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Internal redistribution of radiolabelled silver among tissues of rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*): the influence of silver speciation

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Abstract

Influence of water Ag(I) speciation on pharmacokinetics of Ag(I) during a post-exposure period was investigated in rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*). The rainbow trout is sensitive to waterborne ionic Ag^+ whereas the eel is tolerant. The fish were acclimated to either of two chloride concentrations, 10 or 1200 μM, in synthetic soft water and then exposed to a sublethal 24-h pulse of 1.3 μg l^{-1} of ^{110m}Ag(I) added as $^{110m}AgNO_3$ in these waters. The protocol provided exposures to mainly the free ion Ag^+ (low chloride water) or mainly $AgCl_{aq}$ (high chloride water). Contents and concentrations of ^{110m}Ag(I) in tissues and body fluids were then monitored over a 67-day post-exposure period in Ag(I)-free water of the same chloride levels. Changing the speciation of Ag(I) in the water had no effect on the whole body load of ^{110m}Ag(I), but did result in differences in internal distribution. In trout, changing water Ag(I) speciation significantly altered elimination or accumulation of Ag(I) in several body compartments. Notably, trout exposed to $AgCl_{aq}$ eliminated ^{110m}Ag(I) from the kidney more quickly than trout exposed to Ag(I) primarily as Ag^+. This elimination was matched by higher concentrations of ^{110m}Ag in liver of trout exposed to Ag(I) primarily as $AgCl_{aq}$. In eel, shifting speciation from Ag^+ to $AgCl_{aq}$ hastened elimination of ^{110m}Ag(I) from mid and posterior intestine and increased ^{110m}Ag(I) retention in kidney. While there was no difference between the two fish species in whole body ^{110m}Ag(I) load, most internal body compartments of trout had higher ^{110m}Ag(I) concentrations than those in eel early in the experiment. Because tissue-specific elimination times were longer in eel than in trout, these differences were generally cancelled by the end of the 67-day depuration period. The only exception was the liver, which in trout continued to accumulate ^{110m}Ag(I) throughout the experiment but in eel remained unchanged. The combined effect of ^{110m}Ag(I) movements in the two species was that trout retained all their accumulated ^{110m}Ag(I) through the 67-day period, whereas the body burden of ^{110m}Ag(I) in eel was reduced to half initial values by day 67.

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1. Introduction

While acute toxicity of silver to freshwater fish is relatively well understood, chronic effects have been poorly characterised (Hogstrand and Wood, 1998; Wood et al., 1999). During exposure to the free silver ion, Ag^+, acute toxicity is caused by a net loss of sodium and in some species also chloride. It has been shown that these 'acute' effects occur at relatively low concentrations during long-term exposure (Galvez et al., 1998). However, as the free Ag^+ that causes these effects is virtually non-existent in most natural waters, ionoregulatory disturbances of this kind may not occur in nature. It remains possible that other yet unidentified toxic effects are more important during chronic exposure. It is a reasonable assumption that any such effects might be expected in tissues where silver accumulates. Thus, pharmacokinetics of silver movement through the body can provide an indication of which systems might be affected by silver during long-term exposure.

Acute toxicity to silver in freshwater rainbow trout is only observed during exposure to dissolved Ag^+ (Hogstrand et al., 1996; Wood et al., 1996a,b; Galvez and Wood, 1997; McGeer and Wood, 1998; Galvez et al., 1998; Karen et al., 1999; Bury et al., 1999a,b). Although other forms of silver do not cause the loss of sodium and chloride, which is typical for acute silver toxicity, they are accumulated by fish to various extents (Hogstrand et al., 1996; Wood et al., 1996b; Hogstrand and Wood, 1998). In particular, when present as the uncharged $AgCl_{aq}$ complex silver appears to be taken up readily by the gills of both rainbow trout and eel (Hogstrand et al., 1996; Wood et al., 2002). If long-term accumulation of silver does lead to chronic toxicity, it is conceivable that silver from various sources and routes of exposure could contribute to the effect. Hence, it is of interest to compare pharmacokinetics of silver when presented as different species to the organism. Likewise, from the viewpoint of recovery from previous silver exposure and potential food-chain transfer it is important to establish elimination rates of accumulated silver. The published information on these issues is scattered and inconclusive. Whereas Garnier and co-workers (Garnier et al., 1990; Garnier and Baudin, 1990) found no elimination of silver (^{110m}Ag(I)) in freshwater brown trout (*Salmo trutta*) over a 1-month depuration period, Pentreath (1977) determined the biological half life ($t_{0.5}$) of ^{110m}Ag(I) in the marine plaice to be as short as 9–18 days. This vast difference could be attributed to species differences, but it might also be related to differences in physiology of fish in freshwater (brown trout) and seawater (plaice) as well as contrasts of silver chemistry in the two media. In this context it is interesting to note that the elimination of ^{110m}Ag(I) from a marine elasmobranch, the thornback ray (*Raja clavata*), was much slower ($t_{0.5}$ = 277–1400 days) than in the marine teleost (Pentreath, 1977). Clearly, elimination of silver from fish and its dependence of animal species and water chemistry deserve to be studied in more detail.

Relatively few studies have investigated the distribution of silver in fish following exposure. A review of the literature suggests that the highest concentrations of silver are found in gill, liver, and kidney (Hogstrand and Wood, 1998). In terms of amounts, the liver seems to be the primary tissue for accumulation in rainbow trout and brown trout, accounting for 60–70% of the total body burden (Garnier et al., 1990; Garnier and Baudin, 1990; Galvez et al., 2002). In an experiment where rainbow trout were exposed to a 48-h pulse of waterborne ^{110m}Ag(I), followed by continuous exposure to stable silver isotope, 50% of the radioactivity was localised to the liver 24 h following the pulse, i.e. 72 h after the start of

^{110m}Ag(I) pulse (Galvez et al., 2002). The lack of samples at earlier time points made it impossible to fully characterise the kinetics of silver distribution in the body. The accumulation kinetics of silver in the liver of fish appears to be very rapid, but it must be emphasised that only salmonid species have been investigated, and this group of teleosts is unusually sensitive to silver. Furthermore, the radioactive ^{110m}Ag(I) was 'chased' with stable silver isotope, which may have accelerated the redistribution of ^{110m}Ag(I) in the body.

The aim of the present study was to gather detailed information on redistribution of silver in fish following exposure via the water. Specific objectives included a comparison of silver pharmacokinetics between the silver-sensitive rainbow trout and the silver-tolerant European eel (*Anguilla anguilla*). We also set out to investigate possible differences in distribution kinetics of Ag(I) accumulated from the highly toxic Ag^+ ion and the much less toxic neutral aqueous chloride complex ($AgCl_{aq}$). In a parallel report, we demonstrated that the branchial elimination profile of ^{110m}Ag(I) depended on whether the fish had been exposed to Ag^+ or $AgCl_{aq}$ (Wood et al., 2002). We therefore studied whether redistribution of Ag(I) in the body is dependent upon the speciation of silver in the water. To achieve these objectives, rainbow trout and European eel were exposed to Ag(I), added as $^{110m}AgNO_3$, in water with either low (10 μM) or high (1200 μM) chloride content. In this manner the effect of speciation can be tested because the low chloride water allows the predominance of Ag^+ and the high-chloride water promotes formation of $AgCl_{aq}$. Following a 24-h exposure to ^{110m}Ag(I) the radioactivity in different tissues was followed over a 67-day period, during which no Ag(I) was added to the water. Conducting this study at a nuclear research facility had the advantages of allowing rather considerable activities of ^{110m}Ag(I) to be used and the employment of extremely sensitive high-throughput Geiger-type counters. The present study comprises ^{110m}Ag(I) measurements on a total of 5960 samples, a daunting task in most laboratories. This protocol allowed for detailed quantification of internal redistribution in the two fish species, and possible elimination during the depuration period.

2. Materials and methods

2.1. Experimental animals and acclimation

Juvenile rainbow trout (*Oncorhynchus mykiss*; 20–30 g) and European eel (*Anguilla anguilla*; 45–75 g, all yellow stage, non-migratory) were obtained, fed, and acclimated to laboratory conditions as outlined by Wood et al. (2002). For 16-days prior to the experimental period (December 1997–February 1998), approximately 200 individuals of each species were acclimated, to flowing low chloride (10 μM) soft water and 200 to high chloride (1200 μM) soft water, in separate 150-l PVC tanks. Chloride was added as KCl from a concentrated stock solution via a peristaltic pump.

2.2. Experimental protocol

The experimental design and exposure conditions have previously been described in detail (Wood et al., 2002). Eighty trout and 80 eels were exposed for 24 h to 1.3 $\mu g\,l^{-1}$ of ^{110m}Ag(I) (470 GBq g^{-1} added as $^{110m}AgNO_3$) in 200-l glass aquaria containing either low chloride or high chloride soft water (14 °C). Modelling of ^{110m}Ag(I) speciation, using the geochemical equilibrium program Mineql+ 4.01, suggested that the increase in water $[K^+]$ and $[Cl^-]$ from 10 to 1200 mM resulted in a decrease in the concentration of ionic Ag^+ from 0.78 to 0.23 $\mu g\,l^{-1}$ and a reciprocal increase in the uncharged $AgCl_{aq}$ species from 0.02 to 0.51 $\mu g\,l^{-1}$ (Wood et al., 2002). This corresponded to a relative distribution of the major Ag(I) species, Ag^+, $AgCl_{aq}$, and Ag-DOC ($[DOC] = 1.3$ mgC l^{-1}; Wood et al., 2002) of 60, 1.5, and 38%, respectively, in the low $[Cl^-]$ water and 18, 39, and 38% in the high $[Cl^-]$ water. No other changes in water physicochemistry were evident. Silver presented as Ag-DOC has been shown to have low uptake in fish (Janes and Playle, 1995; Bury et al., 1999a; Luoma et al., 2002). Therefore, for convenience and clarity the low $[Cl^-]$ condition is referred to as 'Ag^+'

exposure and the high [Cl^-] condition as exposure to '$AgCl_{aq}$'.

The two species were exposed in separate tanks. Radioactivity declined by no more than 20% over the 24-h exposure due to uptake by the fish and adhesion to surfaces. All fish were exposed for exactly 24 h, after which they were transferred to the depuration tanks and sampled at the appropriate times up to 67 days post-exposure.

From the ^{110m}Ag(I) exposure, the fish of one species were placed into one of the four identical glass depuration tanks (volume = 80 l), which received vigorous aeration and a one-pass flow-through (rate = 0.71 min^{-1}) of the appropriate low chloride or high chloride soft water at 14 ± 0.5 °C. No detectable silver was present in the water. At each sampling time (3 h and 1, 2, 4, 8, 15, 32, and 67 days post-exposure), 10 fish were sacrificed from each treatment, except for trout at Day 67 where $N = 4$ and 5 at low and high chloride respectively. Samples of gill, blood, kidney, anterior intestine, mid and posterior intestine, liver, bile, and carcass were taken at all sampling points, and of skin and white muscle at days 1, 4,15, 32, and 67.

2.3. Sampling

At sampling, fish were rapidly anaesthetised and then rinsed in ^{110m}Ag-free and anaesthetic-free water. A 0.5-ml blood sample was drawn by caudal puncture after which the fish was euthanised by either a cephalic blow (trout) or cutting the spinal cord (eels). The gills were removed and prepared as described in Wood et al. (2002). Fish were then opened by a longitudinal ventral incision and the bile collected using a syringe. Liver, whole intestine, and kidneys were dissected out. A 1-cm^3 sample of dorsal muscle with covering skin was excised. Skin was separated from the muscle using a scalpel and spread out on a pre-weighed planchet for radioactivity counting. The remaining carcass was placed between moist paper towels while the collected samples were prepared for counting. Blood and bile samples were plated individually on tared planchets, which were then weighed to record exact volumes. Liver samples were homogenised in a glass-Teflon® homogeniser with approximately two volumes of deionised water added and the subsamples of the homogenates were then plated onto pre-weighed planchets. Muscle samples were manually homogenised by mincing with scissors, diluted with two volumes of water and spread out evenly on the pre-weighed planchet. The gastrointestinal tract was divided into 'anterior intestine' and 'mid and posterior intestine' by cutting just posterior to the pylorus. Both parts of the intestine were then opened up longitudinally and spread out on separate pre-weighed planchets. The carcass was homogenised in a food blender and a sample of the carcass was smeared on a pre-weighed planchet. All tissues were dried to a constant weight at room temperature and humidity, weighed, and counted for radioactivity as described below. Thus, as the proportion of total body weight represented by each tissue or body fluid was known (Table 1), the total amount of radioactivity in the whole body and in each individual tissue could be calculated, as well as the relative contribution of each tissue to whole body radioactivity. In turn this could be converted to an absolute silver concentration,

Table 1
Relative sizes of rainbow trout and European eel body compartments used to calculate distribution of ^{110m}Ag(I)

Tissue	Relative compartment sizes (%)	
	Rainbow trout	European eel
Gill filaments	0.51 ± 0.12	0.61 ± 0.06
Whole blood	5.58	5.58
Kidney	0.88 ± 0.20	0.86 ± 0.10
Anterior intestine	1.60 ± 0.38	1.19 ± 0.08
Mid and posterior intestine	1.13 ± 0.31	1.84 ± 0.12
Liver	1.10 ± 0.10	1.65 ± 0.09
Bile	0.2	0.2
Carcass	42.67 ± 1.13	33.25 ± 1.97
Muscle	46.85 ± 2.28	53.33 ± 2.77
Skin[a]	1.36	2.28
Haematocrit[b]	23.5 ± 6.38	37.6 ± 4.55

Compartments are expressed as percentage of the total body weight, with the exceptions for skin and haematocrit, which were given as cm g^{-1} and percent red blood cells of total blood volume, respectively. Values are means ± 1 S.E.M., $n = 5$.

[a] Expressed as cm^2 skin per gram skin.

[b] Expressed weight of red blood cells in percentage of whole blood.

based on the known specific activity of the ^{110m}Ag(I) used. The specific activity of ^{110m}Ag(I) did not change appreciably during the passage through the gill tissue, because the background concentration of stable silver isotope in gills was two orders of magnitude lower (Grosell et al. 2000) than the silver accumulated as ^{110m}Ag(I) during the 24-h exposure (Wood et al., 2002).

2.4. Analytical techniques

^{110m}Ag(I) emits both β and γ radioactivity. For measurement of ^{110m}Ag(I) radioactivity in the majority of samples, water samples and tissue homogenates were dried onto planchets as described above, then counted for β-decay to 99% statistical accuracy using two custom-built low background Geiger-type multi-sample counters (Bøtter-Jensen and Nielsen, 1989). For this type of counting, quench correction is not an issue, but uniform sample geometry is a concern, so care was taken to ensure the latter as described above. For some liver samples, radioactivity was great enough to saturate the β-counters; these samples were therefore recounted by traditional γ-counting, using appropriate standards to cross-calibrate the two detection systems. Quality control revealed that there was no detectable contribution by ^{109}Cd to radioactivity in any of the tissues included in the present study. This was in contrast to the gills of the same fish, which accumulated ^{109}Cd as a trace contaminant of the ^{110m}Ag source (Hansen et al., 2002). Further details of the radioanalytical procedures used can be found in Hansen et al. (2002).

Water samples were taken periodically during the exposure and the following depuration period and analysed for sodium, chloride, potassium, magnesium, calcium, pH, dissolved organic carbon (DOC), and total silver as described in Wood et al. (2002). These measurements allowed for calculation of specific activity of ^{110m}Ag(I) and speciation of silver in the exposure water (Wood et al., 2002).

2.5. Statistical analyses

Data have been expressed as means ± 1 S.E. (N) where N represents the number of different fish contributing to the mean. Differences between treatments, and/or between species at the same sample time were evaluated by Student's unpaired two-tailed t-test. The influence of time and chloride concentration on loading during the exposure period, and on depuration during the post-exposure period were evaluated by two-way ANOVA for each species. If significant double interaction between time and chloride concentration was observed, both chloride and time were tested against the significant interaction rather than the residual. All percentage data were normalised by arc–sin transformation prior to statistical tests. Data of ^{110m}Ag(I) depuration from tissues were modelled as exponential decay, using Sigmaplot 5.00 (SPSS) software. A biexponential depuration equation yielded better fit than a monoexponental equation in all cases where data could be explained by exponential decay. A significance level of $P \leqslant 0.05$ was employed throughout.

3. Results

3.1. Overview

Considerable amounts of ^{110m}Ag(I) were found in the body of both fish species at the first sampling 3 h after transfer of the animals to water without any ^{110m}Ag(I) added (i.e. 27 h after the beginning of ^{110m}Ag exposure). At this time point the ^{110m}Ag(I) activity in the gills was already reduced from maximum values by 98–99% for trout and 93–95% for eels (Wood et al., 2002). The general trend was a statistically significant elimination of ^{110m}Ag(I) from most body compartments including blood plasma (both species, Fig. 1A), red blood cells (rainbow trout, Fig. 1B), intestine (both species, Fig. 2), kidney (rainbow trout, Fig. 3), carcass (both species, Fig. 3B), and bile (rainbow trout, Fig. 4B). Liver of rainbow trout (Fig. 4A) and bile of eel (Fig. 4B) were the only compartments where there was a significant increase in accumulation of ^{110m}Ag(I) with time. No time-dependent changes in ^{110m}Ag(I) levels were detected for red blood cells (Fig. 1B), kidney (Fig. 3B), or liver (Fig. 4A) of eels. Elimination of ^{110m}Ag(I) from tissues fitted best to an exponential

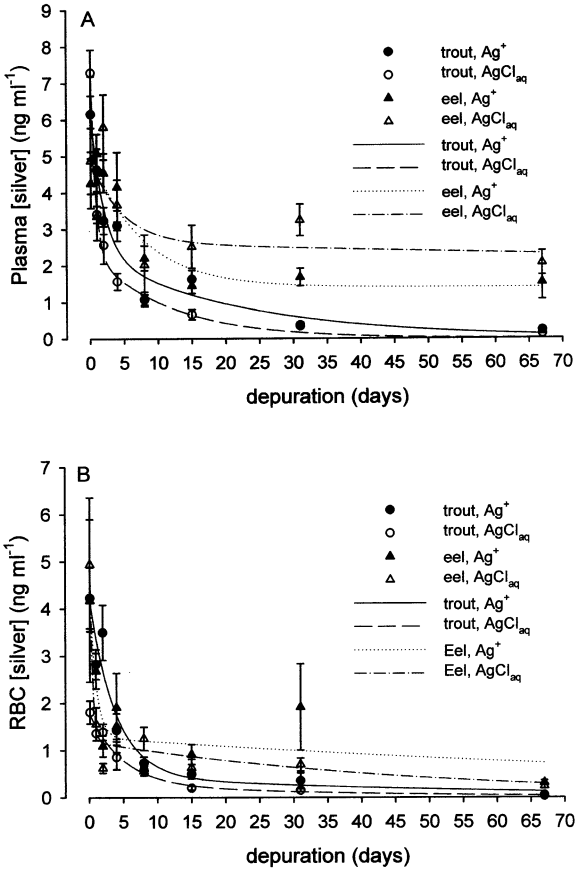

Fig. 1. Depuration over 67 days post-exposure, of Ag(I) in (A) blood plasma and (B) red blood cells (RBC) of rainbow trout and European eel exposed for 24 h to 1.3 μg l^{-1} Ag(I) in either low chloride water (silver mainly as Ag^+) or high chloride water (silver mainly as $AgCl_{aq}$). Means ±1 SEM ($N = 10$ at each sample point except day 67, where $N = 4$ for Ag^+ and $N = 5$ for $AgCl_{aq}$ treatments). The overall effect (by two-way ANOVA) of chloride was significant ($P \leqslant 0.05$) for both plasma and RBC in rainbow trout, but only for plasma in eel. The overall effect (by two-way ANOVA) of time was significant for plasma in both fish species and for RBC in rainbow trout.

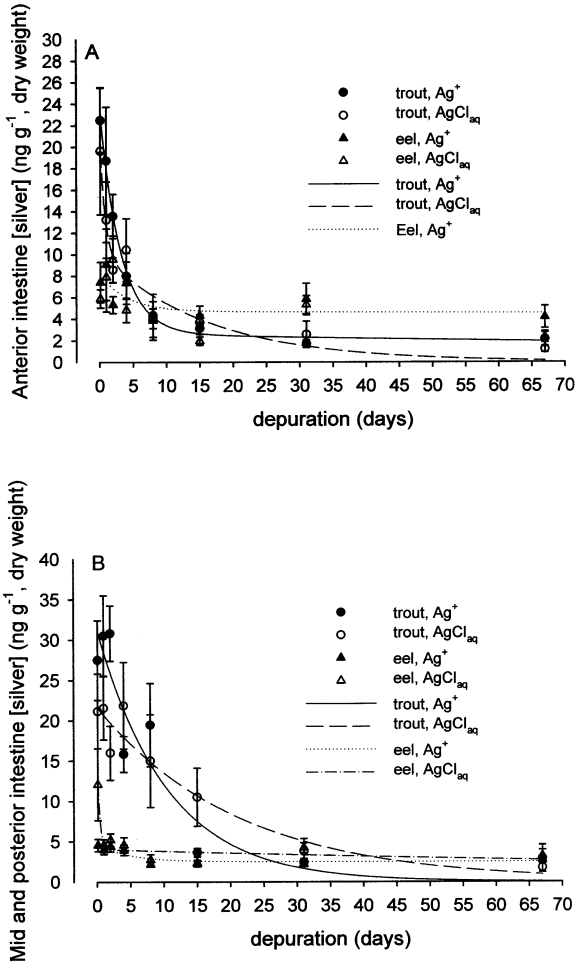

Fig. 2. Depuration over 67 days post-exposure, of Ag(I) in (A) anterior intestine and (B) mid and posterior intestine. Details as in Fig. 1. The overall effect (by two-way ANOVA) of chloride was significant ($P \leqslant 0.05$) for mid and posterior intestine in rainbow trout and eel. The overall effect (by two-way ANOVA) of time was significant for both sections of intestine in rainbow trout and eel.

model with two compartments, a rapidly and a slowly eliminating fraction (REF and SEF; Tables 2 and 3). The elimination half-times, $t_{1/2}$, of the REF of ^{110m}Ag(I) in different tissues were generally short, ranging from 5 to 15 days and accounting for 22–89% of the ^{110m}Ag(I) initially accumulated in the tissue (Tables 2 and 3). The calculated $t_{1/2}$ for the SEF of the different tissues were extremely variable and ranged from 7 days to infinity. Depending on tissue, water [Cl^-], and fish species, 11–78% of the accumulated ^{110m}Ag(I) partitioned to the SEF.

The liver was by far the dominant compartment for ^{110m}Ag(I) accumulation for both fish species (Fig. 4A; Tables 4–7). Already 3 h after transfer to depuration tanks with water without ^{110m}Ag(I), 18% (eel in water with high [Cl^-]) to 75% (trout in water with high [Cl^-]) of all ^{110m}Ag(I) in the body was present in the liver (Tables 4–7). In trout, the

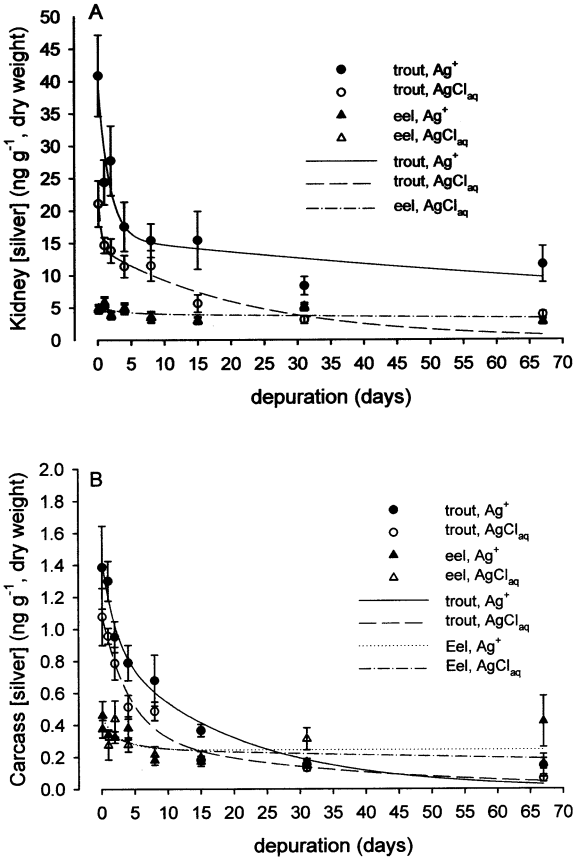

Fig. 3. Depuration over 67 days post-exposure, of Ag(I) in (A) kidney and (B) carcass. Details as in Fig. 1. The overall effect (by two-way ANOVA) of chloride was significant ($P \leqslant 0.05$) for both kidney and carcass in rainbow trout, but only for kidney in eel. The overall effect (by two-way ANOVA) of time was significant for both kidney and carcass in rainbow trout, but only for carcass in eel.

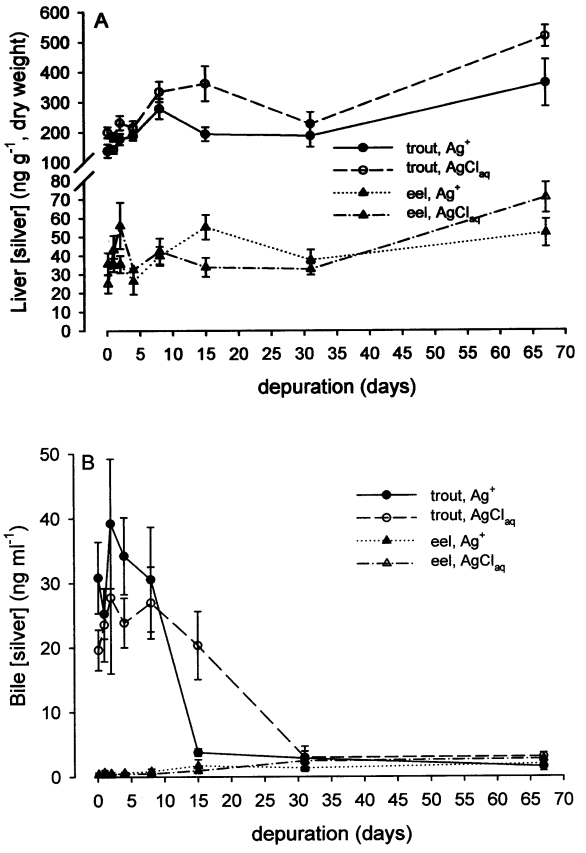

Fig. 4. Depuration over 67 days post-exposure, of Ag(I) in (A) liver and (B) bile. Details as in Fig. 1. The overall effect (by two-way ANOVA) of chloride was significant ($P \leqslant 0.05$) for liver in rainbow trout only. The overall effect (by two-way ANOVA) of time was significant for liver and bile in rainbow trout, but only for bile in eel.

liver continued to accumulate ^{110m}Ag(I) throughout the experiment and by the end of the 67-day depuration period 93–97% of the ^{110m}Ag(I) body burden was found in the liver. The corresponding figures were 72–77% for eel, but this relative increase was caused by reduction of ^{110m}Ag(I) load in other tissues (principally the gills; Wood et al., 2002) rather than an increase in hepatic ^{110m}Ag(I) content. Initially, high concentrations of ^{110m}Ag(I) were also found in bile of rainbow trout, but unlike the situation in the liver, ^{110m}Ag(I) in bile of trout decreased precipitously later in the experiment (Fig. 4B). In contrast, ^{110m}Ag(I) in bile of eel started at very low concentrations and increased slowly through the experimental period (Fig. 4B). Muscle of either species contained less than 0.50 ng g^{-1} (dry weight) of ^{110m}Ag(I) at any time (0.02–0.50 ng g^{-1} dry weight) and the concentration of ^{110m}Ag(I) in skin was an order of magnitude lower than this (0.002–0.020 ng g^{-1} dry weight).

3.2. *Effects of water chloride concentration on ^{110m}Ag(I) pharmacokinetics in rainbow trout*

Shifting ^{110m}Ag(I) speciation from Ag^+ to $AgCl_{aq}$ in the exposure water had influence on

Table 2
Elimination of ^{110m}Ag(I) from tissues in rainbow trout exposed to ^{110m}Ag(I) primarily as Ag^+ or $AgCl_{aq}$ as fitted to a biphasic exponential elimination equation

Tissue and main Ag(I) species	REF (%)	REF $t_{1/2}$ (days)	SEF (%)	SEF $t_{1/2}$ (days)	r^2	$P <$
Blood plasma						
Ag^+	63	1.3±0.5	37	15.3+7.0	0.963	0.002
AgCl	71	0.5±0.07	29	8.0±1.7	0.996	0.001
Red blood cells						
Ag^+	89	2.6±0.9[b]	11	37.3±30.3	0.938	0.007
AgCl	85	3.4+0.9[b]	15	25.5±16.8	0.984	0.001
Anterior intestine						
Ag^+	89	2.2±0.2	11	147.5+92.4	0.996	0.001
AgCl	53	0.5±0.2[a]	47	11.1±4.0[a]	0.947	0.005
Mid and posterior intestine						
Ag^+	49	7.3±7.2	51	7.3±7.2	0.890	0.022
AgCl	50	14.7±14.7	50	14.6±14.6	0.927	0.010
Kidney						
Ag^+	61	1.2±0.4	39	95.0±51.1	0.905	0.016
AgCl	38	0.3±0.2	62	15.7±4.1[a,b]	0.939	0.007
Carcass						
Ag^+	39	1.5±0.7	61	13.6±4.3	0.975	0.001
AgCl	69	2.9±0.9	31	23.7±11.7	0.979	0.001
Muscle						
Ag^+	–	–	–	–	–	–
AgCl	79	3.6±1.3	21	46.2±28.2	0.990	0.127

The two phases were designated the REF and SEF, respectively. ^{110m}Ag(I) in each of these fractions is expressed as percentage of total silver concentration in the tissue. $t_{1/2}$ is the biological half life of each fraction, r^2 the regression coefficient, and P the statistical probability of the curve fit. An 'a' denotes a statistically significant difference between Ag^+ and $AgCl_{aq}$ conditions; differences between corresponding values in rainbow trout and European eel are shown with a 'b' sign. –, indicates that data could not be explained mathematically as exponential decay.

pharmacokinetics of ^{110m}Ag(I) in trout, affecting its elimination or accumulation in liver (Fig. 4A), kidney (Fig. 3A), carcass (Fig. 3B), mid and posterior intestine (Fig. 2B), red blood cells (Fig. 1B), and skin (data not shown). Among tissues, the most substantial effect of speciation was found in the kidney (Fig. 3A) where the increase in water $[Cl^-]$ reduced the REF of ^{110m}Ag(I) and decreased retention times for both REF and SEF (Table 2). Thus, ^{110m}Ag(I) eliminated more quickly from the kidney of the trout that were exposed to $AgCl_{aq}$ in the water than in the ones that were exposed to Ag^+. Trout exposed to $AgCl_{aq}$ also had lower concentration of ^{110m}Ag(I) in the kidney at the first sampling point ($P < 0.05$; Fig. 3A), but the percentage of whole body ^{110m}Ag(I) present in the kidney at this time was the same for both groups of trout (Tables 4 and 5). In contrast to the trout kidney, an increased water $[Cl^-]$ concentration resulted in a tendency for slower elimination of ^{110m}Ag(I) from carcass and mid and posterior intestine (Table 2). $AgCl_{aq}$-exposed rainbow trout also differed from Ag^+-exposed trout in terms of accumulation of ^{110m}Ag(I) in the liver (Fig. 4A). Specifically, rainbow trout exposed to $AgCl_{aq}$ in the water accumulated higher concentrations of ^{110m}Ag(I) in the liver than the trout that were exposed to Ag^+. Furthermore, a higher percentage of the total body burden of ^{110m}Ag(I) was found in the liver of these fish early on in the experiment as compared with Ag^+-exposed trout ($P < 0.05$), indicating that ^{110m}Ag(I) moved more quickly to the liver in trout exposed to $AgCl_{aq}$ (Tables 4 and 5).

Table 3
Elimination of ^{110m}Ag(I) from tissues in European eel exposed to ^{110m}Ag(I) primarily as Ag^+ or $AgCl_{aq}$ as fitted to a biphasic exponential elimination equation

Tissue and main Ag(I) species	REF (%)	REF $t_{1/2}$ (days)	SEF (%)	SEF $t_{1/2}$ (days)	r^2	$P <$
Blood plasma						
Ag^+	72	5.3±2.3	28	†	0.895	0.020
AgCl	47	3.2±2.1	53	364.8±323.8	0.544	0.325
Red blood cells						
Ag^+	72	0.6±0.3[b]	28	83.5±56.6	0.801	0.069
AgCl	82	0.2±0.1[b]	18	33.2±14.4	0.962	0.003
Anterior intestine						
Ag^+	42	2.8±1.7	58	1155.2±1089.2	0.564	0.299
AgCl	–	–	–	–	–	–
Mid and posterior Intestine						
Ag^+	47	3.2±1.7	53	†	0.750	0.107
AgCl	73	0.3±0.l[a]	27	115.5±64.2	0.913	0.013
Kidney						
Ag^+	–	–	–	–	–	–
AgCl	22	2.1±1.6	78	239.0.±174.8[b]	0.360	0.576
Carcass						
Ag^+	45	1.9±1.3	55	†	0.396	0.525
AgCl	33	2.7±1.9	67	165.0±122.8	0.485	1.402
Muscle						
Ag^+	64	5.1±1.3	36	26.1±8.2	0.999	0.041
AgCl	–	–	–	–	–	–

The two phases were designated the REF and SEF, respectively. ^{110m}Ag(I) in each of these fractions is expressed as percentage of total silver concentration in the tissue. $t_{1/2}$ is the biological half life of each fraction, r^2 the regression coefficient, and P the statistical probability of the curve fit. An 'a' denotes a statistically significant difference between Ag^+ and $AgCl_{aq}$ conditions; differences between corresponding values in European eel and rainbow trout are shown with a 'b' sign. –, Indicates that data could not be explained mathematically as exponential decay. †, There was no detectable elimination from the SEF.

3.3. Effects of water chloride concentration on ^{110m}Ag(I) pharmacokinetics in European eel

With the exception of blood (i.e. plasma and red blood cells), the concentrations of ^{110m}Ag(I) in body compartments of eel did not vary appreciably during the depuration period (Figs. 1–4). Consequently, these data fitted relatively poorly to the biphasic exponential elimination model (Table 3). However, this model was still used where possible because other models of elimination fitted even less well to the data. Changing the [Cl^-] of the water also influenced pharmacokinetics of ^{110m}Ag(I) in eels albeit not to the same extent as in rainbow trout. As in trout, water ^{110m}Ag(I) speciation had statistically significant effects on ^{110m}Ag(I) elimination from mid and posterior intestine and from kidney (Fig. 2BFig. 3A), but in contrast to trout no other body compartments showed differences in ^{110m}Ag(I) pharmacokinetics that could be related to the two water chemistry conditions. It was not possible to evaluate how elimination of ^{110m}Ag(I) from kidney differed between Ag^+-exposed and $AgCl_{aq}$-exposed eel because renal elimination of ^{110m}Ag(I) in the eels exposed to Ag^+ could not be fitted to any commonly used regression model (Table 3). The effect of water Cl^- concentration on ^{110m}Ag(I) levels in eel kidney was minute, but because renal ^{110m}Ag concentrations were consistently higher in eel exposed to the $AgCl_{aq}$ condition, compared with those subjected to the Ag^+ condition, and variability between individuals was very low, the overall effect was statistically significant ($P < 0.05$,

Table 4
Concentration (ng g^{-1} wet weight) of whole body ^{110m}Ag(I) and relative distribution (%) of ^{110m}Ag(I) among body compartments in rainbow trout exposed to waterborne Ag(I) primarily as Ag^{+}

Depuration time (days)	Whole body [Ag] (ng g^{-1})	Gills[a] (%)	Blood (%)	Kidney (%)	Anterior intestine (%)	Mid and posterior intestine (%)	Liver (%)	Bile (%)	Carcass (%)	Muscle (%)	Skin (%)	Carcass (muscle and skin) (%)
0.12	16.66±2.19	8.38± 0.77	3.45± 0.64	8.85± 1.26	2.07±0.13	1.71±0.24	40.95± 1.90	2.64± 0.60	31.96± 2.12	–	–	–
1	11.66±0.86	6.89± 0.76	3.04± 0.30	6.63± 0.70	2.63±0.60	3.10±0.52	48.60± 1.70	2.19± 0.36	26.92± 2.24	6.14± 0.33	0.74± 0.06	20.04±2.44
2	12.80±1.09	5.11± 0.52	2.78± 0.39	7.02± 1.16	1.83±0.34	2.83±0.36	54.87± 3.61	3.07± 0.85	25.04± 2.39	–	–	–
4	11.94±0.90	4.46± 0.60	1.64± 0.35	4.54± 0.80	1.03±0.24	1.55±0.23	63.29± 2.71	2.84± 0.55	20.65± 1.94	4.80± 1.07	0.76± 0.12	15.09+1.67
8	15.52±1.68	2.47± 0.64	0.50± 0.09	2.97± 0.60	0.68±0.33	1.86±0.33	71.37± 5.12	1.34± 0.43	18.82± 4.34	–	–	–
15	11.09±1.13	5.66± 1.20	0.83± 0.15	3.24± 0.81	0.42±0.10	0.34±0.06	80.76± 3.16	0.37± 0.09	8.37± 1.77	1.93± 0.26	0.34± 0.04	6.10±1.69
32	8.41±0.66	2.78± 0.88	0.39± 0.10	3.47± 1.03	0.33±0.10	0.38±0.09	85.51± 2.67	0.14± 0.04	7.00± 1.60	1.91± 0.57	0.49± 0.14	4.60±1.29
67	15.61±3.35	1.43± 0.31	0.08+ 0.02	2.72± 0.58	0.22±0.05	0.17±0.07	92.59± 0.10	0.11± 0.04	2.68± 0.76	0.36± 0.06	0.10± 0.02	2.27±0.75

The fish were exposed to ^{110m}Ag during 24 h followed by a 67-day depuration period as indicated in the table. Numbers are given as mean±1 S.E.M. and were based on 10 fish, except for data from day 67, which was based on four fish.

[a] Data from Wood et al. (2002).

Table 5
Concentration (ng g^{-1} wet weight) of whole body ^{110m}Ag(I) and relative distribution (%) of ^{110m}Ag(I) among body compartment in rainbow trout exposed to waterborne Ag(I) primarily as $AgCl_{aq}$

Depuration time (days)	Whole body [Ag] (ng g^{-1})	Gills[a] (%)	Blood (%)	Kidney (%)	Anterior intestine (%)	Mid and posterior intestine (%)	Liver (%)	Bile (%)	Carcass (%)	Muscle (%)	Skin (%)	Carcass (muscle and skin) (%)
0.12	10.90±1.05	8.34±0.61	0.20±0.02	6.67±1.34	2.31±0.48	2.71±0.73	77.06±1.74	1.95±0.31	46.02±3.99	–	–	–
1	9.59±0.53	5.68±0.69	0.31±0.02	6.88±0.64	3.18±0.83	3.48±0.65	73.16±2.47	4.01±1.17	44.90±1.70	9.13±0.76	1.05±0.06	34.72±1.24
2	11.46±0.83	4.02±0.80	0.19±0.01	4.98±0.61	1.61±0.25	1.55±0.36	84.81±1.72	1.29±0.67	22.29±4.95	–	–	–
4	10.30±0.92	3.18±0.52	0.30±0.03	5.15±0.80	2.28±0.58	3.35±0.65	79.07±1.48	3.87±0.82	23.00±3.31	6.07±1.04	0.70±0.13	17.48±13.70
8	14.20±2.03	1.23±0.35	0.16±0.02	1.58±0.14	0.41±0.06	0.44±0.14	93.09±0.78	1.43±0.38	10.53±2.14	–	–	–
15	15.08±2.21	2.29±0.53	0.24±0.04	1.41±0.32	0.72±0.22	1.34±0.55	89.77±1.84	2.37±0.81	6.98±1.64	1.50±0.33	0.29±0.06	5.19±1.46
32	10.36±1.74	0.76±0.20	0.26±0.09	1.89±0.99	0.56±0.33	0.53±0.25	91.73±2.02	0.23±0.04	4.59±0.78	1.46±0.33	0.35±0.07	2.78±1.03
67	20.30±1.37	0.47±0.24	0.14±0.01	0.60±0.10	0.13±0.04	0.14±0.01	97.32±0.39	0.21±0.05	1.48±0.46	0.29±0.03	0.08±0.01	1.11±0.45

The fish were exposed to ^{110m}Ag during 24 h followed by a 67-day depuration period as indicated in the table. Numbers are given as mean±1 S.E.M. and were based on 10 fish, except for data from day 67, which was based on four fish.

[a] Data from Wood et al. (2002).

Table 6
concentration (ng g^{-1} wet weight) of whole body ^{110m}Ag(I) and relative distribution (%) of ^{110m}Ag(I) among body compartments in European eel exposed to waterborne Ag(I) primarily as Ag^{+}

Depuration time (days)	Whole body [Ag] (ng g^{-1})	Gills[a] (%)	Blood (%)	Kidney (%)	Anterior intestine (%)	Mid and posterior intestine (%)	Liver (%)	Bile (%)	Carcass (%)	Muscle (%)	Skin (%)	Carcass (muscle and skin) (%)
0.12	9.93±2.63	52.74± 3.96	3.21± 0.62	2.02± 0.32	1.52±0.49	0.94±0.10	23.23± 3.48	0.02± 0.01	16.31± 1.65	–	–	–
1	8.16±0.68	52.41+ 3.65	3.16± 0.27	2.10± 0.11	1.30±0.45	0.83±0.10	26.93± 2.78	0.06± 0.03	13.20± 0.85	5.47± 0.56	0.49± 0.06	7.42±0.87
2	3.21±4.13	42.92± 8.43	2.46± 0.55	1.35± 0.34	0.66±0.15	0.87±0.20	25.91± 6.18	0.02± 0.01	25.81± 12.3	–	–	–
4	6.71±0.53	51.99± 5.00	3.00± 0.74	2.62± 0.49	1.26±0.31	1.01±0.18	21.73± 4.88	0.07+ 0.02	18.32± 3.55	10.44± 2.09	1.02± 17	6.87±3.74
8	5.37±0.63	36.29± 5.11	1.87± 0.24	2.10± 0.28	1.07±0.34	0.74±0.10	46.57± 4.35	0.11± 0.05	11.24± 1.49	–	–	–
15	5.58±0.61	24.95± 4.73	1.46± 0.19	4.24± 0.72	0.91±0.15	0.74±0.07	57.82± 0.22	0.22± 0.11	9.66± 0.71	8.22± 1.46	1.42± 0.25	0.02±1.63
32	4.00±0.34	17.49± 2.87	3.71± 1.58	7.62± 1.09	2.23±0.89	1.00±0.20	54.16± 4.82	0.27± 0.12	13.51± 2.08	7.68± 1.30	1.78± 0.31	4.05±1.49
67	4.34±0.55	6.11± 0.95	1.64± 0.48	4.67± 0.64	1.21±0.30	1.38±0.32	71.78± 4.81	0.35± 0.12	12.85± 4.01	14.23+ 1.67	0.27± 0.06	1.64±3.90

The fish were exposed to ^{110m}Ag during 24 h followed by a 67-day depuration period as indicated in the table. Numbers are given as mean±1 S.E.M. and were based on 10 fish.

[a] Data from Wood et al. (2002).

Table 7
Concentration (ng g^{-1} wet weight) of whole body ^{110m}Ag(I) and relative distribution (%) of ^{110m}Ag(I) among body compartments in European eel exposed to waterborne Ag(I) primarily as $AgCl_{aq}$

Depuration time (days)	Whole body [Ag] (ng g^{-1})	Gills[a] (%)	Blood (%)	Kidney (%)	Anterior intestine (%)	Mid and posterior intestine (%)	Liver (%)	Bile (%)	Carcass (%)	Muscle (%)	Skin (%)	Carcass (muscle and skin) (%)
0.12	8.12±1.46	52.75±4.15	6.31±1.87	1.94±0.19	1.03±0.31	3.51±2.09	18.45±4.20	0.02±0.01	15.99±3.70	–	–	–
1	9.82±1.60	55.86±1.81	2.24±0.27	1.70±0.35	1.18±0.54	0.85±0.14	24.96±1.60	0.02±0.004	13.18±1.03	4.58±0.51	0.44±0.04	8.15±0.86
2	7.58±1.37	57.52±4.69	4.08±0.62	1.88±0.27	1.84±0.46	1.38±0.17	12.98±5.39	0.04±0.01	20.28±2.66	–	–	–
4	5.28±0.84	53.32±7.44	5.75±1.35	3.48±0.83	1.08±0.21	1.64±0.33	12.96±5.54	0.12±0.07	21.64±5.85	10.97±2.58	1.48±0.45	9.18±3.26
8	5.39±0.35	34.64±4.40	3.44±0.65	1.95±0.41	0.76±0.32	0.80±0.16	46.33±6.68	0.05±0.02	12.02±2.41	–	–	–
15	3.70±0.44	20.92±3.56	3.98±0.89	2.78±0.54	0.65±0.14	1.09±0.21	52.32±6.04	0.14±0.05	18.09±4.40	7.28±1.81	2.02±0.48	8.79±2.26
32	3.89±0.37	14.84±2.47	4.50±0.52	4.25±0.35	1.78±0.37	1.88±0.25	47.70±4.21	0.33±0.09	24.67±3.47	13.70±2.40	2.70±0.47	8.26±1.67
67	5.14±0.49	6.60±1.99	1.88±0.44	1.69±0.26	0.56±0.16	0.86±0.14	77.43±4.88	0.28±0.09	10.66±3.47	13.53±3.84	0.36±0.16	3.22±1.56

The fish were exposed to ^{110m}Ag during 24 h followed by a 67-day depuration period as indicated in the table. Numbers are given as mean±1 S.E.M. and were based on 10 fish.

[a] Data from Wood et al. (2002).

two-way ANOVA). The overall influence of high water chloride on ^{110m}Ag(I) kinetics in the mid and posterior portions of the intestine was a speeding up of ^{110m}Ag(I) elimination (Table 3). Specifically, adding Cl^- to the water seemed to increase the REF and substantially reduce the $t_{1/2}$ times for both the REF and the SEF. This effect was reflected in the distribution of ^{110m}Ag(I) in mid and posterior intestine expressed in percentage of the total body burden of ^{110m}Ag(I) (Tables 6 and 7). Three hours after cessation of ^{110m}Ag(I) exposure, eels exposed to $AgCl_{aq}$ had a three times higher proportion of the whole body ^{110m}Ag(I) burden in the mid and posterior intestine than eels exposed to ^{110m}Ag(I) predominantly as Ag^+. In contrast, after the 67-day depuration period there was no longer any difference between the groups in relative ^{110m}Ag(I) content of the mid and posterior intestine.

3.4. Comparisons between rainbow trout and European eel

There were pronounced differences in ^{110m}Ag(I) pharmacokinetics between the silver-sensitive rainbow trout and the silver-tolerant eel. In spite of trout having higher concentrations of ^{110m}Ag(I) in several body compartments than eel early in the experiment, there was no significant overall difference in whole body ^{110m}Ag(I) loads between trout and eel throughout the 67-day depuration period. This was partly due to the fact that gills of both species had accumulated high amounts of ^{110m}Ag(I), which in eel, but not in trout, eliminated slowly (Wood et al., 2002). While the REF $t_{1/2}$ in various body compartments were similar for the two species, there was a tendency for the SEF of ^{110m}Ag(I) in most tissues of eel to have much longer $t_{1/2}$ than those of rainbow trout (Tables 2 and 3). In some cases (i.e. mid and posterior intestine, blood plasma, and carcass of eel exposed to ^{110m}Ag(I) as $AgCl_{aq}$), $t_{1/2}$ was too slow to be determined and the computer model indicated that there was no elimination of ^{110m}Ag(I) from the SEF of these body compartments. By comparing all measurable $t_{1/2}$ for the SEF of trout and eel, it can be shown that, in general, the SEF did eliminate more slowly in eel than in trout ($P <$ 0.01). The generally longer $t_{1/2}$ of the SEF in eel than in trout resulted in ^{110m}Ag(I) concentrations on day 67 for all body compartments except for liver to be higher than or no different from those in trout (Figs. 1–4), This further contributed to the lack of overall statistical difference in whole body ^{110m}Ag(I) concentration between the two species (Tables 4–7). In spite of the generally longer $t_{1/2}$ of the SEF in eel than in trout, there was no overall elimination of ^{110m}Ag(I) from whole body in trout while the eel lost 37–56% of the accumulated ^{110m}Ag(I) during the course of the 67-day experiment (Tables 4–7). On a net basis, this was mainly due to loss of ^{110m}Ag from the gills of eels (Tables 6 and 7).

Overall, differences in ^{110m}Ag(I) concentrations between rainbow trout and eel were statistically significant for mid and posterior intestine (Fig. 2B), kidney (Fig. 3A), liver (Fig. 4A), bile (Fig. 4B), muscle (data not shown), and skin (data not shown). Mid and posterior intestine, kidney, and carcass showed similar patterns with eel initially having much lower ^{110m}Ag(I) concentration than trout, with little difference between species later in the experiment. As described above, this could be explained for mid and posterior intestine and carcass by the much longer $t_{1/2}$ for the SEF in eel than in trout (Tables 2 and 3). In kidney of eel there was no statistically significant elimination of ^{110m}Ag(I) whereas in rainbow trout, ^{110m}Ag(I) was lost very rapidly from the REF of the kidney ($t_{1/2} = 0.3$–1.2 days; Table 2). In extra-branchial tissues, the most profound differences between rainbow trout and eel in internal distribution of ^{110m}Ag(I) were the levels of ^{110m}Ag(I) in liver and bile (Fig. 4). Hepatic ^{110m}Ag(I) concentrations were one order of magnitude higher in trout, than in eel (Fig. 4A). Furthermore, they continued to increase with time in trout whereas, statistically, they remained the same in eel throughout the 67 days of post-exposure. The differences in ^{110m}Ag(I) levels of the bile between the species were even more marked (Fig. 4B). The ^{110m}Ag(I) concentration of bile in trout was very high at the 3-h sampling point, but decreased over time to reach a relatively constant low level after 15–30 days of the depuration period. This occurred despite of the fact that hepatic ^{110m}Ag(I) increased concomi-

tantly. In contrast, the eel had very low levels of ^{110m}Ag(I) in the bile at the first sampling 3 h post-exposure, but as the depuration period progressed there was a slight increase in bilary ^{110m}Ag(I) levels.

4. Discussion

4.1. Effects of water Ag(I) speciation on pharmacokinetics of ^{110m}Ag(I)

In the present study we demonstrate that the speciation of ^{110m}Ag(I) in the water column is consequential to the pattern of ^{110m}Ag(I) distribution in the body of rainbow trout and European eel. The effects of water ^{110m}Ag(I) speciation was clear in rainbow trout, in which 6 out of the 10 body compartments analysed for the present study showed statistically significant influence of water [Cl^-] on tissue ^{110m}Ag(I) concentrations. We have previously shown that the water [Cl^-] concentration also has profound effects on loading and depuration of ^{110m}Ag(I) in the gills of both trout and eel (Wood et al., 2002). After 24 h of exposure to ^{110m}Ag(I), the concentration of ^{110m}Ag(I) in gill tissue was three to five times higher in fish exposed in water with high [Cl^-] as compared with those exposed to ^{110m}Ag(I) in low [Cl^-] water (Wood et al., 2002).

In the present study, the effects of shifting the speciation from Ag^+ to $AgCl_{aq}$ on extra-branchial ^{110m}Ag(I) pharmacokinetics was complex and varied between species and tissues. In rainbow trout, arguably the most notable differences were a quicker elimination of ^{110m}Ag(I) from kidney and a greater accumulation of ^{110m}Ag(I) in liver of $AgCl_{aq}$-exposed fish as compared with those exposed to Ag^+. In eel, only kidney and mid and posterior intestine showed ^{110m}Ag(I) concentrations that were significantly different between the two exposure conditions. Overall, there were relatively small changes in ^{110m}Ag(I) concentrations in extra-branchial tissues of eel over time; with the consequence that tissue-specific elimination/accumulation of ^{110m}Ag(I) did not fit well to mathematical models. Nevertheless, the general tendency was for $AgCl_{aq}$ exposure to result in more rapid elimination of ^{110m}Ag(I) from tissues than after exposure to Ag^+. The main point, however, is that in both fish species the pharmacokinetics of ^{110m}Ag(I) depuration was influenced by the original speciation of ^{110m}Ag(I) in the water column.

The above findings are remarkable considering that the stability of the $AgCl_{aq}$ complex ($\log K = 3.3$; Mineql+ 4.01 database) is much less than those between silver and gill surface ($\log K = 10.0$; Janes and Playle, 1995) or silver and intracellular ligands, such as glutathione ($\log K = 12.3$; Adams and Kramer, 1998) and metallothionein ($\log K \sim 18$; Kagi and Kojima, 1987). Thus, once inside the gills, former speciation of ^{110m}Ag(I) in the water should be totally irrelevant and ^{110m}Ag(I) should bind to available reduced sulphur, S(II-), sites (Luoma et al., 2002). Indeed, subcellular fractionation of gills from rainbow trout used in the present study showed that 65–95% of the ^{110m}Ag(I) bound to the gills sedimented together with nuclei and mucus material and there was no difference in subcellular distribution of ^{110m}Ag(I) in gills of fish from either water quality (Wood et al., 2002).

The most reasonable explanation for the observed differences in pharmacokinetics of Ag(I), related to Ag(I) speciation in the water, is that Ag^+ and $AgCl_{aq}$ enter the gill epithelium through different routes. Bury and Wood (1999) showed that the apical entry of Ag^+ is via a phenamil-inhibitable, H^+-ATPase dependent pathway, suggestive of a Na^+ channel-like transporter. Thus, the uptake of Ag^+ is relatively specific and will largely be limited to epithelial cells expressing this Na^+ channel-like transporter. The mitochondria-rich chloride cells would be likely candidates for such activity because these are dedicated ion-transporting cells. The observed speciation-specific patterns of Ag(I) pharmacokinetics could be explained if $AgCl_{aq}$ were to enter non-specifically across the whole surface of the gill and end up more or less evenly distributed across the epithelium. This hypothesis is supported by the fact that $AgCl_{aq}$ is many orders of magnitude less toxic to rainbow trout than is Ag^+ (Hogstrand et al., 1996), indicating that Ag(I) accumulated from

$AgCl_{aq}$ is not concentrated at the site of toxicity, the ion-transporting cells.

The effects of water [Cl^-] on extra-branchial pharmacokinetics of ^{110m}Ag(I) may partly be a consequence of the associated differences in ^{110m}Ag(I) depuration from gill tissue (Wood et al., 2002). This, in turn, would be explained by a possible uneven distribution of basolateral Ag(I) transporters (Bury et al., 1999c) between gill cell types, such that Ag(I) accumulated in ion transporting cells is removed from the gills more quickly than from other gills cells. Furthermore, the molecular exit site of Ag(I) from the gills could perhaps influence Ag(I) speciation in the blood plasma. For example, the basolateral ferrous iron transporter, IREG1, is believed to deliver Fe^{2+} directly to ceuroloplasmin (Donovan et al., 2000; McKie et al., 2000). We propose that the basolateral Ag-dependent ATPase (Bury et al., 1999c) may be located primarily in ion transporting cells and that this enzyme donates Ag(I) relatively specifically to a plasma protein. Further, movement of Ag(I) across the basolateral membrane of other gill cells may be less specific in nature and in terms of transport sites and delivery of Ag(I) to the extracellular space. Such a difference in speciation of Ag(I) in the plasma could, then, influence distribution between internal tissues. Thus, at least three factors: cellular site of uptake, rate of depuration from the gills, and speciation in blood plasma could play roles in changing Ag(I) pharmacokinetics as a function of original speciation of Ag(I) in the water column. Without molecular evidence, any model attempting to explain the demonstrated differences in Ag(I) pharmacokinetics following exposure to Ag(I) mainly as Ag^+ or $AgCl_{aq}$ remains speculative. However, differences in uptake pathways for Ag(I) and subsequent speciation in blood plasma would be consistent with the patterns observed in the present study and by Wood et al. (2002).

4.2. Comparison between species

The European eel is three times more tolerant than rainbow trout to Ag^+ in the low [Cl^-] condition used in the present study (Grosell et al., 2000). The eel also handled Ag(I) very differently from rainbow trout (Wood et al., 2002; present study). Over the 67-day depuration period there was no loss of ^{110m}Ag(I) from rainbow trout while eels lost 50% of the accumulated ^{110m}Ag(I). Gradual loss of ^{110m}Ag(I) from the gills that did not appear elsewhere in the body accounted for most of this elimination (Wood et al., 2002). The apparent lack of elimination of whole body ^{110m}Ag(I) in trout confirms and extends data from a previous study in which no loss of ^{110m}Ag(I) was observed during three weeks following exposure to waterborne ^{110m}Ag(I) (Galvez et al., 2002). In a comparative experiment on retention of dietary ^{110m}Ag(I) between thornback ray (*Raja clavata*) and plaice (*Pleuronectes platessa*) it was found that the ray did not eliminate any ^{110m}Ag(I) over a 94-day period whereas less than 0.05% of the ingested ^{110m}Ag(I) remained in the body of plaice 46 days after the dose (Pentreath, 1977). Clearly, there are profound differences in whole body elimination time of Ag(I) between fish species, but these do not necessarily reflect 96-h LC50 of a certain species to Ag(I).

Pharmacokinetics specific to the various body compartments were also very different in rainbow trout and eel. At the first sampling point (3 h post exposure) ^{110m}Ag(I) concentrations in blood plasma, intestine (all sections), kidney, carcass, bile, and skin were much higher in rainbow trout than in eel, but after 67 days of depuration there were little or no differences in ^{110m}Ag(I) concentrations between species for these tissues. Thus, while there was initially less ^{110m}Ag(I) in many compartments of the eel, they eliminated ^{110m}Ag(I) more slowly than did the same compartments of the trout. The kinetic model for ^{110m}Ag elimination did not fit well to most data from eel, but for the data available, there were much longer SEF in eel than in trout (Table 2 versus Table 3). While ^{110m}Ag(I) in most tissue eliminated more slowly in eel than in trout, the fate of this eliminated ^{110m}Ag(I) again seemed to be different. In trout there was a successive accumulation of ^{110m}Ag(I) in the liver as it left the other tissues, whereas in eel the concentration of hepatic ^{110m}Ag(I) did not change from the beginning to the end of the depuration period. Thus, the ^{110m}Ag(I) that eliminated from various tissues of the eel (particularly

the gills) seemed to have contributed to the loss of total ^{110m}Ag(I) from the body. This is further supported by the slow, but steady increase of ^{110m}Ag(I) concentration and content in bile of eel, suggesting some hepato-biliary excretion. In the trout, ^{110m}Ag(l) did not leave the body but redistributed and accumulated in the liver. Interestingly, calculated excretion of ^{110m}Ag(I) via the bile over the 67-day depuration period, based on biliary ^{110m}Ag(I) content and assumed co-ordination between gall bladder emptying and feeding (Grosell et al., 2001), was higher in trout (10–21% of initial whole body ^{110m}Ag) than in eel (3–4%). This may suggest reabsorption of ^{110m}Ag(I) across the intestine of trout, because there was no concomitant reduction in whole body ^{110m}Ag(I) content. It should be noted that in the tissues most important for human consumption, muscle and skin, the ^{110m}Ag(I) concentrations were very low in both species and combined (muscle and skin) they never contributed to more than 14% of the total body burden (0.4–10% in trout; 5–14% in eel).

Although ^{110m}Ag(I) concentrations in several tissues were higher in trout than in eel and although only eel were able to eliminate some of the whole body ^{110m}Ag(I) during the experiment, these differences probably do not account for the differences between these species in acute sensitivity to Ag(I). Instead the difference in acute toxicity is likely reflected in the differences in Na^+ and Cl^- turnover between trout and eel (Grosell et al., 2000). Ag^+ causes acute damage to fish by potent and specific inhibition of Na^+/K^+-ATPase in the gills, thereby blocking Na^+ and in some species also Cl^- uptake from the water (Wood et al., 1996a; Morgan et al., 1997). This leads to net Na^+ and Cl^- losses, followed by a chain of events ending in cardiovascular collapse (Hogstrand and Wood, 1998). Ag(I) is equally potent in inhibiting branchial Na^+/K^+-ATPase in eel and trout, but because eel rely less than trout on uptake of Na^+ and not at all on Cl^- uptake across the gills, blockage of branhial Na^+/K^+-ATPase has less physiological impact on the Ag(I) tolerant eel (Grosell et al., 2000). However, the possible mechanisms of chronic toxicity are much less well understood (Wood et al., 1999, 2002; Luoma et al., 2002). Differences between species in ^{110m}Ag(I) handling as those described herein, may play a part in defining sensitivity to Ag(I) on a chronic basis.

4.3. General patterns of Ag(I) pharmacokinetics in fish

General patterns of pharmacokinetics of ^{110m}Ag(I) in trout and eel in the present study, agree reasonably well with what has previously been described for rainbow trout and other species of fish (Pentreath, 1977; Garnier et al., 1990; Galvez et al., 2002). ^{110m}Ag(I) eliminates very rapidly from the gills of rainbow trout and stabilises at constant low levels after a about a month at the conditions used in the present study (Wood et al., 2002) or in less than 3 days if the ^{110m}Ag(I) is chased with stable Ag(I) isotope (Galvez et al., 2002). Similar to the present study, the liver has been found to be the major accumulatory organ for Ag(I) in a range of fish species and typically accounts for more than 70% of the whole body Ag(I) burden within a week after start of Ag(I) exposure (Pentreath, 1977; Garnier et al., 1990; Hogstrand and Wood, 1998; Webb and Wood, 2000; Galvez et al., 2002). In all fish species investigated, the Ag(I) contents of skin and muscle are extremely low (Pentreath, 1977; Garnier et al., 1990; Hogstrand and Wood, 1998; Webb and Wood, 2000; Galvez et al., 2002).

Galvez et al. (2002) exposed rainbow trout for 2 days to waterborne ^{110m}Ag(I) and continued after this pulse of radiolabelled Ag(I) with exposure to stable Ag(I) for 3 weeks. In contrast to the present study, Galvez et al. (2002) found a large but transient accumulation of ^{110m}Ag(I) in the intestine, which at the most contributed to 35% of the total ^{110m}Ag(I) content of the fish. This can be compared with the present study in which maximally 6 and 4% of whole ^{110m}Ag(I) body burden that was contained in intestine of rainbow trout and eel, respectively. The reason for this discrepancy is not clear, but may relate to that the study by Galvez et al. (2002) involved 'chasing' ^{110m}Ag(I) with 'cold' Ag(I) whereas the present study did not. In both studies it is believed that ^{110m}Ag(I) initially entered across the gills because of the impossibly high drinking rates that would

have been required to account for the amount of ^{110m}Ag(I) that did accumulate, because of the very high Ag(I) loads initially found in gills during several studies (Coleman and Cearley, 1974; Garnier et al., 1990; Bury and Wood, 1999; Wood et al., 2002), and because of the discovery of efficient carrier mediated transport of Ag(I) across both apical and basolateral membranes of rainbow trout gill (Bury and Wood, 1999; Bury et al., 1999c).

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