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Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout 2: tissue specific metal accumulation

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Abstract

Tissue specific metal accumulations (gills, liver, kidney and whole body) in rainbow trout (*Oncorhynchus mykiss*) were compared during chronic exposure (up to 100 days) to sublethal levels of waterborne Cd (3 $\mu g \cdot l^{-1}$), Cu (75 $\mu g \cdot l^{-1}$) or Zn (250 $\mu g \cdot l^{-1}$) in moderately hard water (hardness of 140 $mg \cdot l^{-1}$, pH 8.0). A general pattern of tissue metal increase and stabilization was evident for all three metals, although the degree and time course of accumulation varied. The exception to this general pattern was a lack of Zn accumulation in the liver and kidney although small amounts did accumulate in the gills and whole body. Accumulation of Cu occurred primarily in the liver while for Cd the kidney was the major organ of accumulation. Exponential modeling was employed to compare and contrast the saturation concentration and time to half saturation of various tissues. Accumulation of essential metals (Cu and Zn), if it occurred, was rapid and increases were relatively low. For example the time to half saturation during Cu exposures was always less than 2 weeks and the maximum level of accumulation was less than four times background levels. For non-essential Cd, time to half saturation for the liver and kidney was always longer than 5 weeks and modeled saturation concentrations were up to 80-fold higher than background. The response to Cu and Zn suggested an active regulation of tissue burdens while that of Cd appears to be more passive, resulting in continuous metal accumulation over an extended time course. While the initial patterns of accumulation for each metal were generally consistent with the damage, repair and acclimation pattern from concurrent physiological measurements it was clear that tissue metal accumulation was not a good indicator of either exposure of physiological impact.

Keywords: Rainbow trout; Cadmium; Copper; Zinc; Accumulation; Modeling; Tissue burden; Physiology

1. Introduction

In the last few years there has been interest in developing predictive models for acute toxicity based on the short-term binding of waterborne metals to fish gills ('biotic ligand model'; Playle,

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1998). Recently, extension of this approach to predictive models for chronic toxicity based on longer term tissue-specific metal residues has been advised (Bergman and Dorward-King, 1997). Tissue specific accumulation of metal has been proposed as a key indicator of chronic exposure (Bergman and Dorward-King, 1997) and an understanding of the toxicokinetics of accumulation of a metal during chronic sublethal exposure is a critical element in establishing links between toxicity and exposure in risk assessment (McCarty and MacKay, 1993). Tissue specific kinetic modeling of metal accumulation has been described for metals such as Cd, Zn, Cr and Ni (Calamari et al., 1982; Wicklund Glynn, 1991; Thomann et al., 1997) and the overall process of pharmokinetic modeling in aquatic animals was reviewed by Barron et al. (1990).

Some progress has been made in understanding accumulation – response relationships on a short term basis in fish chronically exposed to sublethal metals (e.g. Alsop et al., 1999; Hollis et al., 1999; Taylor et al., 2000). However, much less is known about the pattern of metal accumulation over the longer term during chronic sublethal exposure, and its possible association with chronic physiological disturbances. The relationships among tissue metal burdens and overall toxic response during chronic exposure is complicated by the fact that metals accumulate differentially in tissues. An additional complication is that some metals, for example Cu and Zn, are essential minerals with cellular and tissue levels subject to metabolic regulation (Cousins, 1985; Vallee and Falchuk, 1993).

The general physiological response to chronic metal exposure follows the damage-repair model of McDonald and Wood (1993) with initial physiological disruption, particularly of ion regulation, followed by a recovery during sublethal exposure to Cd, Cu or Zn (see McGeer et al., 2000). The initial site of impact of waterborne metals in fresh water fish is the gill and accumulations of metal on or in the gill are assumed to be causative of the initial damage. A major feature of the recovery phase is the internal mobilization of metal binding proteins such as metallothionein (McCarter and Roch, 1983; Bradley et al., 1985; Hogstrand and Wood, 1996) which are assumed to act as detoxification and storage mechanisms. As a result, deleterious effects of long term accumulation of metal during chronic exposure may be minimal.

Our work describes the time course of accumulation of metals in target tissues (gills, liver, kidney) of rainbow trout during chronic sublethal exposures. Three different metals, Cu, Cd and Zn were individually assessed and then compared and contrasted. This work was done in combination with an examination of other physiological measures that are described in a companion paper (McGeer et al., 2000). We were particularly interested in whether the tissue specific metal accumulation could be described by simple exponential models (Wicklund Glynn, 1991) and whether this modeling was useful in terms of understanding the chronic effects of metals. Modeling was evaluated on its ability to describe the accumulation process and whether it improved our understanding of the physiological processes that occur during sublethal exposure. The levels of exposure for each metal were chosen to be environmentally relevant, to induce resistance to acute challenges and to cause few mortalities (see McGeer et al., 2000). Fish were exposed to either 3 $\mu g \cdot l^{-1}$ Cd, 75 $\mu g \cdot l^{-1}$ Cu or 250 $\mu g \cdot l^{-1}$ Zn (nominal concentrations) in moderately hard dechlorinated Hamilton tap water from Lake Ontario.

2. Methods

2.1. Exposures, series 1 and series 2

These exposures were performed on the same group of fish as in McGeer et al., (2000). Rainbow trout (1–2 g) were obtained from a local commercial supplier (Humber Springs, Orangeville, ON) and maintained in dechlorinated Hamilton tap water ('hard water'-ionic composition: Ca^{2+}, 1.0 mM; Mg^{2+}, 0.2 mM; Na^{+}, 0.6 mM; K^{+}, 0.2 mM; Cl^{-}, 0.7 mM; hardness, 140 $mg \cdot l^{-1}$ as $CaCO_3$ and pH 8) for at least 1 month prior to experimentation. Trout were exposed to waterborne treatments of 3 $\mu g \cdot l^{-1}$ Cd (as $Cd(NO_3)_2 \cdot 4H_2O$) or 75 $\mu g \cdot l^{-1}$ Cu (as $Cu(SO_4) \cdot 5H_2O$) or 250 $\mu g \cdot l^{-1}$ Zn (as

$Zn(SO_4)_2 \cdot 7H_2O$) along with a control group, similarly treated but not exposed to metal. Measured concentrations of metals in untreated water were 0.057 ± 0.005 μg $Cd \cdot l^{-1}$ (mean $\pm$ 1 S.E.M., $n = 34$), 1.6 ± 0.3 μg $Cu \cdot l^{-1}$ ($n = 35$) and 2.4 ± 0.5 μg $Zn \cdot l^{-1}$ ($n = 11$).

Two series of exposures were conducted. In series 1, fish were exposed to Cd, Cu or Zn for 65 days on a flow through basis. Note that for series 1 the Zn exposure ended on day 13 as a result of a mechanical failure. In series 2 the time course of metal loading into tissues was further explored and trout were exposed to Cu or Cd for 100 days. Exposures are described in detail by McGeer et al., (2000) and therefore only in brief here. Feeding regime, temperature, duration, initial fish weight and measured water Cu, Cd or Zn concentrations for series 1 and 2 are shown in Table 1.

2.2. Tissue sampling

At various times during exposure, trout ($n \geq 6$) were non-selectively netted from each exposure tank (plus controls), anaesthetized (200 $mg \cdot l^{-1}$ buffered MS222) and killed. Gill, liver and kidney were then removed, weighed and saved, as was the remaining carcass. Gills were vigorously rinsed in deionized water and blotted dry immediately after removal. In series 1, sampling was at 1, $2\frac{1}{2}$, 4, $5\frac{1}{2}$, 10, 16 and 65 days of exposure. During series 2, fish were sampled on days 0, 1, 3, 7, 10, 14, 20, 30, 52, 70, 80 and 100.

Table 1
Description of exposure conditions for chronic sublethal rainbow trout in series 1 and 2[a].

	Exposure details	
	Series 1	Series 2
Duration (days)	65	100
Temperature (°C)	14 ± 1	17 ± 1.5
Total n [n/tank]	600 [150]	900 [300]
Initial weight (g)	3.2 ± 0.1 (600)	3.3 ± 0.1 (50)
Ration (biomass/d)	Satiation and 4%	1.5%
Cu conc. ($\mu g \cdot l^{-1}$)	73.7 ± 2.1 (12)	75.8 ± 1.8 (23)
Cd conc. ($\mu g \cdot l^{-1}$)	2.91 ± 0.10 (12)	3.07 ± 0.19 (21)
Zn conc. ($\mu g \cdot l^{-1}$)	234 ± 5.0 (5)	None

[a] Measured values are presented as mean $\pm$ S.E.M. (n) except for temperature where mean $\pm$ range are shown. Full details are provided in McGeer et al., (2000).

2.3. Tissue sample analysis

Gill, liver and kidney tissue samples as well as remaining carcass were digested in approx. 5 vol. of 1 N HNO_3 (Trace Metals Grade, Fisher Sci., Nepean ON: Janes and Playle, 1995) and measured for total metal concentration. Cd, Cu and Zn content of water as well as Cu and Cd content of digested tissues and carcass were measured by graphite furnace atomic absorption spectrophotometry (AA1275 and GTA-95, Varian, Austr.). Remaining carcass Zn content was measured by atomic absorption spectrophotometry (AA1275 Varian).

2.4. Calculations and statistics

Metal burden was expressed as $\mu g \cdot g^{-1}$ wet weight and calculated using measured concentrations and tissue weights, with whole body values representing the sum of measured tissue and carcass contents. To described the time-dependent pattern of Cd, Cu or Zn metal accumulation in gill, liver and kidney, metal accumulation was modeled (Barron et al., 1990; Wicklund Glynn, 1991) using a simple equation which describes an exponential rise to a maximum. The equation yields variables that describe the maximum concentration and the time to 50% of the maximum accumulation. The equation used was,

$$[f(x) = C_0 + C_s * (1 - \exp^{-(\ln 2/(t\frac{1}{2}) * x)})]$$

where '$f(x)$ is the metal content at day 'x', 'C_s' describes the maximum accumulation (saturation) above ground and '$t\frac{1}{2}$' is the time to half C_s. The background metal content of the tissue, 'C_0' was calculated as the pooled mean of all values for unexposed trout and was subtracted from exposure values before modeling. Therefore saturation concentration for each tissue was calculated as $C_0 + C_s$. Accumulation above background for metals in tissues were calculated by expressing the modeled saturation concentrations as the % increase above background ($100 * C_s/C_0$). Whole

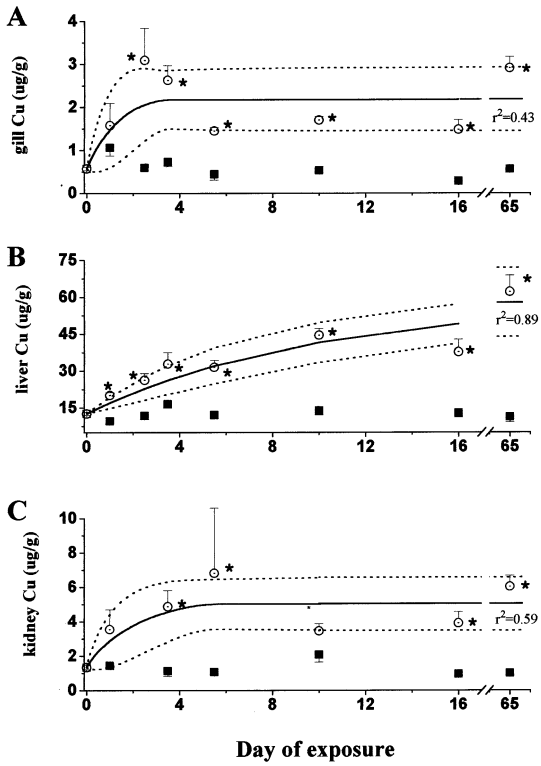

Fig. 1. Copper accumulation in (A) gills, (B) liver and (C) kidney of rainbow trout exposed to 75 $\mu g \cdot l^{-1}$ Cu (open circles) for up to 65 days (series 1). An unexposed control group is also shown (filled squares). Mean $\pm$ 1 S.E.M. ($N \geq 6$) and * indicates significant difference ($P < 0.05$) from the control mean at that time. Included on the graphs are the best fit lines from the exponential model (solid line, with r^2 value) and associated 95% confidence interval (dotted lines).

body bioconcentration factor (BCF) was also calculated, for control and exposed fish. BCF calculation was done by dividing modeled saturation concentrations or mean background concentrations ($\mu g \cdot kg^{-1}$, for exposed or control fish respectively) by the waterborne concentration ($\mu g \cdot l^{-1}$). Modeling of data was accomplished with the software package SigmaPlot (SPSS Inc., Chicago, IL).

Metal tissue burdens were compared to the corresponding control value at each sampling time by Student's *t*-test. Pre-exposure metal burden data were not collected in series 1 exposures, so a pooled mean of all control values has been used to represent the Day 0 level. In all cases, $\alpha \leq 0.05$ was the accepted level of significance, and Duncan's New Multiple Range Test was used for multiple comparisons.

3. Results

3.1. Series 1

All rainbow trout exposed to Cd or Cu accumulated significant amounts of their respective metal in all measured tissues (Figs. 1 and 2, Table 2). Fish exposed to Cu showed a rapid loading of Cu into the gills, followed by a partial clearance, then stabilization of gill Cu content (Fig. 1A). Note however that gill Cu was again elevated at day 65, suggesting a biphasic pattern. In the kidney (Fig. 1C), liver (Fig. 1B) and whole body (Table 2), levels increased and then more or less stabilized. Whole body Cu subsequently declined towards control levels by day 65 (Table 2).

Cd exposure resulted in significant and rapid accumulation, then stabilization in the gill (Fig. 2A) with slower progressive buildup in liver (Fig. 2B) and kidney (Fig. 2C), patterns which were approximately linear over time up to day 16. Whole body Cd burden increased and then remained relatively constant over the 65th day of exposure (Table 2). The major organ of accumulation for Cu was the liver (Fig. 1B) while for Cd, it was the kidney (Fig. 2C).

Tissue accumulations of Zn were less dramatic than for the other two metals, becoming significant only at day 10 for gills (Fig. 3A) and liver (Fig. 3B). These increases were small relative to the high background levels of this essential metal already present in the tissues. Interestingly, the increased whole body Zn levels (Table 2) at day 1 were accompanied by decreased Zn levels in the various tissues measured, indicating significant accumulation in another tissue(s).

3.2. Series 2

Trout exposed to 3 Cd or 75 $\mu g \cdot l^{-1}$ Cu in series 2 showed similar tissue-specific accumulations as were observed in the series 1 experiments. Cu exposure resulted in significant accumulation of metal in the gill and liver (Fig. 4A and B). Again,

there was evidence of a biphasic pattern in the gill with branchial Cu peaking on day 7, falling on days 10 and 14, then rising again thereafter. Kidney (Fig. 4C) and whole body (Table 3) Cu accumulations were variable and increases were not always significant relative to controls. For Cd-exposed fish, the gill Cd concentration increased to a constant level, and the kidney was again the major organ of accumulation (Fig. 5A and C). Accumulations of Cd in the liver and kidney (Fig. 5B and C) were significantly greater than controls after the first few days of exposure, and as in series 1, the accumulation patterns were more or less linear with time. Whole body Cd content also increased significantly (Table 3).

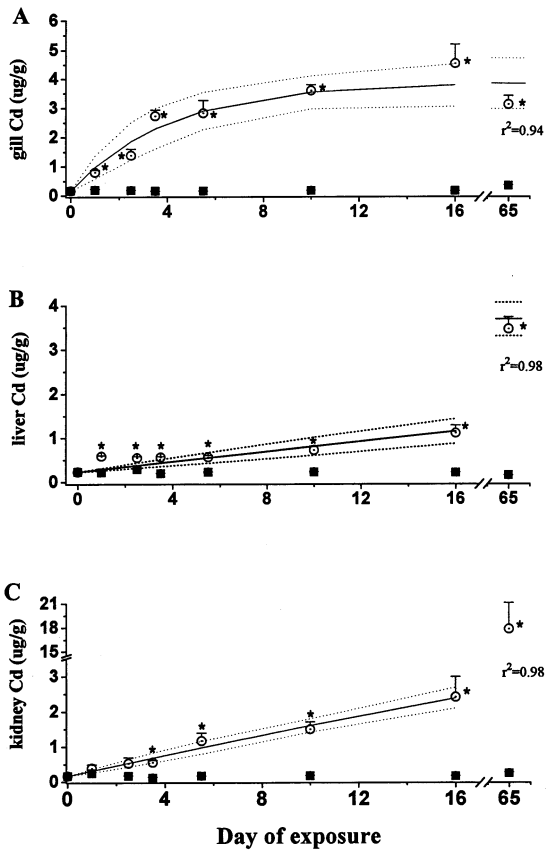

Fig. 2. Cadmium accumulation in (A) gills, (B) liver and (C) kidney of rainbow trout exposed to 3 $\mu g \cdot l^{-1}$ Cd (open circles) for up to 65 days (series 1). An unexposed control group is also shown (filled squares). Mean ± 1 S.E.M. ($N \geq 6$) and * indicates significant difference ($P < 0.05$) from the control mean at that time. Included on the graphs are the best fit lines from the exponential model (solid line, with r^2 value) and associated 95% confidence interval (dotted lines). Note that for kidney at day 65, the confidence interval is not shown due to the y-axis break, but ranged from 12.4–14.6 $\mu g\ Cd \cdot g^{-1}$ wet weight of kidney.

3.3. Modeling metal accumulation

When Cd, Cu and Zn tissue accumulation data for series 1 and 2 were each fitted to a simple exponential model the output matched reasonably well with the observed responses (see fitted lines with 95% CI in Figs. 1–5). In general, the accumulation factor in the tissues of exposed fish was highest for Cd, intermediate for Cu and low for Zn (Table 4).

Time course modeling for tissue Cu accumulation in trout exposed to 75 $\mu g \cdot l^{-1}$ Cu showed similar saturation concentrations for series 1 and 2 (Table 4). The $t_{\frac{1}{2}}$ to Cu saturation was also similar across the two exposure series except for gills where it was longer in series 2 (Table 4). Calculated accumulation factors showed that all tissues tended to be approximately the same (except for the kidney in series 2) but clearly the vast majority of Cu was retained in the liver (Table 4, Figs. 1 and 4). BCF for all three metals declined as a result of exposure (Table 5).

A comparison of modeling results for gill Cd accumulation for series 1 versus series 2 shows saturation concentrations were similar but $t_{\frac{1}{2}}$ was longer for the series 2 exposure (Table 4) for the gills. Predicted saturation concentrations for kidney Cd were in agreement but those for liver Cd were not. In both exposures, the $t_{\frac{1}{2}}$ values for liver and kidney Cd accumulation were much longer than the $t_{\frac{1}{2}}$ for gills, reflecting the more linear nature of the uptake curve for the former two tissues. In general, predictions based on the data from series 2 were better with smaller S.E.M. and higher r^2 values (Table 4, Figs. 1, 2, 4 and 5), thus reflecting the greater number of data points, especially at later times, in series 2.

Modeling Zn accumulation in trout exposed to 250 $\mu g \cdot l^{-1}$ Zn illustrated that accumulation oc-

Table 2
Whole body Cd, Cu or Zn content (μg g^{-1} wet weight) of rainbow trout of series 1 exposed to either 3 μg·l^{-1} Cd or 75 μg·l^{-1} Cu or 250 μg·l^{-1} Zn or control conditions (unexposed)[a]

Day	Whole body Cd content		Whole body Cu content		Whole body Zn content	
	Unexposed control	Cd exposed (3 μg l^{-1})	Unexposed control	Cu exposed (75 μg l^{-1})	Unexposed control	Zn exposed (250 μg l^{-1})
0	0.21 ± 0.006		1.15 ± 0.06		27.5 ± 0.6	
1	0.22 ± 0.047	0.57 ± 0.035*	0.93 ± 0.22	2.71 ± 0.58*	26.4 ± 1.9	37.4 ± 2.9*
2.5	0.20 ± 0.006	0.57 ± 0.022*	1.61 ± 0.10	2.40 ± 0.32*	30.1 ± 0.8	31.6 ± 2.8
3.5	0.21 ± 0.002	0.54 ± 0.005*	1.58 ± 0.05	2.78 ± 0.11*	26.4 ± 1.4	35.5 ± 1.6*
5.5	0.22 ± 0.004	0.55 ± 0.025*	1.00 ± 0.04	2.16 ± 0.21*	28.4 ± 1.3	33.0 ± 1.3*
10	0.21 ± 0.004	0.68 ± 0.013*	1.13 ± 0.05	2.23 ± 0.14*	25.3 ± 1.3	32.5 ± 0.8*
16	0.21 ± 0.004	0.69 ± 0.008*	0.92 ± 0.06	1.86 ± 0.10*		
65	0.31 ± 0.026	0.69 ± 0.071*	0.82 ± 0.19	1.19 ± 0.18		

[a] Mean ± S.E.M. are shown, *n* is at least 6.
* Indicates a significant difference from the control value on that day.

curred in the gills (Fig. 3) with saturation occurring at about 34% above background levels (Table 4). Fitting the liver and kidney Zn data to the exponential model proved impossible because significant accumulations did not occur (Fig. 3B and C).

4. Discussion

All fish exposed to either Cd or Cu experienced significant accumulations of metal in all measured tissues with a pattern of metal loading onto the gill accompanied by distribution through to the liver and kidney. Half-times for gill loading were generally lower than for internal tissues. Trout exposed to 250 μg·l^{-1} Zn also accumulated Zn but to a lower degree and only in the gills and whole body (Fig. 3, Table 2). As illustrated in McGeer et al., (2000), the physiological effects of these chronic sublethal exposures to Cu, Cd or Zn followed a pattern of damage, recovery and acclimation. For Cd-exposed fish, the initial damage phase (reduced appetite and blood ion loss) was

Table 3
Whole body Cd or Cu content (μg g^{-1} wet weight) of rainbow trout of series 2 exposed to either 3 μg·l^{-1} Cd or 75 μg·l^{-1} Cu or control conditions (unexposed)[a]

Day	Whole body Cd content		Whole body Cu content	
	Unexposed control	Cd exposed (3 μg l^{-1})	Unexposed Control	Cu exposed (75 μg l^{-1})
0	0.34 ± 0.07	—	2.27 ± 0.12	—
1	0.31 ± 0.01	0.42 ± 0.01*	1.86 ± 0.14	2.24 ± 0.30
3	0.29 ± 0.01	0.40 ± 0.01*	1.77 ± 0.09	2.59 ± 0.36
7	0.35 ± 0.01	0.76 ± 0.03*	2.19 ± 0.22	3.11 ± 0.26*
10	0.31 ± 0.01	0.73 ± 0.06*	1.98 ± 0.11	2.98 ± 0.16*
14	0.31 ± 0.02	0.57 ± 0.01*	1.71 ± 0.21	3.02 ± 0.56
20	0.27 ± 0.02	0.59 ± 0.03*	1.87 ± 0.09	2.46 ± 0.27
52	0.33 ± 0.01	0.73 ± 0.07*	0.98 ± 0.08	2.35 ± 0.49*
70	0.17 ± 0.01	1.12 ± 0.13*	0.95 ± 0.25	1.87 ± 0.19*
100	0.18 ± 0.01	0.95 ± 0.02*	0.94 ± 0.07	2.37 ± 0.34*

[a] Mean ± S.E.M. are shown, *n* is at least 5.
* Indicates a significant difference from the control value on that day.

associated with metal accumulation, particularly in the gill (Figs. 2 and 5). For trout exposed to Cu, the time course of metal accumulation (Figs. 1 and 3) also corresponded with the disruption in ionic regulation (see Figs. 2 and 4 of McGeer et al., 2000). Ion loss was also evident in trout exposed to Zn (see Fig. 2 of McGeer et al., 2000) but the association with metal accumulation in the gills was less clear (Fig. 3).

Subsequent to the accumulation during the initial damage phase there was a stabilization of tissue metal levels (Figs. 1–4 and 5, Table 2 and Fig. 3), relatively quickly for Cu and Zn, but much more slowly for Cd exposed fish. The relatively rapid stabilization of Cu accumulation profiles (Figs. 1 and 4; Table 4) was associated with a similar pattern of short-lived ion loss and recovery to pre-exposure levels during the same exposures (see Fig. 2 and Fig. 4 of McGeer et al., 2000). In comparison, the time course of Cd loading into tissues was relatively lengthy (Fig. 2 and Fig. 5) as was the re-establishment of ionic

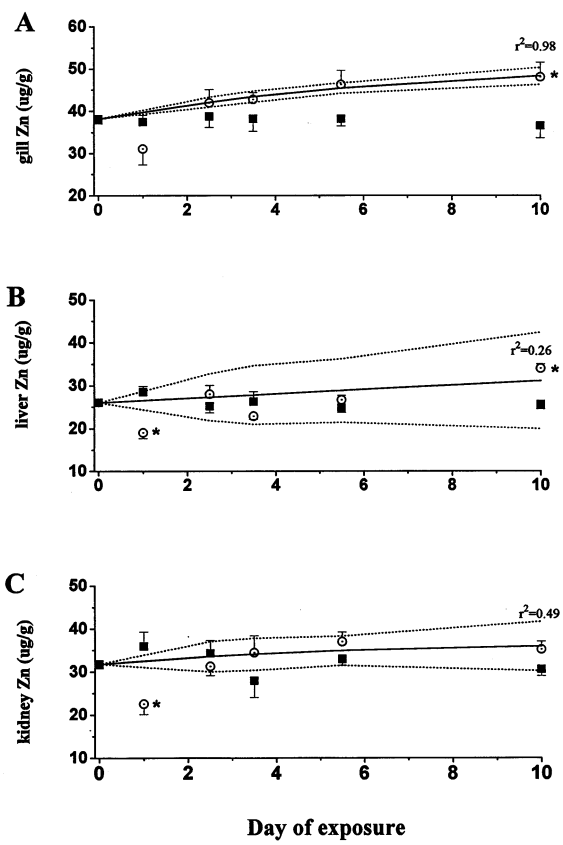

Fig. 3. Zinc content in (A) gills, (B) liver and (C) kidney of rainbow trout exposed to 250 $\mu g \cdot l^{-1}$ Zn (open circles) for up to 10 days (series 1). An unexposed control group is also shown (filled squares). Mean $\pm$ 1 S.E.M. ($N \geq 6$) and * indicates significant difference ($P < 0.05$) from the control mean at that time. Included for gill Zn is the best fit line from the exponential model (solid line, with r^2 value) and associated 95% confidence interval (dotted lines) and parameters are also given for kidney and liver Zn even though no significant accumulation occurred.

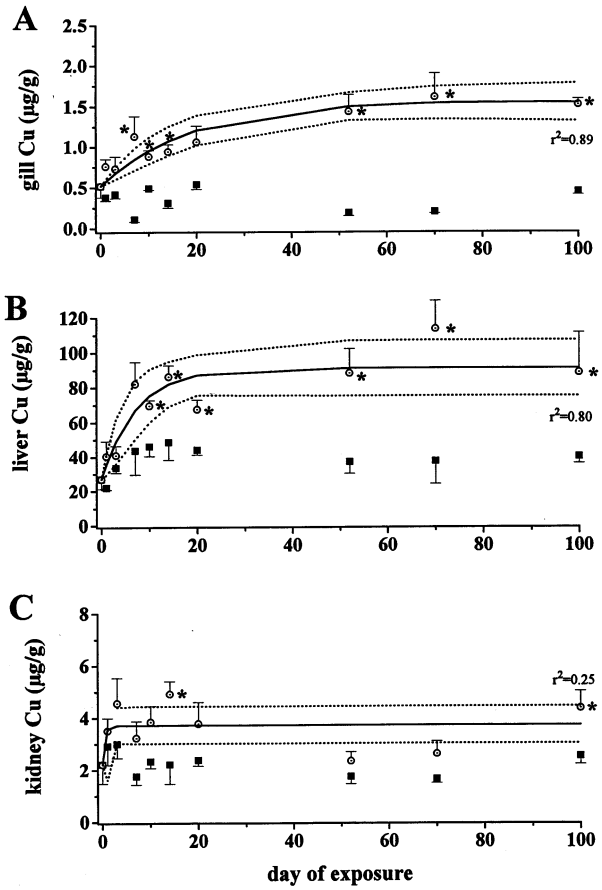

Fig. 4. Copper accumulation in (A) gills, (B) liver and (C) kidney of rainbow trout exposed to 75 $\mu g \cdot l^{-1}$ Cu (open circles) for up to 100 days (series 2). An unexposed control group is also shown (filled squares). Mean $\pm$ 1 S.E.M. ($N \geq 5$) and * indicates significant difference ($P < 0.05$) from the control mean at that time. Included on the graphs are the best fit lines from the exponential model (solid line, with r^2 value) and associated 95% confidence interval (dotted lines).

balance, particularly blood Ca^{2+} (see Fig. 2C and Fig. 4B of McGeer et al., 2000). Therefore, the initial physiological disruption and subsequent recovery during sublethal Cu and Cd exposure was generally linked with the time course of loading of metal onto the gills and subsequent stabilization of tissue burdens. While an association between metal accumulation and physiological effect has been demonstrated by this study in combination with McGeer et al., (2000) a full understanding of the mechanistic links among these responses will require further investigation.

The relatively rapid saturation of all tissues in trout exposed to waterborne Cu (short $t_{\frac{1}{2}}$ and low accumulation factor, Table 4) may reflect the essential nature of Cu and pre-existing mechanisms for uptake, regulation and elimination of Cu. The ability of trout to respond rapidly to Cu and re-establish physiological control (homeostasis) has been previous shown (e.g. Laurén and McDonald, 1987a,b). Similar patterns of tissue specific Cu accumulation have been observed in previous chronic Cu exposure studies (Dixon and Sprague, 1981; Laurén and McDonald, 1987a,b; Grosell et al., 1997, 1998). As well, the biphasic profile of Cu loading into the gill with increases during the initial days of exposure followed by subsequent declines and stabilization at levels above controls has been shown by Laurén and McDonald (1987b) and Grosell et al. (1997). This biphasic pattern of gill Cu accumulation suggests an active regulation of gill Cu content (Grosell et al., 1997, 1998) and is not the pattern expressed in the exponential model. By its nature, the nonlinear model assumed an exponential rise to a maximum and therefore may not capture more complex patterns of accumulation that involve physiological responses directed at regulation of tissue metal levels, such as elimination. Active regulation of internal Cu levels in response to sublethal waterborne Cu, specifically an up-regulation of hepatic turnover and enhanced elimination of Cu via the bile has been demonstrated by Grosell et al. (1997, 1998). It is possible that physiological mechanisms related to Cu metabolism may have contributed to the metabolic 'costs' associated with Cu acclimation as illustrated by reduced Ucrit and increased MO_2 (see McGeer et al., (2000)).

The Zn data (Fig. 4), though limited, indicate that this essential metal is also homeostatically regulated (Alsop et al., 1999), perhaps to a even more precise level than Cu. The mechanisms of Zn regulation appear different, with only slight buildup on the gills and negligible internal accumulation (Spry et al., 1988; Alsop et al., 1999), which likely reflects the reduced branchial Zn uptake rate identified by Hogstrand et al. (1995, 1998) as an important part of the acclimation mechanism.

In contrast to Cu, the pattern of response to Cd was very different with the gills clearly providing a barrier to internal uptake, (Figs. 2 and 5, Table 4). Elevated long term liver and kidney accumulation of Cd agrees with other studies (Roberts et al., 1979; Calamari et al., 1982; Giles, 1988; Hollis et al., 1999) and is suggestive of a process that is not metabolically regulated.

The ability of trout gills to absorb and store large amounts of Cd in a short time may be important in reducing the amount available for transfer into the blood and then to other organs, thus explaining the extended $t_{\frac{1}{2}}$ for Cd accumulation in the liver and kidney (greater than 5 weeks; Table 4). Long times to establishment of modeled steady state Cd levels in the kidney and liver were also noted by Calamari et al. (1982). Other than gill storage and possibly gill Cd elimination, there does not appear to be a mechanism for eliminating internal concentrations of Cd as Wicklund Glynn (1991) demonstrated that depuration of liver and kidney Cd was extremely slow. Even if elimination pathways via the kidney or liver are enhanced in the trout during our chronic exposures, clearly they would not be enough to regulate internal Cd levels. Accumulation $t_{\frac{1}{2}}$'s were over 5 weeks for liver and kidney and final predicted accumulation factors for Cd were very high (1000–8000%, Table 4). Therefore, the physiological response to Cd appears to be somewhat more passive, involving storage, particularly in the kidney, rather than active regulation and elimination.

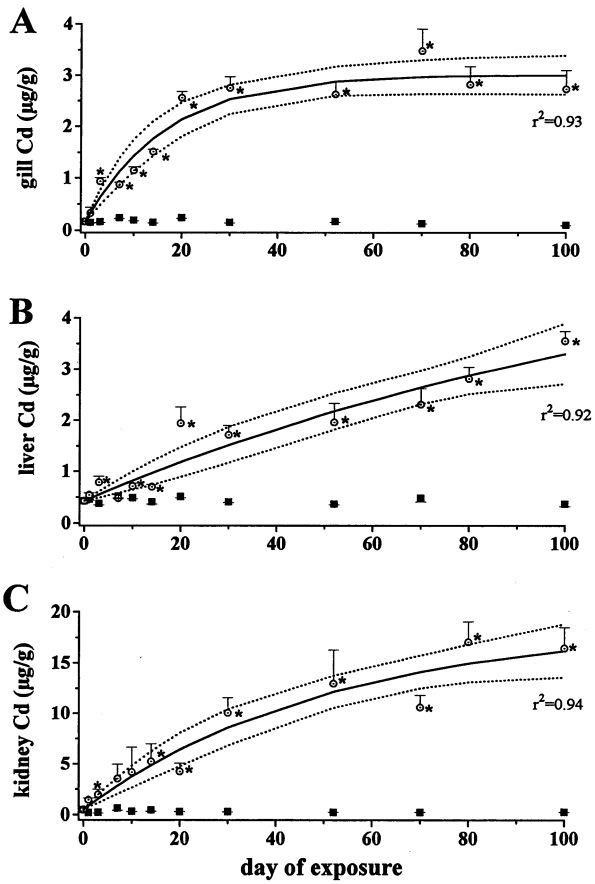

Fig. 5. Cadmium accumulation in (A) gills, (B) liver and (C) kidney of rainbow trout exposed to 3 $\mu g \cdot l^{-1}$ Cd (open circles) for up to 100 days (series 2). An unexposed control group is also shown (filled squares). Mean ± 1 S.E.M. ($N \geq 5$) and * indicates significant difference ($P < 0.05$) from the control mean at that time. Included on the graphs are the best fit lines from the exponential model (solid line, with r^2 value) and associated 95% confidence interval (dotted lines).

Complementing this response was the relatively slow recovery of ionic balance, particularly Ca^{2+} (see Figs. 2 and 4, McGeer et al., 2000) and no significant metabolic costs, as estimated by Ucrit and MO_2 (see Fig. 5 McGeer et al., 2000).

The results of this study show that for some metals, tissue specific accumulation of metal is not a good indicator of chronic exposure or long term physiological impact. This was particularly true for the nutritive metals Cu and Zn, where tissue burdens in exposed fish became independent of exposure relatively quickly. In contrast tissue Cd levels in the kidney and liver continued to reflect the exposure for a prolonged period. Thus, the proposal to use tissue metal burden as a biomarker of exposure (Bergman and Dorward-King, 1997) may only apply in the case of non-nutritive metals. In addition, the BCF value, which is generally considered to be an indicator directly related to exposure, was actually inversely related to exposure. In spite of metal accumulation, the BCF value was higher for unexposed controls than for exposed trout (Table 5), thus illustrating the fact that metals are a natural component in all waters and that nutritional metals, by requirement undergo bioaccumulation.

This study also showed that while there was an association between physiological response and tissue accumulation during the initial period of exposure, tissue burden did not serve as a good index of long term physiological impact. For example, during the recovery and acclimation phases of exposure, physiological disruption was reduced towards control levels but tissue burdens tended to remain elevated or continued to rise. The fundamental differences between the accumulation of the three metals observed in this study and the consequences of exposure observed in McGeer et al., (2000) demonstrate the unique and complicated natures of Cu, Cd and Zn toxicokinetics and question the use of tissue burdens as indicators of impact and exposure.

Overall, the general agreement between series 1 and 2 in tissue accumulation patterns (Figs. 1 and 2 vs. Figs. 4 and 5) and modeling results (Table 4) despite different feeding regimes and exposure temperature, indicates that the data are robust. However, comparison of modeled data for liver Cd in series 1 versus 2 illustrates that for tissues with long $t_{\frac{1}{2}}$, an extended sampling time course is required for accurate estimation of the saturation concentration. As such, the data from series 2 were much more powerful than series 1 where only one data point was collected beyond 16 days. Modeling the data for liver Cd from series 1 gives a predicted saturation concentration of 18.3 µg Cd g^{-1} (Table 4) even though the last data point (day 65) is less than 4 $\mu g \cdot g^{-1}$ (Fig. 2). The series 2 modeled saturation concentration for liver Cd was 6 $\mu g \cdot g^{-1}$ (Table 4, Fig. 5) and is undoubtedly a more accurate estimate of the true value.

While the general patterns of accumulation for each metal have been demonstrated previously, the present study is the first to directly compare the patterns of chronic Cu, Cd and Zn accumulation, measured simultaneously, under identical exposure conditions. Modeling of the accumulation data to derive t_2^1 and saturation concentrations proved useful in making general comparisons among metals and tissues and revealed features of tissue specific responses to chronic metal exposure. In combination with the associated study of physiological responses (McGeer et al., 2000), the damage–repair–acclimation process for Cu was relatively rapid and carried a metabolic cost that was suggestive of active regulation. In comparison the damage–repair–acclimation process for Cd occurred over a longer time frame and did not appear to involve either active regulation or metabolic cost.

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Table 4
Predicted saturation concentration ($\mu g \cdot g^{-1}$) and associated time to half saturation (t_2^1) for gill, liver and kidney for rainbow trout exposed to either 3 $\mu g \cdot l^{-1}$ Cd or 75 $\mu g \cdot l^{-1}$ Cu or 250 $\mu g \cdot l^{-1}$ Zn[a]

Treatment	Tissue	Saturation concentration ($\mu g \cdot g^{-1}$)	t_2^1 (days)	Accumulation factor %
3 $\mu g \cdot l^{-1}$ Cd				
Series 1	Gills	3.9 ± 0.4	2.8	2235
	Liver	18.3 ± 28.4	213	7891
	Kidney	13.5 ± 22.7	61	7983
	Whole body	0.78 ± 0.09	2.3	256
Series 2	Gills	3.0 ± 0.2	11.8	1686
	Liver	6.0 ± 3.6	95	1216
	Kidney	18.8 ± 3.1	36	5271
	Whole body	0.95 ± 0.1	12.8	198
75 $\mu g \cdot l^{-1}$ Cu				
Series 1	Gills	2.2 ± 0.3	0.4	283
	Liver	57.8 ± 5.8	6.8	358
	Kidney	5.0 ± 0.6	0.7	276
	Whole body	2.2 ± 0.2	0.05	87
Series 2	Gills	1.6 ± 0.1	13.0	220
	Liver	91.0 ± 6.9	5.0	238
	Kidney	3.7 ± 0.3	0.3	68
	Whole body	2.7 ± 0.1	1.7	67
250 $\mu g \cdot l^{-1}$ Zn				
Series 1	Gills	51.5 ± 2.3	4.9	34
	Liver	nc	nc	nc
	Kidney	nc	nc	nc
	Whole body	33.3 ± 1.2	0.7	21

[a] For Cd and Cu, prediction variables are calculated for two different exposure experiments, series 1 being 65 days in length and series 2 being 100 days. Means for data (see Fig. 1 through 5) were fitted to a simple exponential model. For Zn, day 1 data were excluded. The accumulation factor shows the predicted saturation concentration as a % increase from the background levels (unexposed controls) and nc indicates the variables could not be calculated as no significant accumulation occurred.

Table 5
Whole body bioconcentration factor (BCF) for Cu, Cd and Zn in rainbow trout exposed to either 3 $\mu g \cdot l^{-1}$ Cd or 75 $\mu g \cdot l^{-1}$ Cu or 250 $\mu g \cdot l^{-1}$ Zn, or not exposed to metals (unexposed controls)[a]

	Whole body BCF	
Metal	Unexposed controls	Metal exposure
Cd		
Series 1	3684	268
Series 2	5088	309
Cu		
Series 1	719	30
Series 2	1063	36
Zn		
Series 1	11458	142

[a] Predicted saturation concentration (see Table 4) and the average over all sampling times were used as estimates of whole body metal concentrations in exposed and control fish, respectively. BCF is shown for two exposure experiments, series 1 being 65 days in length and series 2 being 100 days.

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