regular intervals through the course of a day. Control exposures (NOM-free) exhibited up to a 10-fold variation in silver accumulation with time of day. At 8 a.m. the control accumulation was $0.82 \pm 0.31$ ng mg wet weight$^{-1}$, which fell to $0.08 \pm 0.01$ ng mg wet weight$^{-1}$ during the 4 p.m. trial. While NOM exposures exhibited less variability, the effect of NOM on silver accumulation with respect to the corresponding control fluctuated considerably with time. NOM exposure elicited a significant decrease in silver accumulation during the 8 a.m. trial, but was without significant effects on silver body burden at other time intervals.

Experiments employing gut dissection of animals exposed to either light or dark conditions were conducted to assess the possibility that variation in accumulation may have resulted from changes in gut silver accumulation, as a possible consequence of altered feeding behaviour. *D. magna* that were assayed in the dark and in the absence of NOM, had a significantly greater (1.8–3.3-fold) silver accumulation than corresponding NOM-exposed animals, and/or animals exposed to light conditions throughout the assay. Conversely, while control-exposed gut contribution

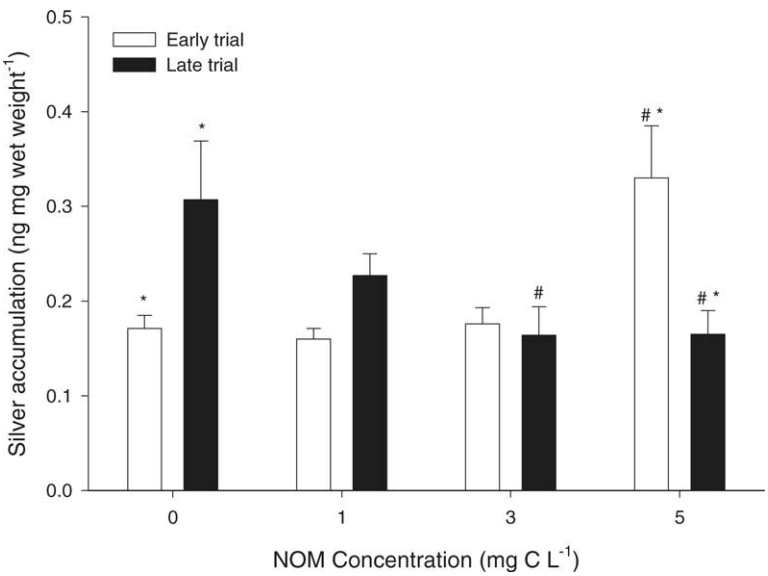


Fig. 2. Silver accumulation (ng mg wet weight$^{-1}$) in adult *Daphnia magna* following 1 h exposure to silver (1 μg $L^{-1}$) in the absence or presence of Suwannee River NOM (SRN; 0, 1, 3 or 5 mg C $L^{-1}$), during trials conducted in the morning (early trial; white bars) or in the afternoon (late trial; black bars) in animals derived from the same holding conditions. Plotted values represent means (±S.E.M.) of five individuals. Significant differences from controls (0 SRN) within trials ($^{\#}$), and between trials at identical concentrations ($^{*}$) were tested at the $\alpha = 0.05$ level by two-way ANOVA, with post-hoc L.S.D. analysis.

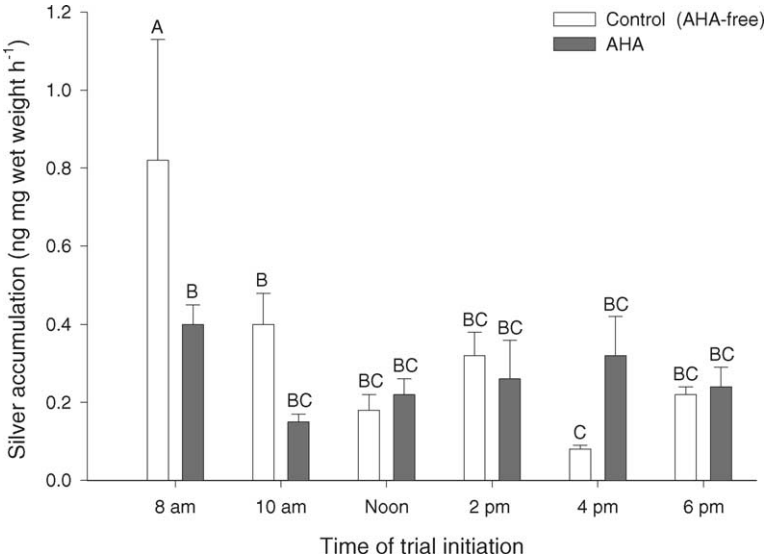


Fig. 3. Effect of time of day on silver accumulation (ng mg wet weight $h^{-1}$) in adult *Daphnia magna* following 1 h exposure to silver (1 μg $L^{-1}$) in the presence (grey bars) or absence (white bars) of Aldrich humic acid (AHA; 8 mg C $L^{-1}$). Plotted values represent means (±S.E.M.) of five to six individuals. Bars sharing letters are not significantly different as determined by one-way ANOVA with post-hoc LSD analysis at the $\alpha = 0.05$ level.

to total silver burden remained constant, there was a significant decrease in the proportion of silver in the gut in exposures performed in the light relative to those performed in the dark

Silver elimination profiles for both AHA-exposed and control animals followed a two-compartment, first order kinetic model (Fig. 5; Newman, 1998; Neumann et al., 1999; Gillis et al., 2004, 2005):

$$S_{TB} = S_F\, e^{(-k_F t)} + S_S\, e^{(-k_S t)} \quad (1)$$

where $S_{TB}$ is the total silver burden at time $t$, $S_F$ the silver burden of the fast depurating compartment, and $S_S$ the silver burden of the slowly depurating compartment, each of which is expressed as a percentage of initial total silver burden. For both rapidly and slowly depurating compartments elimination rate constants ($h^{-1}$), denoted by $k_F$ and $k_S$, respectively, were derived. The various parameter values determined from this model are exhibited in Table 1. Statistical analyses revealed that there were no significant differences between control- and AHA-exposed animals when individual parameter values were compared, but the area under the curve was statistically distinct. The half-life of silver (calculated as $0.693/k_x$) was 1.64 and 1.16 h in the rapidly depurating compartment in the absence of or presence of NOM, respectively. The half-life of silver in the more slowly depurating compartment was 37.06 and 24.40 h for NOM-free and NOM conditions, respectively. Elimination occurred so rapidly that it was not necessary to account for radiolabel decay (c.f. Fraysse et al., 2002). Owing to the frequent handling,

Table 1
Parameters describing the elimination of silver from *Daphnia magna* in the absence or presence of AHA (8 mg C $L^{-1}$) as determined using a two-compartment first order kinetic model

| | $S_F$ (% of total) | $k_F$ ($\times 10^{-3}$, $h^{-1}$) | $S_F$ 1/2 (h) | $S_S$ (% of total) | $k_S$ ($\times 10^{-3}$, $h^{-1}$) | $S_S$ 1/2 (h) | $r^2$ | AUC (% × h) |
|---|---|---|---|---|---|---|---|---|
| AHA-free | 39 ± 10 | 423 ± 231 | 1.64 | 61 ± 9 | 19 ± 6 | 37.06 | 0.977 | 503 ± 98 |
| AHA | 52 ± 6 | 598 ± 164 | 1.16 | 48 ± 5 | 28 ± 7 | 24.40 | 0.993 | 258 ± 59[a] |

Refer to Eq. (1), and discussion of calculations in results for further information and for description of abbreviations. $S_x$ 1/2 is the half-life of silver in each compartment and AUC is the area under the curve. Values given represent mean ± S.E.M.

[a] Statistical differences between AHA-free and AHA conditions were determined via Mann–Whitney *U* analysis at the $\alpha = 0.05$ level.

some animals perished during these experiments. Data obtained from these animals were excluded from analysis.

The shed exoskeletons and neonates of animals that moulted during elimination studies were monitored as potential sources of silver elimination, but were found to have silver concentrations equivalent to dissolved silver concentrations in the elimination medium (data not shown). Consequently moulting was not considered to be a significant route of loss.

## 4. Discussion

### *4.1. Geochemical speciation dictates silver accumulation*

The complexation of silver by NOM reduces the toxic free ion form of the metal and thus reduces its ability to interact with the site of toxic action, the sodium uptake pathway (Janes and Playle, 1995). Consequently a decrease in silver accumulation would be expected. This was observed for *D. magna* in the current study at high concentrations of NOM following a 24 h exposure to silver (Fig. 1). At lower concentrations of NOM, however, there appeared to be a qualitatively different effect. At a NOM level of 2 mg C $L^{-1}$, NRN actually facilitated silver accumulation. Even at higher levels of NOM, NRN appeared to be a less successful inhibitor of silver uptake than AHA.

Certain NOM sources are known to offer better amelioration of silver toxicity to freshwater fish and invertebrates than others (e.g. Van Genderen et al., 2003; Glover et al., in review). However, the two sources used here, AHA and NRN, exhibit similar protective abilities against silver toxicity to *Daphnia* (Glover et al., in review). This suggests that differences in accumulation profiles are not the reflective of the relative toxicity of silver in the presence of these NOM sources.

The different silver accumulation profiles are instead likely to reflect differences in water chemistry. Reconstituted NRN contains a five-fold higher chloride content than does AHA on a per milligram of carbon basis (Glover, unpublished data). The neutral $AgCl_0$ complex formed by silver in the presence of chloride is capable of diffusion across biological membranes, and thus can promote enhanced accumulation (e.g. Wood et al., 2002a). The mean enhancement of silver accumulation by NRN over the silver accumulation in the presence of AHA at the same organic carbon content was 5.1-fold (Fig. 1). This suggests the presence of two competing speciation-related events determine silver accumulation; the decrease in silver bioavailability promoted by complexation with NOM, and the enhanced bioavailability caused by increased neutral $AgCl_0$ formation. While having different effects on accumulation, the formation of both these silver species acts to decrease silver toxicity.

This chloride effect may also explain why some previous investigations have failed to exhibit a consistent relationship between silver burden and toxicity in the presence of NOM. Richards et al. (2001) noted that although a variety of naturally-isolated NOM sources were protective against the toxicity of a mixed metal exposure including silver, to rainbow trout, this was not reflected in gill binding. Naturally-isolated NOM sources offer many advantages over the commercially-available NOM samples used in the present study (see Malcolm and MacCarthy, 1986). They are often more realistic of the NOM of natural waters as they have been isolated by procedures that do not substantially alter their physicochemical and biological properties. However, these samples are often collected via reverse osmosis, an isolation procedure that concentrates the NOM, but also may act to concentrate other ions, such as chloride. Furthermore in an attempt to purify these samples they may often be passed through cation exchange columns to remove associated metals, a process that may also introduce chloride contamination of the sample. Consequently the distorted relationship between toxicity and accumulation is likely a function of chloride content. Both laboratory and field studies therefore need to account carefully for the potential confounding influence of chloride on silver accumulation.

### *4.2. High variability of 1 h accumulation assays*

Considerable differences in patterns of silver accumulation were observed between assays of 1 h versus 24 h duration. While differences between NOM sources were noted in longer trials (Fig. 1), the primary differences in shorter trials were the time-dependent variations in accumulation with the same NOM source (e.g. Fig. 2). This phenomenon was investigated by a series of experiments that examined silver

accumulation as a function of time and light conditions. Silver accumulation was shown to be highly time-dependent, even in the absence of NOM in the water (Fig. 3). The large variation in control accumulation suggested that a physiological process was responsible for the heterogeneity observed, as opposed to a geochemical explanation related specifically to the presence of NOM.

Daphnids exposed to NOM in the dark exhibited significantly enhanced gut silver burdens, indicative of a feeding-type response (Fig. 4). In natural waters *Daphnia* exhibit filtration patterns that vary with light conditions. For example elevated filtering rates were observed in daphnids following initiation of a dark phase (Starkweather, 1975), similar to the conditions that would be encountered under dark assay conditions here. The light entrainment of feeding rhythm appears to be a phenomenon of larger daphnids (Haney, 1985), possibly explaining why previous studies (e.g. Bianchini and Wood, 2003) may not have observed such a phenomenon.

The colloidal and particulate nature of silver–NOM complexes (Wen et al., 2002) may likely account for the differences between gut accumulation in the absence and presence of NOM. These colloids could potentially reach a size (~0.45 μm; Brendelberger, 1991) whereby they are trapped by the filter mesh of the daphnid feeding apparatus, and treated as food particles. Certainly there is evidence suggesting that the formation of silver sulfide colloids results in an enhanced gut accumulation of silver in *Daphnia* (Bianchini et al., 2002a, 2002b). Consequently increased feeding behaviour could differentially favour the uptake of silver by the gut in the presence, but not the absence of NOM (Fig. 5).

In the absence of NOM, the proportion of silver in the gut remained constant between light and dark experiments, but the actual amount of accumulated silver varied (Fig. 4). This suggests that there is change in a factor not related to gut association that mediated fluctuating silver uptake patterns. In recent studies examining sodium uptake in *Daphnia* it has been shown that sodium uptake parameters can vary considerable with time (Glover et al., 2005). Factors such as fed state, for example, are known to influence waterborne sodium uptake in *Daphnia* (Stobbart et al., 1977). Given that the mechanism of silver uptake in *Daphnia* is via the sodium uptake pathway (Bianchini and Wood, 2003) it is reasonable to assume that fluctuations such as these may extend to silver uptake. It is likely that such variations are only observed in the absence of NOM, as when NOM is present there is less free silver ion available to exploit the altered sodium uptake pathway. This would explain why in the presence of NOM in animals assayed in the dark, total silver burden is not enhanced despite increased gut accumulation.

Total silver burden in daphnids was almost identical following a 1 h or 24 h silver accumulation period (Fig. 1 versus Fig. 4A, dark). This suggests that peak silver burden is rapidly attained and maintained through prolonged exposure, a result noted previously by the kinetic analysis of Bianchini and Wood (2003). Two mechanisms would explain such a pattern. Either silver uptake is regulated, or elimination of silver is rapidly initiated to limit accumulation. There is experimental evidence suggesting that *Daphnia* can acclimate to pulsed exposures of silver in laboratory conditions (Glover and Wood, 2004). The data displayed here also support the alternate hypothesis, and suggest a role for silver elimination in controlling silver body burden.

### 4.3. Rapid elimination of silver

Silver is eliminated very rapidly from *D. magna*, especially in the presence of NOM. Modelling of elimination showed that there were two main compartments from which silver was depurated. The rapid component was likely to consist of silver that was loosely associated with the carapace and/or that which was eliminated by the passage of gut contents (Gillis et al., 2004). The slower depurating compartment likely represented silver which had been incorporated, and subsequently must be actively eliminated. The nature of NOM-facilitation of elimination is unknown. It is possible that the ingestion of organic matter, that has a high affinity for silver, can act as a depurating ligand, facilitating the removal of silver from epipodite and gut surfaces exposed to the ambient water via filtering and drinking processes. Alternatively it is possible that NOM may act to modify physiological processes, such as feeding that may act to stimulate water flow over depurating surfaces, thus facilitating silver elimination.

Depuration in the presence of NOM yielded half-lives of 1.16 and 24.4 h from the rapid and slowly eliminating compartments, respectively. This is similar to a previous finding for the same species in water containing moderate DOC levels (3 mg $L^{-1}$; Adam et

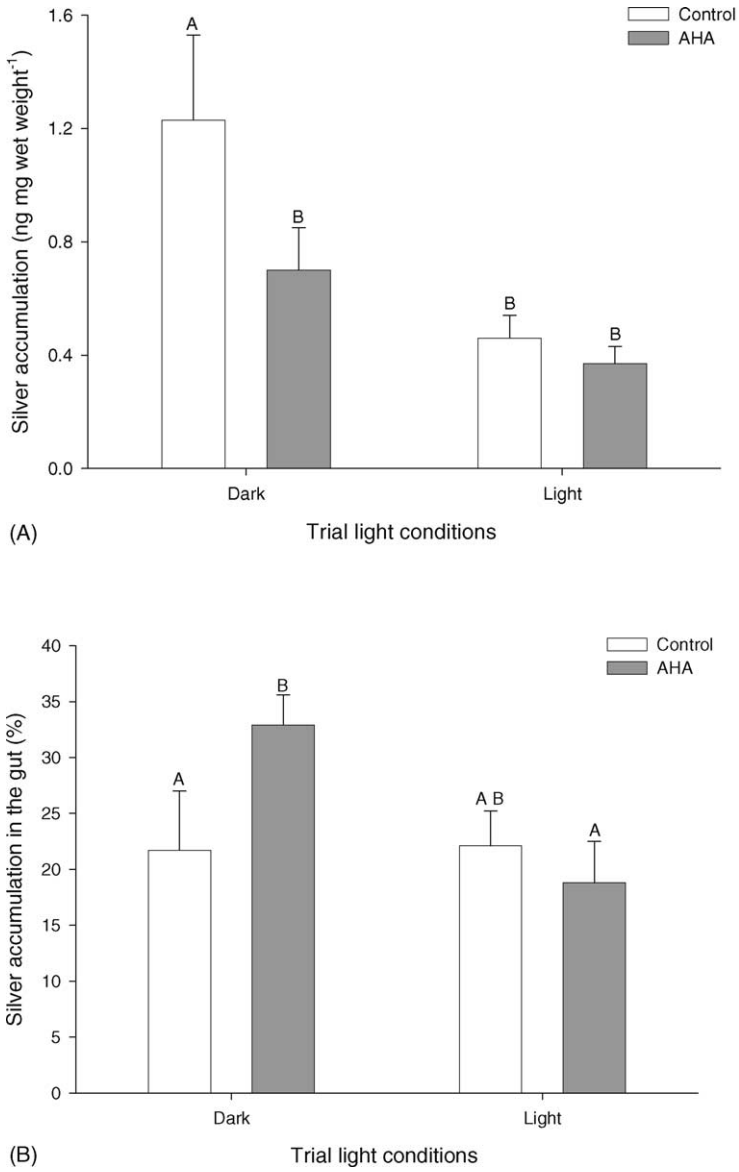


Fig. 4. Effect of accumulation trial light conditions on (A) total silver accumulation (ng mg wet weight$^{-1}$) and (B) gut silver accumulation (% of total accumulation) in adult *Daphnia magna* following 1 h exposure to silver (1 μg L$^{-1}$) in the absence or presence of Aldrich humic acid (AHA; 8 mg C L$^{-1}$). Plotted values represent means (±S.E.M.) of five to six individuals. Bars sharing letters are not significantly different ($p < 0.05$) as determined by one-way ANOVA, with post-hoc L.S.D. analysis. The gut data were arc-sine transformed prior to statistical analysis.

al., 2001). In this study two compartments for $^{110m}$Ag depuration were also described with half-lives of 20 min and 1 day.

In the present investigation animals were unfed for the first 24 h of elimination. A recent report (Gillis et al., 2005) showed that gut clearance of *Daphnia* is much slower in the absence of food, suggesting that the relatively rapid elimination rates discerned may actually underestimate the rate in natural, nutrient-rich waters. In contrast to its rapid elimination in *Daphnia*, the elimination of silver in other invertebrate species examined is considerably slower. For the molluscs

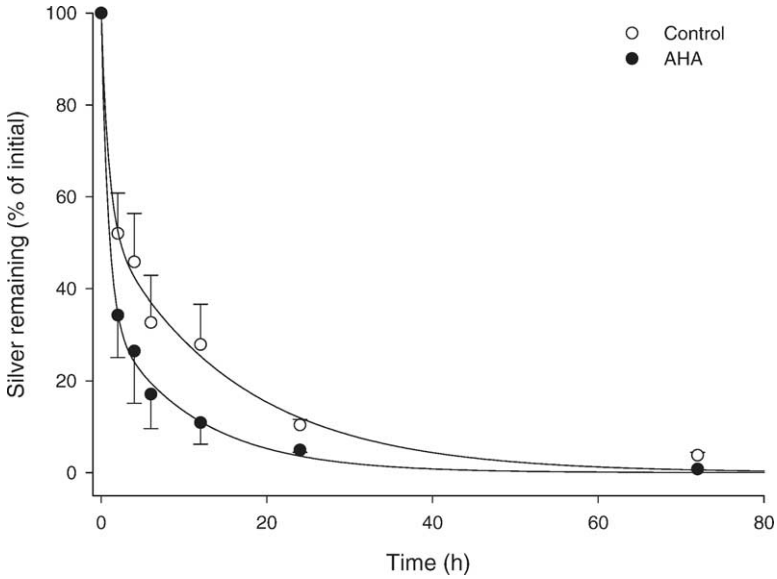


Fig. 5. Elimination of silver (% of initial accumulation) from adult *Daphnia magna* in the presence (black circles) or absence (white circles) of Aldrich humic acid (AHA; 8 mg C $L^{-1}$). Plotted values represent the mean (±S.E.M.) of 8–10 individuals. Curves represent those fitted using a two-component, first order kinetic model (Sigmaplot ver. 8.0.2; SPSS Inc.). $r^2$ values of 0.94 were obtained for both control and NOM curves.

*Dreissena polymorpha* and *Corbicula fluminea* the biological half-life of silver has been calculated in the order of 8–22 days (Roditi and Fisher, 1999; Fraysse et al., 2002). Recent data show that fish species also depurate whole body silver relatively slowly (Bertram and Playle, 2002; Hogstrand et al., 2003; Nichols and Playle, 2004). Even taking into account experimental variation between these studies, these results highlight the rapid elimination of silver from *Daphnia*.

Another study from fish suggests that this difference may be related to the way in which *Daphnia* deal with a toxic insult. Wood et al. (2002a) showed that trout gills exhibited a relatively rapid decline in silver (half-life ~2 h), as the toxic metal was eliminated from the sensitive site to storage sites such as the liver (Hogstrand et al., 2003). In the liver of fish silver is effectively detoxified by metallothionein, which has a high affinity for silver and acts to sequester the metal from sensitive cellular sites (Hogstrand et al., 1996). While *Daphnia* are capable of synthesising a metallothionein-like protein in response to metal exposure (Stuhlbacher et al., 1992), the induction response time is unknown (De Coen and Janssen, 1997). In addition there may be metabolic constraints preventing the synthesis of an energetically costly protein, and instead detoxification is concentrated towards the elimination of silver as opposed to sequestration of biologically inert metal.

The significant action of NOM on elimination suggests that the ameliorative effects of these ubiquitous components of aquatic systems may be manifold. Not only do these substances protect by binding silver and preventing uptake, but may also facilitate a more rapid removal of silver from the animals, thus potentially minimising toxic effects in this manner.

### *4.4. Implications for environmental modelling and laboratory testing*

The accumulation of silver in the presence of NOM is highly influenced by a number of geochemical and physiological factors. Silver burden will fluctuate in response to both water chemistry (i.e. chloride content, NOM concentration) and as a result of regulation of sodium uptake pathways and feeding behaviours. Changes in elimination profiles associated with different water chemistries would also appear to have an impact upon silver accumulation. Given the rapid loss of silver from the animals, and the ability to acclimate to silver exposure, body burden may be a poor indicator of toxicity. This is especially

true when it is considered that whole body burden measures include both silver that has been absorbed, and that which is ingested. These two sources of burden may show opposite patterns of accumulation depending upon the time of collection, thus potentially distorting the relationship between accumulation and toxicity. Furthermore, the absorption of neutral silver complexes such as that formed in the presence of chloride will additionally complicate the relationship.

It is of interest to note that Muyssen and Janssen (2002) have also recorded rapid changes in metal burdens in *Daphnia* over short time intervals upon zinc exposure. This suggests that rapid fluctuations in metal content are likely not restricted to silver. The caveats discussed herein are therefore likely to apply to studies of metal metabolism in cladocerans in general.

## Acknowledgements

This work was supported by a NSERC Collaborative Research and Development Grant Project (CRDPJ 257740-02) with co-funding from Kodak Canada Inc. CMW is supported by the Canada Research Chair program. Dr. Patricia Gillis is thanked for assistance with elimination rate calculations.

## References

Adam, C.R., Baudin, J.P., Garnier-Laplace, J., 2001. Kinetics of $^{110m}$Ag, $^{60}$Co, $^{137}$Cs, and $^{54}$Mn bioaccumulation from water and depuration by the crustacean *Daphnia magna*. Water Air Soil Pollut. 125, 171–188.

Bertram, B.O.B., Playle, R.C., 2002. Effects of feeding on waterborne silver uptake and depuration in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 59, 350–360.

Bianchini, A., Wood, C.M., 2003. Mechanisms of acute silver toxicity in *Daphnia magna*. Environ. Toxicol. Chem. 22, 1361–1367.

Bianchini, A., Bowles, K.C., Brauner, C.J., Gorsuch, J.W., Kramer, J.R., Wood, C.M., 2002a. Evaluation of the effect of reactive sulfide on the acute toxicity of silver (I) to *Daphnia magna*: Part 2: Toxicity results. Environ. Toxicol. Chem. 21, 1294–1300.

Bianchini, A., Rouleau, C., Wood, C.M., 2002b. Silver accumulation in *Daphnia magna* in the presence of reactive sulfide. Aquat. Toxicol. 72, 339–349.

Brendelberger, H., 1991. Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnol. Oceanogr. 36, 884–894.

Bury, N.R., Galvez, F., Wood, C.M., 1999b. Effects of chloride, calcium and dissolved organic carbon on silver toxicity: comparison between rainbow trout and fathead minnows. Environ. Toxicol. Chem. 18, 56–62.

Bury, N.R., McGeer, J.C., Wood, C.M., 1999a. Effects of altering freshwater chemistry on physiological responses of rainbow trout to silver exposure. Environ. Toxicol. Chem. 18, 49–55.

Bury, N.R., Shaw, J., Glover, C., Hogstrand, C., 2002. Derivation of a toxicity based model to predict how water chemistry influences silver toxicity to invertebrates. Comp. Biochem. Physiol. C 133, 259–270.

Bury, N.R., Wood, C.M., 1999. Mechanism of branchial apical silver uptake by rainbow trout is via the proton-coupled $Na^+$ channel. Am. J. Physiol. 277, R1385–R1391.

De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna* toxicity testing. 2. Digestive enzyme activity in *Daphnia magna* exposed to sublethal concentrations of cadmium, chromium and mercury. Chemosphere 35, 1053–1067.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. 20, 2383–2396.

Erickson, R.J., Brooke, L.T., Kahl, M.D., Vende Venter, F., Harting, S.L., Markee, T.P., Spehar, R.L., 1998. Effects of laboratory test conditions on the toxicity of silver to aquatic organisms. Environ. Toxicol. Chem. 17, 572–578.

Fraysse, B., Baudin, J.-B., Garnier-Laplace, J., Adam, C., Boudou, A., 2002. Effects of Cd and Zn waterborne exposure on the uptake and depuration of $^{57}$Co, $^{110m}$Ag and $^{134}$Cs by the Asiatic clam (*Corbicula fluminea*) and the zebra mussel (*Dreissena polymorpha*)—whole organism study. Environ. Pollut. 118, 297–306.

Gillis, P.L., Chow-Fraser, P., Ranville, J.F., Ross, P.E., Wood, C.M., 2005. *Daphnia* need to be gut cleared too: the effect of exposure to and ingestion of metal-contaminated sediment on the gut clearance patterns of *D. magna*. Aquat. Toxicol. 71, 143–154.

Gillis, P.L., Dixon, D.G., Borgmann, U., Reynoldson, T.B., 2004. Uptake and depuration of cadmium, nickel, and lead in laboratory-exposed *Tubifex tubifex* and corresponding changes in the concentration of a metallothionein-like protein. Environ. Toxicol. Chem. 23, 76–85.

Glover, C.N., Pane, E.F., Wood, C.M., 2005. Humic substances influence sodium metabolism in the freshwater crustacean, *Daphnia magna*. Physiol. Biochem. Zool. 78, 405–416.

Glover, C.N., Playle, R.C., Wood, C.M., in review. Heterogeneity of natural organic matter (NOM) amelioration of silver toxicity to *Daphnia magna*: effect of NOM source and silver-NOM equilibration time. Environ. Toxicol. Chem.

Glover, C.N., Wood, C.M., 2004. Physiological interactions of silver and humic substances in *Daphnia magna*: effects on reproduction and silver accumulation following an acute silver challenge. Comp. Biochem. Physiol. C 139, 273–280.

Grosell, M., Brauner, C.J., Kelly, S.P., McGeer, J.C., Bianchini, A., Wood, C.M., 2002. Physiological response to acute silver exposure in the freshwater crayfish (*Cambarus diogenes diogenes*)—a model invertebrate? Environ. Toxicol. Chem. 21, 369–374.

Haney, J.F., 1985. Regulation of cladoceran filtering rates in nature by body size, food concentration, and diel feeding patterns. Limnol. Oceanogr. 30, 397–411.

Hogstrand, C., Galvez, F., Wood, C.M., 1996. Toxicity, silver accumulation and metallothionein induction in freshwater rainbow

trout during exposure to different silver salts. Environ. Toxicol. Chem. 15, 1102–1108.

Hogstrand, C., Grosell, M., Wood, C.M., Hansen, H., 2003. Internal redistribution of radiolabelled silver among tissues of rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*): the influence of silver speciation. Aquat. Toxicol. 63, 139–157.

Janes, N., Playle, R.C., 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 14, 1847–1858.

Karen, D.J., Ownby, D.R., Forsythe, B.L., Bills, T.P., La Point, T.W., Cobb, G.B., Klaine, S.J., 1999. Influence of water quality on silver toxicity to rainbow trout (*Oncorhynchus mykiss*), fathead minnows (*Pimephales promelas*), and water fleas (*Daphnia magna*). Environ. Toxicol. Chem. 18, 63–70.

Malcolm, R.L., MacCarthy, P., 1986. Limitations in the use of commercial humic acids in water and soil research. Environ. Sci. Technol. 20, 904–911.

Morgan, I.J., Henry, R.P., Wood, C.M., 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.

Morgan, T.P., Grosell, M., Gilmour, K.M., Playle, R.C., Wood, C.M., 2004. Time course analysis of the mechanism by which silver inhibits active $Na^+$ and $Cl^-$ uptake in gills of rainbow trout. Am. J. Physiol. 287, R234–R242.

Morgan, T.P., Wood, C.M., 2004. A relationship between gill silver accumulation and acute silver toxicity in the freshwater rainbow trout: support for the acute silver biotic ligand model. Environ. Toxicol. Chem. 23, 1261–1267.

Muyssen, B.T.A., Janssen, C.R., 2002. Accumulation and regulation of zinc in *Daphnia magna*: links with homeostasis and toxicity. Arch. Environ. Contam. Toxicol. 43, 492–496.

Neumann, P.T.M., Borgmann, U., Norwood, W., 1999. Effect of gut clearance on metal body burden concentrations in *Hyalella azteca*. Environ. Toxicol. Chem. 18, 976–984.

Newman, M.C., 1998. Fundamentals of Ecotoxicology. Sleeping Bear. Farmington Hills, MI, USA.

Nichols, J.W., Playle, R.C., 2004. Influence of temperature on silver accumulation and depuration in rainbow trout. J. Fish Biol. 64, 1638–1654.

Richards, J.G., Curtis, P.J., Burnison, B.K., Playle, R.C., 2001. Effects of natural organic matter source on reducing toxicity to rainbow trout (*Oncorhynchus mykiss*) and on metal binding to their gills. Environ. Toxicol. Chem. 20, 1159–1166.

Roditi, H.A., Fisher, N.S., 1999. Rates and routes of trace element uptake in zebra mussels. Limnol. Oceanogr. 44, 1730–1749.

Roditi, H.A., Fisher, N.S., Sañudo-Wilhelmy, S.A., 2000. Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature 407, 78–80.

Shafer, M.M., Overdier, J.T., Armstrong, D.E., 1998. Removal, partitioning, and fate of silver and other metals in wastewater treatment plants and effluent receiving streams. Environ. Toxicol. Chem. 17, 630–641.

Starkweather, P.L., 1975. Diel patterns of grazing in *Daphnia pulex* Leydig. Verh. Int. Verein. Limnol. 19, 2851–2857.

Stobbart, R.H., Keating, J., Earl, R., 1977. A study of sodium uptake by the water flea *Daphnia magna*. Comp. Biochem. Physiol. A 58, 299–309.

Stuhlbacher, A., Bradley, M.C., Naylor, C., Calow, P., 1992. Induction of cadmium tolerance in two clones of *Daphnia magna* Straus. Comp. Biochem. Physiol. C 101, 571–577.

Van Genderen, E.J., Ryan, A.C., Tomasso, J.R., Klaine, S.J., 2003. Influence of dissolved organic matter source on silver toxicity to *Pimephales promelas*. Environ. Toxicol. Chem. 22, 2746–2751.

Wen, L.-S., Santschi, P.H., Gill, G.A., Tang, D., 2002. Silver concentrations in Colorado, USA, watersheds using improved methodology. Environ. Toxicol. Chem. 21, 2040–2051.

Wood, C.M., Grosell, M., Hogstrand, C., Hansen, H., 2002a. Kinetics of radiolabelled silver uptake and depuration in the gills of rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*): the influence of silver speciation. Aquat. Toxicol. 56, 197–213.

Wood, C.M., Hogstrand, C., Galvez, F., Munger, R.S., 1996. The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*). 1. The effects of ionic $Ag^+$. Aquat. Toxicol. 35, 93–109.

Wood, C.M., La Point, T.W., Armstrong, D.E., Birge, W.J., Brauner, C.J., Brix, K.V., Call, D.J., Crecelius, E.A., Davies, P.H., Gorsuch, J.W., Hogstrand, C., Mahoney, J.D., McGeer, J.C., O'Connor, T.P., 2002b. Biological effects of silver. In: Andren, A.W., Bober, T.W. (Eds.), Silver in the Environment: Transport, Fate, and Effects. Society of Enviromental Toxicology and Chemistry Press, Pensacola, FL, USA.

Zimmermann, S., Alt, F., Messerschmidt, J., von Bohlen, A., Taraschewski, H., Sures, B., 2002. Biological availability of traffic-related platinum-group elements (palladium, platinum, and rhodium) and other metals to the zebra mussel (*Dreissena polymorpha*) in water containing road dust. Environ. Toxicol. Chem. 21, 2713–2718.