Arch. Environ. Contam. Toxicol. 51, 615–625 (2006)
DOI: 10.1007/s00244-005-0212-7

ARCHIVES OF
Environmental
Contamination
and Toxicology


# Dietary Pb Accumulation in Juvenile Freshwater Rainbow Trout (*Oncorhynchus mykiss*)

L. C. Alves,[1] C. N. Glover,[2] C. M. Wood[1]

[1] Department of Biology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1
[2] National Institute of Nutrition and Seafood Research, Postboks 2029 Nordnes, 5817 Bergen, Norway

Received: 18 August 2005 / Accepted: 12 March 2006

**Abstract.** Three different diets amended with lead (Pb) nitrate $Pb(NO_3)_2$ (7, 77, and 520 μg Pb/g dry weight) and a Pb-free control diet (0.06 μg Pb/g dry weight) were fed to juvenile freshwater rainbow trout for 21 days. Accounting for measured food consumption, the calculated doses per fish were 0.02, 3.7, 39.6, and 221.5 μg/day, for the control, low, intermediate, and high Pb treatments, respectively. The patterns of Pb accumulation over time were determined in various tissues (gills, liver, kidney, intestine, carcass), red blood cells (RBC), and plasma, as well as feeding, growth, hematological, and ionoregulatory parameters. Pb accumulation occurred in a dose-dependent manner in all tissues except the plasma, where accumulation was minimal. Overall, when fed the highest Pb diet, the intestine exhibited the greatest Pb burden (17.8 μg Pb/g tissue wet weight), with high concentrations also found in the kidney (2.4 μg Pb/g tissue wet weight) and liver (1.9 μg Pb/g) at the highest dietary Pb treatment by day 21. The RBCs accumulated a substantial amount of Pb (1.5 μg Pb/g) when compared to the plasma (0.012 μg Pb/g) in the high treatment group. The percentage of Pb retained in the fish decreased with increasing dietary Pb concentrations. Growth, survival, plasma protein, and hematocrit were not significantly affected by dietary Pb. Plasma $Ca^{2+}$ levels decreased at the beginning of the experiment, whereas $Mg^{2+}$ levels decreased during the middle of the experiment in both the intermediate and high dietary treatments. Both the $Ca^{2+}$ and $Mg^{2+}$ levels stabilized by day 21. Branchial $Ca^{2+}$ and $Na^{+}$ influx rates were not affected by dietary Pb, except on day 8 where $Na^{+}$ influx rates were significantly elevated. The results of this study show that Pb does accumulate internally from the diet when present at levels within the range reported in contaminated benthic invertebrates in nature. We further identify the intestine as a potential target site of chronic toxicity of Pb via the diet, and RBCs as a reservoir of dietary Pb.

Lead (Pb) is a non-nutrient metal found in the earth's crust, and thus may enter the aquatic environment through natural processes of geological weathering and volcanic emissions (Demayo *et al*. 1982). The background concentration of Pb in uncontaminated surface water has been estimated to be 0.02 μg Pb/L (Flegal *et al*. 1987). Pb may also enter the aquatic environment through anthropogenic practices such as the mining, refining, and smelting of Pb (Sorensen 1991; World Health Organization 1995).

In fish, the primary site of acute waterborne Pb toxicity is at the gills where inhibitory actions of Pb on $Ca^{2+}$, $Na^{+}$, and $Cl^{-}$ uptake have been documented (Varanasi and Gmur 1978; Rogers *et al*. 2003, 2005; Rogers and Wood 2004). Nevertheless, there is evidence for a specific chronic toxic action of waterborne Pb on erythrocyte function in fish. Hodson *et al*. (1978) found that chronic waterborne Pb exposure (13 μg Pb/L; 32 weeks) to rainbow trout resulted in significant increases in red blood cell (RBC) numbers, decreases in RBC volumes, and δ-aminolevulinic acid dehydratase (ALAD) activity, an enzyme that catalyses the formation of porphobilinogen from aminolevulinic acid in the heme synthesis pathway. This suggests that fish exposed chronically to waterborne Pb may be at risk of anemia.

Nevertheless, Pb toxicity may also occur via the gastrointestinal tract (Sorensen 1991). Crespo *et al*. (1986) showed that rainbow trout that were orally administered with Pb in the diet (10 μg Pb/g dry weight (dw)/fish/day) had morphological alterations of the intestinal brush border, which resulted in an impairment of intestinal NaCl absorption. Studies have demonstrated that fish fed diets containing metals (As, Cd, Cu, Pb, and Zn) have reduced feeding activity, growth, and survival rates (Farag *et al*. 1994; Woodward *et al*. 1994, 1995). Farag *et al*. (1994) reported that cutthroat trout (*Oncorhynchus clarki*) fed benthic macroinvertebrates containing high levels of metals (As, Zn, Cd, and Pb) from different contaminated sites along the Coeur d' Alene River, Idaho, had a higher concentration of these metals in the stomach and pyloric ceca when compared to the gills and kidney. Normal Pb levels in uncontaminated benthic invertebrates are usually less than 1 μg/g dw. However, body burdens up to 792 μg/g dw have been reported in benthic invertebrates in the South Fork of the Coeur d' Alene River (Farag *et al*. 1994, 1999). While these studies using naturally contaminated diets have displayed evidence of dietary Pb uptake and toxic effects to fish, the

*Correspondence to:* L. C. Alves; *email:* lara.alves@ec.gc.ca

laboratory study of Hodson *et al*. (1978) reported that rainbow trout fed diets contaminated with up to 118 μg Pb/g did not take up any Pb into internal tissue.

Although acute waterborne Pb interactions at the gills have been well studied (Davies *et al*. 1976; Holcombe *et al*. 1976; Hodson *et al*. 1978, Rogers *et al*. 2003, 2005; Rogers and Wood 2004), the effects of dietary Pb on toxicological and physiological parameters have received very little attention. The initial objective of this study was to assess whether three different Pb-contaminated diets (7, 77, 520 μg Pb/g dw) fed to juvenile rainbow trout for 21 days resulted in Pb accumulation in specific internal tissues: liver, kidney intestine, gills, carcass, and blood. These concentrations were specifically selected to include the range of doses used by Hodson *et al*. (1978; 4–118 μg Pb/g dw) and the concentrations found in benthic invertebrates (Farag *et al*. 1994, 1999; 0–792 μg/g dw). If gastrointestinal Pb uptake occurred, a second objective was to evaluate the pattern of tissue-specific accumulation over time, and to look for any evidence of regulation or depuration. In light of the known actions of waterborne Pb on RBC function, a third objective was to test for possible hematological abnormalities in terms of plasma protein and hematocrit. A final objective was to examine if there were any disruptions in growth and survival rates, plasma $Ca^{2+}$ and $Mg^{2+}$ regulation, and $Na^{+}$ and $Ca^{2+}$ influx rates from the water, since many of these parameters are affected by waterborne Pb.

## Materials and Methods

### *Fish*

Juvenile rainbow trout (*Oncorhynchus mykiss*) (N = 368) weighing 3–6 g were obtained from Humber Springs Trout Hatchery (Orangeville, Ontario). Upon arrival at McMaster University, Hamilton, Ontario, fish were randomly selected and placed in eight 200-L flow-through, aerated holding tanks (46 fish per tank). Each tank was supplied with 0.8 L/min of dechlorinated Hamilton water (in mM: $Na^{+}$ 0.6 mM; $Cl^{-}$ 0.7; $Ca^{2+}$1.0; $Mg^{2+}$ 0.2; $K^{+}$ 0.05, water hardness in the form of $CaCO_3$ = 140 ppm) and allowed to acclimate to ambient conditions (11–13°C and pH 7.5–8.0) for at least 7 weeks prior to the start of the experiment. During the acclimation period no deaths occurred.

Fish were fed commercial salmon fry pellets (Nelson's Silver Cup fish feed; Murray, Utah, USA; 52% crude protein [min.]; 14% crude fat [min.]; 3% crude fiber [max.]; 12% ash [max.]; and 1% sodium [actual]) once daily to satiation from the third day of arrival until the start of the experiment. By this time, fish weighed about 10–13 g. Each tank was assigned to one of four treatment groups, with each treatment replicated. The four treatment groups included a nominally Pb-free diet (control), a low Pb diet (nominally 10 μg Pb/g dw), an intermediate diet (nominally 100 μg Pb/g dw), and a high Pb diet (nominally 500 μg Pb/g dw). The actual measured mean concentrations were 0.06 ± 0.004, 7.2 ± 0.9, 76.5 ± 6.7, and 519.8 ± 50.0 μg Pb/g dw.

Two weeks into the acclimation period, eight fish were randomly selected from each of the eight tanks and given a passive integrative transponder (PIT) tag, placed into the peritoneal cavity under MS-222 (3-aminobenzoic acid ethylester; 0.5 g/L) anesthesia. This was done to establish specific growth rates over the 21-day experiment.

### *Diet Preparation*

Pb-enriched diets were made by adding lead nitrate ($Pb(NO_3)_2$) into the same commercial salmon fry food (Silver Cup Feed, Murray, Utah, USA) as used during the acclimation period. The commercial food was pulverized into a fine powder using a household blender for approximately 2 minutes. Then, 500 g of the fine powder was hydrated with 40% v/w of double distilled water (NANOpure II; Sybron/Barnsted, Boston, MA, USA) containing different proportions of dissolved $Pb(NO_3)_2$ and blended in a commercial pasta maker for 1.5 h in order to achieve the intended doses of 10, 100, and 500 μg Pb/g dw food. The paste was then passed through a cutter where small strands were broken into small pellets. The control diet was prepared the same way except Pb was not added. The food pellets were first air-dried for 48 h, then dried in a 60°C oven to a constant weight, and frozen until further use. The measured concentration of Pb (Table 1) was determined by heat-digesting the food pellets in five volumes of 1N $HNO_3$ at 60°C for 48 h. The supernatant was then diluted with 1% $HNO_3$ and 0.5% ammonium phosphate and measured on a graphite furnace atomic absorption spectrophotometer (AAS; 220 SpectrAA; Varian GTA-110; Varian, Australia) with a detection limit of 0.06 μg Pb/L against a certified multi-element Pb standard (Anachemia Inc., Quebec), employing appropriate blank samples and reference samples. Moisture was measured to be 6% by drying food to a constant weight in a 60°C oven.

### *Feeding, Mean Weight, Growth, and Food Consumption*

A total of four replicated treatments (0, 7, 77, and 520 μg Pb/g dw, Table 1) were tested in this study. On the third day of the experiment, mechanical failure (low aeration) resulted in an alteration in the exposure conditions in one of the replicated 77-μg Pb/g dw diet treatment tanks. This tank was eliminated from this experiment and thus the n value for this treatment was half that of all others. Each group was fed once daily to satiation, by placing 10–15 pellets on the surface of the water every minute until the fish were no longer striking at the pellets. The pellets not eaten on the surface water were removed and the bottom of each tank was siphoned approximately one hour post feeding to control any leaching of Pb from the food and feces. The amount of all food consumed by the fish was recorded in order to determine the voluntary ration. Unfiltered water samples (~10 ml) were taken daily before and after siphoning in order to determine the extent of any waterborne Pb contamination. Water was acidified for metal analysis.

Mean fish weights were calculated every week by bulk weighing all fish from each holding tank. The mean fish weight was determined by dividing the number of fish in each tank by the total biomass. Fish that were tagged were individually weighed and their fork lengths were measured. The condition factor for both tagged and bulk-weighed fish was determined using the formula

$$\mathrm{CF} = (W/L^3)*100 \quad (1)$$

where W is the weight in grams and L is the fork length in centimeters.

Specific growth rates (SGR) expressed on a %/day basis (Ricker 1979) were determined using the least-squares linear regression (SigmaStat, version 3.0) through the natural logarithm of the individual tagged fish weights versus time. The ration of food consumption for bulk-weighed fish at satiation (% body wt/day) was determined using the formula

$$\mathrm{r} = (\mathrm{y}/\mathrm{n}*\mathrm{w})*100 \quad (2)$$

**Table 1.** Total waterborne Pb concentrations (μg/L) for different tank diets throughout the 3-week experiment

| Week | Control (0 μg Pb/g) | Low (7 μg Pb/g) | Intermediate (77 μg Pb/g) | High (520 μg Pb/g) |
|---|---|---|---|---|
| 0 | 1.4 ± 0.2 a | 1.4 ± 0.2 a | 1.4 ± 0.2 a | 1.4 ± 0.2 a |
| 1 | 1.7 ± 0.2 a | 1.7 ± 0.2 a | 2.8 ± 0.5 a | 9.5 ± 1.2 b |
| 2 | 1.4 ± 0.2 a | 1.2 ± 0.1 a | 1.4 ± 0.1 a | 10.1 ± 1.5 b |
| 3 | 1.7 ± 0.2 a | 1.5 ± 0.1 a | 2.4 ± 0.5 a | 11.0 ± 2.0 b |

Data are represented as mean ± 1 SEM, $n = 28$, for all treatments, except the 77-μg Pb/g dry weight treatment, $n = 4$. Values sharing lowercase letters are not significantly different from other treatment values within the same week ($p < 0.05$).

where r is the ration (% body wt/day), y is the total food in grams fed to fish in each tank, n is the number of fish in each tank, and w is the average mean fish wet weight in grams. Food conversion efficiency (FCE) (in %) for each treatment was expressed using the formula

$$\mathrm{FCE} = (\mathrm{SGR_{ave}}/\mathrm{r}) * 100 \quad (3)$$

where $\mathrm{SGR_{ave}}$ is the mean specific growth rate (%/day), and r is the ration (% body wt/day).

## *Tissue Sampling*

At day 0 two fish from each tank, and on days 7, 14, and 21 six randomly selected fish per tank, were sacrificed with 1.0 g/L MS 222. Tagged fish were not sampled. Blood was taken by caudal puncture using an ice-chilled 250-μL gas tight Hamilton syringe pre-rinsed with lithium heparin (50 i.u./mL). Some of the whole blood was used for measurement of hematocrit by capillary tube centrifugation at 13,700 g for 2 min. The hematocrit was read directly from the tube, which was then broken in order to extract plasma for total plasma protein (g/100 mL) by refractometry (American Optical, Buffalo, NY) (Alexander and Ingram 1980). The rest of the whole blood was centrifuged at 10,000 g for 2 min and plasma and RBCs collected, snap-frozen in liquid nitrogen, and stored at −70°C until further analysis for plasma $Ca^{2+}$, $Mg^{2+}$, total plasma Pb, and total RBC Pb concentrations.

The liver, kidney, intestine, and gill baskets were dissected, rinsed, blotted, weighed, and kept at 4°C until analysis for Pb content. The gills were rinsed with double-distilled water and the intestinal tract was flushed out with 0.9% NaCl to remove any source of non-tissue Pb. The pyloric cecae were removed from the anterior intestine and placed with the carcass, which also included the similarly flushed stomach. The presence of food residue was noted in the pyloric cecae.

Plasma $Ca^{2+}$ and $Mg^{2+}$ concentrations were determined by flame atomic absorption spectrophotometry (AAS; Varian 220FS, Australia). Samples were prepared by diluting the plasma with 0.5% $LaCl_3$ and assaying it against similarly diluted known standards. Pb in plasma and water samples were determined in the same manner as outlined for the analysis of Pb in food.

Weighed liver, kidney, intestine, gill, carcass, and RBC samples were digested in 5 volumes of 1 N $HNO_3$ in a 60°C oven for 48 h. After 48 h, these samples were centrifuged at 10,000 g for 20 min and the supernatant was appropriately diluted and measured by graphite furnace AAS for Pb accumulation, as outlined earlier for analysis of Pb in food.

## *$Na^+$ and $Ca^{2+}$ Influx Rate*

On day 0 two randomly selected fish from each tank, and on days 8 and 22 six randomly selected fish from each tank, were used to measure the unidirectional $Na^+$ and $Ca^{2+}$ influx rates from the water. At time 0, 0.1 μCi of $^{22}Na$ and 10 μCi of $^{45}Ca$ were added to 3-L flux chambers containing 2 L of dechlorinated Hamilton tap water, and allowed to mix for 10 min. For the entire flux period (4 h), the flux chambers were partially submerged on a wet table receiving a constant water flow in order to maintain temperature at 11–13°C. After a 10-min isotope equilibration period, fish were added to the individual flux chambers. Two 5-mL water samples were removed at 10 min, 2 h, and 4 h for determination of initial, mid, and final ion concentration and radioactivity measurements.

After 4 h, fish were sacrificed by adding an overdose of MS 222 to the flux chambers. Fish were immediately removed and placed in a plastic bag containing 200 ml of 10 mM NaCl plus 10 mM $CaCl_2{\cdot}2H_2O$ for 10 min, as a cold displacement to remove any loosely surface-bound $^{22}Na$ and $^{45}Ca$. The fish were blotted dry, weighed, and placed in plastic vials to count for $^{22}Na$ on a γ-counter (Canberra-Packard A5000 Minaxi). The γ-counter did not detect $^{45}Ca$ radioactivity. Samples were prepared for scintillation counting of $^{45}Ca$ using a similar protocol to Franklin *et al*. (2005). In short, fish were acid-digested in the same manner as for determination of the Pb tissue burden. The acid-digested samples were centrifuged at 5525g for 10 min (Sigma 4K15C Refrigerated Centrifuge); 2.5 ml of the supernatant was diluted with 10 ml of Ultima Gold AB cocktail (Packard Bioscience, USA). For water samples, 5 ml of aqueous counting scintillation fluor (ACS, Amersham, UK) was added to 2 ml of water. The processed tissue and water were placed in a dark room overnight to reduce chemiluminescence, then counted on a liquid scintillation counter (1217 Rackbeta Liquid Scintillation Counter, LKB Wallac, Finland). Since $^{22}Na$ is a γ/β emitter, the scintillation counter detected both $^{45}Ca$ and $^{22}Na$ radioactivity, so it was necessary to distinguish the radioactivity due to $^{45}Ca$ alone (β emitter) using the protocol of Van Ginneken and Blust (1995).

The $^{45}Ca$ values from tissue digests were then quench-corrected to the same counting efficiency as $^{45}Ca$ found in water samples by the method of external standard ratios (ESR), using a $^{45}Ca$ quench curve that was generated from carcass tissue of varying weights, processed the same as above and counted in the same cocktail.

The unidirectional $Na^+$ and $Ca^{2+}$ influxes (μmol/kg/h) were calculated using the formula

$$J_{in} = \text{Fish CPM}/(\text{MSA.W.t.}) \quad (4)$$

where MSA is the mean specific activity (CPM/μmol) of water in the γ counter for $^{22}Na$ or in the scintillation counter for $^{45}Ca$, W is the weight of the fish (kg), t is the time in hours, and Fish CPM is the total counts per minute of $^{22}Na$ or $^{45}Ca$ accumulated by the fish during the flux period.

## *Statistics*

All statistical tests were performed using the computer software Sigmastat (3.0.) or SYSTAT 10. Data were tested for homogeneity of variances among groups using the Bartlett test. Those that failed were subjected to natural logarithm or square root transformations to obtain homogeneity among groups. Data that could not be normalized were subjected to non-parametric analysis, using the Kruskal-Wallis procedure, where all ranks were corrected for ties. Comparisons between

**Table 2.** Specific growth rates (SGR), voluntary rations, food conversion efficiency (FCE), and condition factors (CF) of juvenile rainbow trout fed to satiation with diets containing different concentrations of Pb

| | Week | 0 μg Pb/g | 7 μg Pb/g | 77 μg Pb/g | 520 μg Pb/g |
|---|---|---|---|---|---|
| SGR (%/day) | | 2.47 ± 0.08 a | 2.47 ± 0.11 a | 2.33 ± 0.13 a | 2.37 ± 0.18 a |
| Ration (% body wt/day) | 1 | 2.33 ± 0.23 a | 2.56 ± 0.14 a | 2.72 ± 0.27 a | 2.51 ± 0.21 a |
| | 2 | 2.69 ± 0.17 a | 2.41 ± 0.10 a | 2.56 ± 0.13 a | 2.40 ± 0.15 a |
| | 3 | 2.09 ± 0.24 a | 2.59 ± 0.17 a | 2.20 ± 0.14 a | 2.54 ± 0.19 a |
| CF | 0 | 1.85 ± 0.04 a | 1.97 ± 0.04 a | 1.94 ± 0.04 a | 1.85 ± 0.04 a |
| | 1 | 1.97 ± 0.05 a | 2.01 ± 0.05 a | 1.98 ± 0.07 a | 1.97 ± 0.06 a |
| | 2 | 2.04 ± 0.08 a | 1.97 ± 0.03 a | 2.05 ± 0.08 a | 2.34 ± 0.12 b |
| | 3 | 1.84 ± 0.05 a | 1.95 ± 0.03 a | 2.01 ± 0.08 a | 1.91 ± 0.07 a |
| FCE (%) | 1 | 106 ± 3 a | 97 ± 4 a | 86 ± 4 a | 94 ± 7 a |
| | 2 | 92 ± 3 a | 102 ± 4 a | 91 ± 5 a | 99 ± 7 a |
| | 3 | 118 ± 4 a | 95 ± 4 b | 106 ± 6 ab | 93 ± 7 b |

SGR and Rations are mean ± SEM. SGR was calculated using the linear regression (SigmaStat version 3) of the natural logarithm of individual tagged fish weight versus time. The estimates for each fish were then averaged. Condition factor (CF) for bulk-weighed fish was measured using the formula $CF = (W/L^3) \times 100$. FCE was calculated by the formula (SGR/average ration during the week) multiplied by 100. Values sharing lowercase letters are not significantly different from other treatment values within the same week ($p < 0.05$).

treatments and days were done using two-way analysis of variance (ANOVA), followed by a Tukey (parametric analysis) or Dunn's test (non-parametric analysis), as appropriate,. Nested data within the replicated tanks within the same treatments and between days were not significantly different, using the general linear model estimate in SYSTAT 10.

## Results

Waterborne Pb values (Table 1) were not significantly above background in the low (7 μg Pb/g) and intermediate (77 μg Pb/g) dietary Pb exposures, but were significantly elevated to about 10 μg/ L in the high (520 μg Pb/g) dietary Pb exposure, presumably due to the leaching from the diet and/or feces. This suggests that Pb accumulation for the 520-μg Pb/g treatment may not be solely based on dietary Pb exposure, but a combination of dietborne and waterborne Pb.

No mortality was associated with the dietary Pb treatments throughout the course of the experiment. Specific growth rates, using tagged fish over 21 days and voluntary rations consumed each week from bulk-weighed fish did not differ among treatment groups (Table 2). Total food eaten over 21 days also did not differ between treatments. Condition factors were extremely high and fish in all tanks were considered overweight both at the start and end of the experiment (Table 2). The food conversion efficiency, calculated based on mean SGR values of tagged fish, did not differ consistently among treatments (Table 2).

At all dietary Pb doses, Pb accumulation was seen in the gills (Figure 1), intestine (Figure 2), liver (Figure 3), kidney (Figure 4), and carcass (Figure 5) during the course of the 21-day experiment. At day 21, the order of Pb concentration in specific tissues was intestine > carcass > kidney > liver > gills. The intestine showed a substantial accumulation with a burden of 17.8 μg Pb/g tissue wet weight in the 520-μg Pb/g exposure, which was 445× greater than the control (0.04 μg Pb/g) on day 21. The intestine (17.8 μg Pb/g tissue wet weight), carcass (2.7 μg Pb/g tissue wet weight), kidney (2.4 μg Pb/g tissue wet weight), and the liver (1.9 μg Pb/g tissue wet weight) all exhibited their highest Pb burdens on day 21 compared to day 0, 7, and 14. In contrast, the gills, at least at the highest dose, had the greatest Pb accumulation on day 7 (8.0 μg Pb/g tissue wet weight) and a much lower Pb burden (2.2 μg Pb/g tissue wet weight) by day 21. Pb burden in the intestine (Figure 2) increased with time in all dietary Pb treatments.

When the percentage distribution of Pb burden in the whole fish is considered (Figure 8), the carcass accumulated about 80%, the intestine about 10%, while the gills, kidney, and liver made up the remaining 10% of the Pb burden. These values are not surprising since the carcass makes up 85–90% of the weight of the fish and it also contained the stomach and the pyloric ceca; the latter contained some food residue. Notably, the biggest change from the % Pb distribution in the non-exposed fish was a much larger % contribution from the intestine in all dietary Pb treatments.

The RBCs accumulated a substantial concentration of Pb by day 21 (Figure 6), with almost no Pb present in the plasma (Table 3). Indeed, only at day 21 at the highest dose level was the plasma Pb significantly elevated. RBC Pb increased from background concentrations of 0.05 μg Pb/g to about 1.5 μg Pb/g in the highest dose level by day 21, about 105 times more than that of the plasma.

There were only a few significant effects of dietary Pb exposure on plasma $Ca^{2+}$ and $Mg^{2+}$ regulation. Plasma $Mg^{2+}$ levels fell significantly in the intermediate and high Pb treatments on day 14, but had stabilized by day 21 (Table 3). A similar pattern was seen with the plasma $Ca^{2+}$ of fish exposed to the intermediate and high diets, where there was a significant decrease in Ca levels on day 7 when compared to the control. The $Ca^{2+}$ levels had recovered by day 14, and thereafter remained stable. The hematocrit (30–40%) and the plasma protein (5.8–6.4 g/100 mL) remained high throughout the experiment and were not affected by the dietary Pb exposure.

$Ca^{2+}$ influx rates from the water were not significantly affected in juvenile rainbow trout and remained stable throughout the experiment (Table 4). However, on day 8, $Na^+$ influx rates were significantly elevated in all treatments, and this occurred to the greatest extent in the high Pb diet treatment (Table 4). These effects had disappeared by day 22.

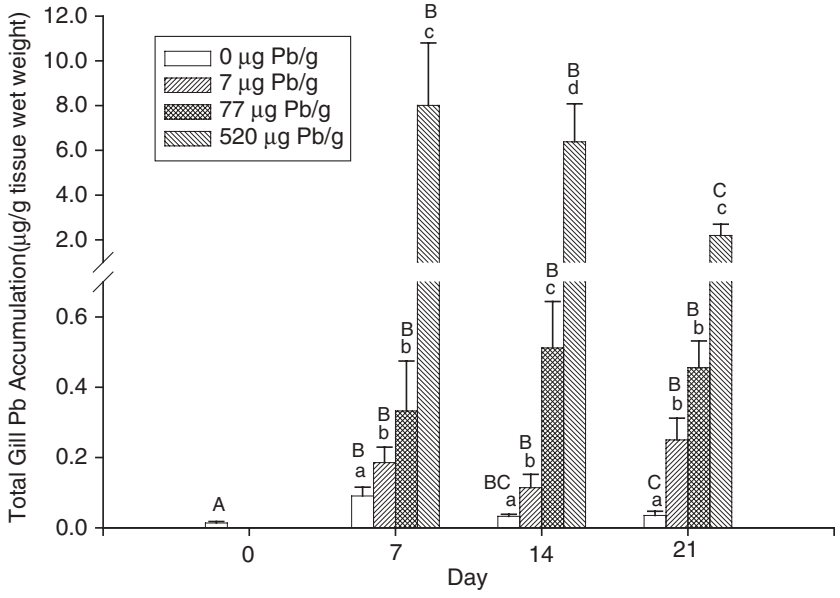


**Fig. 1.** Lead accumulation in the gills of juvenile rainbow trout exposed to different levels of Pb in the diet over 21 days. Pb tissue burden is expressed in µg g/g wet tissue weight. Data represented as mean ± 1 SEM, $n = 12$, except the 77-µg Pb/g treatment, $n = 6$. Values sharing lowercase letters are not significantly different ($p < 0.05$) between treatment means within a day and values sharing uppercase letters are not significantly different between days within treatment means using a two-way ANOVA with a Tukey multiple comparison, or Kruskal-Wallis ANOVA on ranks with a Dunn's multiple comparison test

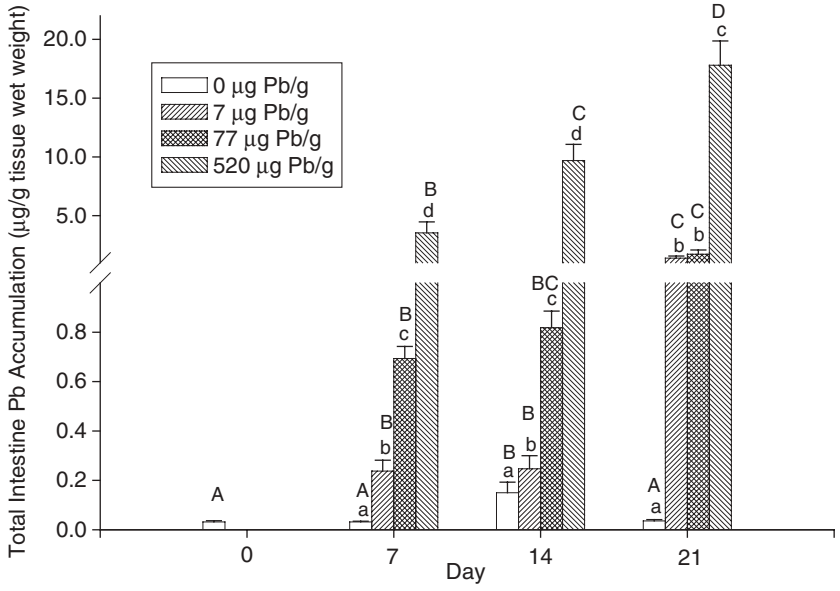


**Fig. 2.** Lead accumulation in the intestine of juvenile rainbow trout exposed to different levels of Pb in the diet over 21 days. The intestine does not include the pyloric ceca. Other details as in legend of Figure 1

## Discussion

Dietary Pb concentrations were chosen to mimic environmentally relevant concentrations in terms of those found in benthic invertebrates at both contaminated and uncontaminated sites in the environment (0–792 µg Pb/g dw; Woodward *et al*. 1994; Farag *et al*. 1999), as well as to cut across the same range (4–118 µg Pb/g dw) as used by Hodson *et al*. (1978) in an earlier dietary study. Dietary exposure to Pb at these measured concentrations ( 0.06, 7, 77, and 520 µg Pb/g) had few apparent adverse effects on rainbow trout during the 21-day experiment. There were no mortalities and no significant differences were observed in specific growth rates and rations among fish fed either the control diet or the various Pb diets. These results are consistent with Mount *et al*. (1994) who reported no effects on survival and growth of rainbow trout fed a dietary concentration as high as 170 µg Pb/g.

In the present study, total waterborne Pb was significantly elevated above background levels in the high dietary treatment tanks (from ~1.5 to 10 µg Pb/L; Table 2). Therefore, it is possible that the Pb accumulation in the tissues, especially in the gill, was attributable to the combination of both dietary and waterborne Pb exposure in this treatment group only. The primary site of acute waterborne Pb uptake in fish is via the gills (Varanasi and Gmur 1978). Rainbow trout exposed to chronic waterborne Pb (13 µg Pb/L for 32 weeks) also showed elevated gill Pb concentrations (approximately 2.5 µg Pb/g) relative to other tissues such as the kidney and liver (Hodson *et al*. 1978). The gills in the present study did accumulate Pb (to about 2.1 µg Pb/g on day 21), mainly in the high dietary Pb treatment. Waterborne Pb levels (Table 2) in the low and intermediate dietary treatments were similar to background levels. This suggests any accumulation that was observed in the gills and in the other tissues of fish in the low and

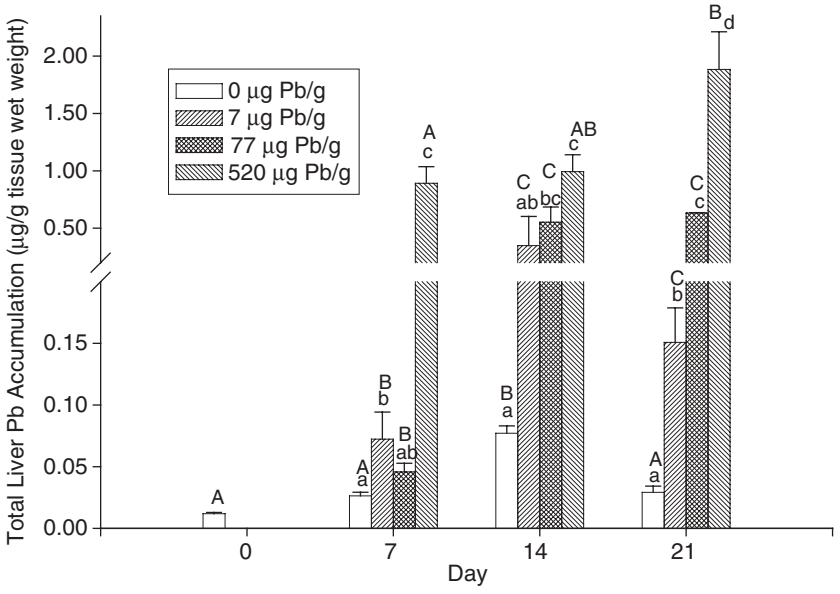


**Fig. 3.** Lead accumulation in the liver of juvenile rainbow trout exposed to different levels of Pb in the diet for 21 days. Other details as in legend of Figure 1

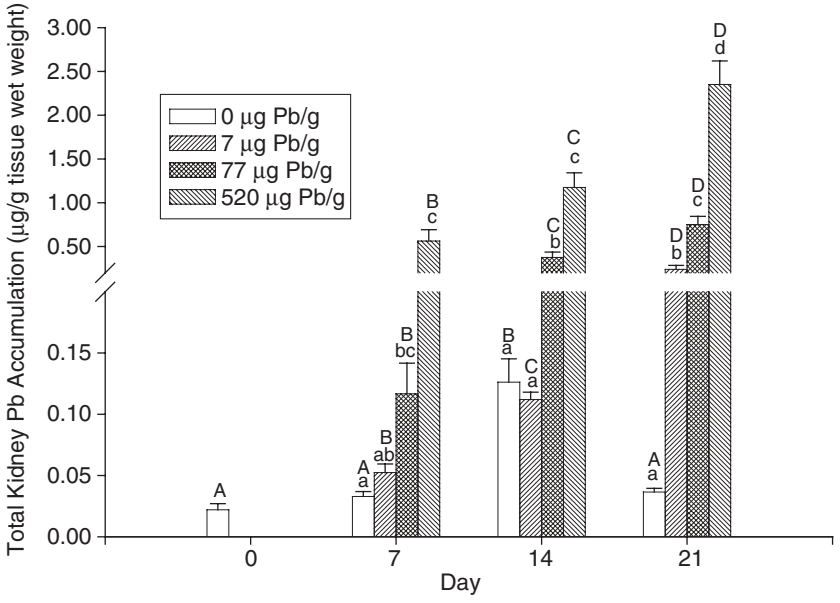


**Fig. 4.** Lead accumulation in the kidney of juvenile rainbow trout exposed to different levels of Pb in the diet for 21 days. Other details as in legend of Figure 1

intermediate treatments was the result of dietary Pb, and not waterborne Pb. Notably, in the present study, the gill Pb burden substantially dropped from 8.0 µg Pb/g tissue wet on day 7 to 2.1 µg Pb/g tissue wet weight on day 21 (Figure 1), despite a relatively constant waterborne Pb level (Table 3). This suggests that the gill Pb burden may have been excreted, or redistributed to other tissues. Pb burdens in the low dietary treatment in all the tissues were close to background control levels, which suggests that a dietary concentration >7 µg Pb/g is needed to see an effect on Pb burden in internal tissues.

Tissue accumulation data (Figures 1–5) show that the intestine had the greatest Pb burden among all the tissues analyzed. This is consistent with Farag *et al*. (1994) who found that adult rainbow trout fed metal-contaminated (As, Cd, Cu, Pb, and Zn) benthic invertebrates from the Clark Fork River, Montana, exhibited substantial metal accumulation in the gut tissues, with the highest concentration in the pyloric ceca and stomach. In the present study, the stomach and pyloric ceca were not analyzed individually, but grouped with the carcass. The presence of these tissues in the carcass may explain the high proportion of lead associated with this tissue, especially since there was some presence of food residue in the pyloric ceca. We hypothesize that just as the gills are the primary site of exposure and the site of waterborne Pb toxicity (Rogers *et al*., 2003, 2005, Rogers and Wood 2004), a similar situation may exist in terms of dietary Pb toxicity at the intestine. Longer-term dietary Pb studies, coupled with physiological and histological measurements of potential sublethal impacts, will be required to confirm or refute this hypothesis.

The high Pb burden in the intestine in this study may be due to binding of Pb from the diet by mucus whose secretion may be stimulated by dietary metals (Glover and Hogstrand 2002).

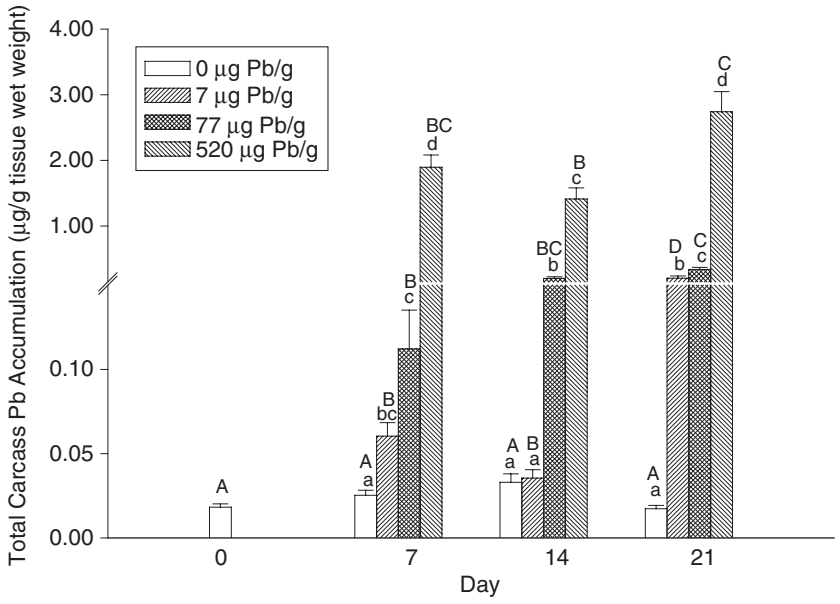


**Fig. 5.** Lead accumulation in the carcass of juvenile rainbow trout exposed to different levels of Pb in the diet for 21 days. Other details as in legend of Figure 1

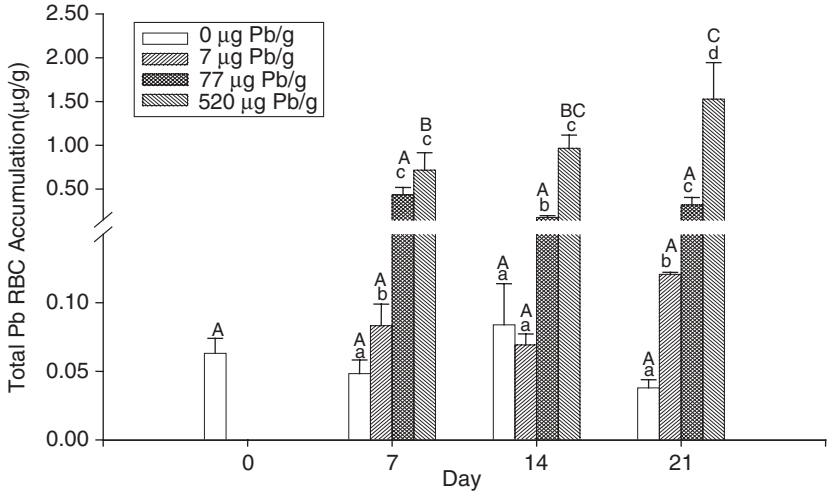


**Fig. 6.** Lead accumulation in the red blood cells of juvenile rainbow trout exposed to different levels of Pb in the diet for 21 days. Other details as in legend of Figure 1

This mucus layer can act to sequester high levels of metals, and thus prevent the exposure of the underlying epithelial tissue to potentially toxic metal levels. Such a scenario has previously been observed in fish exposed to intestinally-perfused zinc (Glover and Hogstrand 2002). The trapped metal burden may subsequently be sloughed off as a result of movement of food through the intestine. As such, the high levels of Pb associated with the intestine, at least in part, may represent Pb that is adsorbed, but not absorbed.

The present results demonstrate that dietary Pb can cross the intestinal epithelium and significantly accumulate in internal soft tissues such as the liver (Figure 3), kidney (Figure 4), and RBCs (Figure 6). When coho salmon (*Oncorhynchus kisutch*) were exposed to 150 µg Pb/L in sea water for 15 days, twice as much Pb was found in the posterior kidney (1.8 µg Pb/g) than in the anterior kidney (0.5 µg Pb/g) (Reichert *et al*. 1979). These findings of significant renal Pb accumulation were confirmed by the present study, where kidney Pb burden data on day 21 in the high dietary Pb treatment was 2.4 µg Pb/g tissue wet weight (Figure 4). Reichert *et al*. (1979) suggested that the high renal Pb concentration may be associated with the excretory/ionoregulatory function of the posterior kidney in fish (Smith and Bell 1976; Reichert *et al*. 1979). In particular, since $Ca^{2+}$ and Pb are believed to be antagonists of one another, these ions may be competing for transport sites at renal tubule cells. This could result in Pb becoming trapped in the tubule cells and result in considerable Pb tissue burden in this tissue.

The lower Pb accumulation of liver (Figure 3), when compared to the intestine, kidney, and gills, suggests that Pb is deposited preferentially into internal soft tissues other than the liver. The low levels of Pb in the liver may be explained by the fact that Pb, in contrast to many other metals, is unable to induce the production of the metal-binding protein metallothionein (MT) in the liver (Reichert *et al*. 1979; Campana *et al*. 2003). Additionally, as mentioned above, Pb may achieve uptake by passage through $Ca^{2+}$ transporters, and thus the presence and quantity of $Ca^{2+}$ uptake pathways may determine tissue-specific Pb distribution.

**Table 3.** Plasma protein, hematocrit (%), and plasma $Ca^{2+}$, $Mg^{2+}$, and Pb concentrations over 21 days in rainbow trout fed different concentrations of Pb in the diet

| Day | Treatment (μg Pb/g dry wt.) | Hematocrit (%) | Plasma Protein (g/100 mL) | Plasma $Ca^{2+}$ (μM) | Plasma $Mg^{2+}$ (μM) | Plasma Pb (μg/L) |
|---|---|---|---|---|---|---|
| 0 | 0 | 39.6 ± 2.4 A | 5.8 ± 0.4 A | 2.1 ± 0.3 A | 0.8 ± 0.1 A | 6.3 ± 1.6 A |
| 7 | 0 | 31.2 ± 1.0 Aa | 5.9 ± 0.3 Aa | 4.4 ± 0.5 Ba | 0.9 ± 0.1 Aa | 7.6 ± 0.5 Ba |
| | 7 | 34.4 ± 1.9 Aa | 5.8 ± 0.3 Aa | 3.6 ± 0.4 Aa | 0.9 ± 0.1 Aa | 5.5 ± 1.1 Aa |
| | 77 | 32.9 ± 4.2 Aa | 5.0 ± 0.4 Aa | 2.9 ± 0.2 Ab | 1.0 ± 0.1 Aa | 4.5 ± 1.0 Aa |
| | 520 | 38.2 ± 3.2 Aa | 5.3 ± 0.5 Aa | 2.8 ± 0.2 Ab | 1.1 ± 0.1 Aa | 12.3 ± 3.1 Aa |
| 14 | 0 | 31.0 ± 2.4 Aa | 6.3 ± 0.2 Aa | 4.4 ± 0.3 Ba | 1.6 ± 0.2 Aa | 16.5 ± 3.1 Ba |
| | 7 | 33.1 ± 1.2 Aa | 6.4 ± 0.6 Aa | 4.4 ± 0.3 Aa | 1.2 ± 0.2 Aa | 6.3 ± 0.9 Ab |
| | 77 | 30.4 ± 2.0 Aa | 5.4 ± 0.6 Aa | 3.1 ± 0.3 Aa | 0.9 ± 0.2 Aab | 10.1 ± 4.0 Aab |
| | 520 | 32.0 ± l.6 Aa | 6.1 ± 0.3 Aa | 4.5 ± 0.3 Ba | 0.7 ± 0.1 Ab | 14.8 ± 3.3 Aa |
| 21 | 0 | 35.9 ± 1.6 Aa | 6.2 ± 0.2 Aa | 4.6 ± 0.2 Ba | 1.3 ± 0.3 Aa | 4.1 ± 0.7 Aa |
| | 7 | 32.8 ± 1.6 Aa | 5.3 ± 0.2 Ab | 3.7 ± 0.3 Aa | 0.9 ± 0.1 Aa | 6.0 ± 0.7 Aab |
| | 77 | 31.4 ± 1.6 a | 5.6 ± 0.2 Aab | 3.8 ± 0.4 Aa | 1.0 ± 0.2 Aa | 7.1 ± 0.9 Ab |
| | 520 | 31.3 ± 1.4 Aa | 6.4 ± 0.3 Aa | 4.1 ± 0.5 Ba | 1.1 ± 0.2 Aa | 11.9 ± 1.8 Ac |

Values sharing lowercase letters are not significantly different from other treatment values within the same week ($p < 0.05$). Values sharing uppercase letters are not significantly different from other treatment values between days.

**Table 4.** $Na^+$ and $Ca^{2+}$ influx rates throughout the course of the experiment

| Day | Treatment (μg Pb/g) | Ca (μmol/kg h) | Na (μmol/kg/h) |
|---|---|---|---|
| 0 | 0 | 128.4 ± 31.8 | 185.9 ± 18.1 |
| 8 | 0 | 156.6 ± 13.9 a | 344.0 ± 37.1 a |
| | 7 | 123.1 ± 16.7 a | 392.1 ± 55.3 ab |
| | 77 | 146.0 ± 14.5 a | 441.7 ± 30.4 ab |
| | 520 | 137.7 ± 26.1 a | 512.1 ± 45.2 b |
| 22 | 0 | 126.2 ± 5.0 a | 186.3 ± 22.8 a |
| | 7 | 145.3 ± 16.0 a | 242.2 ± 29.1 a |
| | 77 | 129.6 ± 11.7 a | 167.4 ± 10.6 a |
| | 520 | 113.4 ± 14.0 a | 195.5 ± 29.8 a |

Values sharing lowercase letters are not significantly different from other treatment values within the same week ($p < 0.05$).

When net Pb retention from the diet over 21 days in the whole fish was calculated, the low Pb diet had the highest net Pb retention (4.8%) followed by the high Pb exposure (1.1%) and the intermediate Pb exposure (0.8%). Therefore, the higher the dietary Pb concentration, the lower the proportion of dietary Pb retained, suggesting that Pb levels may be regulated in terms of reduced Pb absorption and/or an increased Pb excretion. These accumulation results contrast with the study of Hodson *et al*. (1978), who found that dietary Pb (up to 118 μg Pb/g for 32 weeks) was not taken up by juvenile rainbow trout, and did not affect the fish at all. The difference may be that the $Pb(NO_3)_2$ in the Hodson *et al*. (1978) study was added to commercial trout chow combined with beef liver protein. It may be that the beef liver, which is high in iron (Fe), may have reduced Pb bioavailability. Evidence in mammals suggests that Fe deficiency enhances dietary Pb uptake, probably via reduced competition for uptake via the divalent metal ion transporter (Bressler *et al*. 2004). This would explain why Hodson *et al*. (1978) had a significant decrease in blood iron in fish exposed to dietary Pb and not waterborne Pb.

In mammals, under steady state conditions, about 96% of Pb in the whole blood is in the RBCs (World Health Organization 1995). In this study, 99% of Pb was in the RBCs on day 21 in fish fed the 520-μg Pb/g diet. Given that there was an increase in RBC Pb (Figure 6), a portion of Pb accumulation in the various tissues may be a function of vascularization. Figure 7 plots the accumulation of Pb in each tissue (gill, intestine, liver, and kidney) that may be explained by accounting for the amount of Pb present in trapped RBCs in the tissue, using estimates of $^{51}Cr$ RBC spaces from Olson (1992). In all three Pb diet treatments, about 9–14% of the Pb accumulated in the kidney is estimated to be the result of trapped RBCs in this tissue, whereas 86–91% of Pb accumulation in the kidney is truly in the renal tissue. Comparable figures for the liver and gill were only 1–3% in the trapped RBCs. In the intestine, 0.3% of Pb accumulated is the result of vascularization in terms of trapped RBC, while the rest has been built up in the intestinal tissues. These data coupled with the demonstrated capacity of the fish to take up Pb suggests that more studies need to be done in order to determine if exposure to dietary Pb over a longer period of time has a toxic action in fish.

$Ca^{2+}$, a tightly regulated ion in freshwater fish, is continuously absorbed from the water via the gills (Flik and Verbost 1993). A significant decrease in plasma $Ca^{2+}$ levels in the intermediate and high Pb diets occurred on day 7 (Table 3). However, $Ca^{2+}$ levels recovered thereafter. Notably, this recovery occurred without any change in $Ca^{2+}$ influx rates from the water (Table 4). Studies have shown decreased plasma $Ca^{2+}$ levels in rainbow trout exposed to waterborne Pb, indicating the presence of a $Ca^{2+}$/Pb interaction at the gills (Rogers *et al*. 2003, 2005; Rogers and Wood 2004). The significant decrease in plasma $Ca^{2+}$ levels in this study may be the result of a $Ca^{2+}$/Pb interaction at the intestine (see discussion below), which in addition to the gill may act as an important $Ca^{2+}$ uptake route (Flik and Verbost 1993). In addition, the intermediate and high dietary Pb treatment groups exhibited a significant decrease in $Mg^{2+}$ levels on day 14 with stabilization thereafter. This is not surprising since most $Mg^{2+}$ normally comes from the diet in fish (Bijvelds *et al*. 1998), and the presence of metals such as Pb will potentially decrease both $Mg^{2+}$ and $Ca^{2+}$ absorption at the intestine.

Although there was an increase in Pb accumulation in the liver, the organ where the majority of plasma proteins are formed, there were no significant differences in plasma protein concentrations among the treatments. The majority of Pb was bound to the RBCs (Figure 6), but there was a small but significant increase by day 21 in the plasma Pb levels in trout on the high Pb diet (Table 4). Possibly the RBCs may have

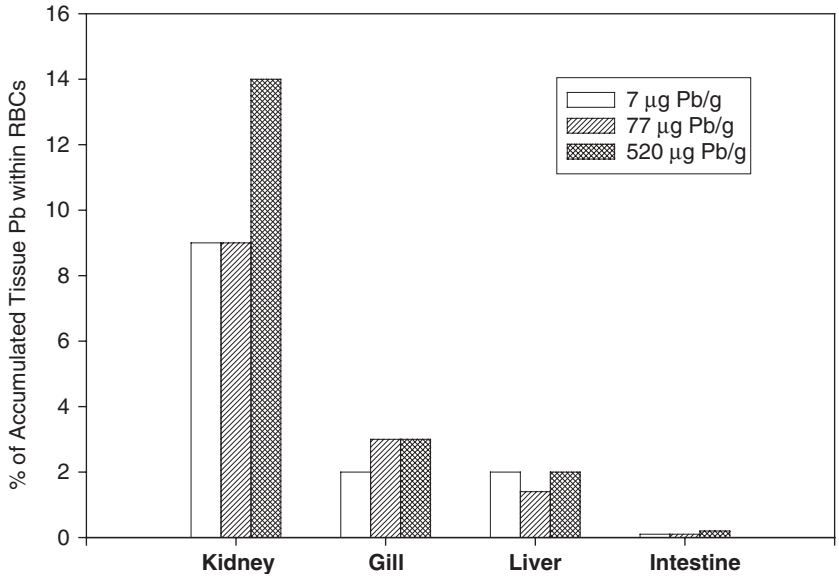


**Fig. 7.** Percentage of accumulated Pb burden explained by Pb within the trapped RBCs on day 21. The intestine does not include the pyloric ceca

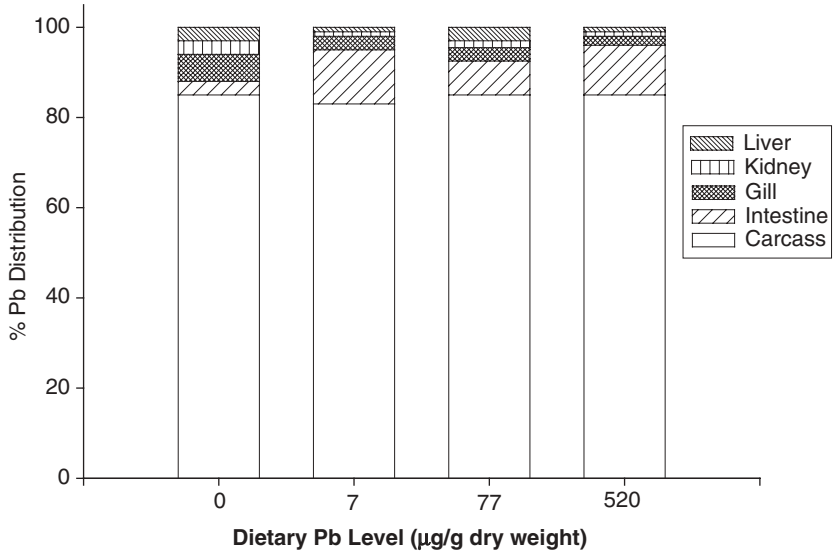


**Fig. 8.** Percent distribution of total Pb burden on day 21 in different tissues of juvenile rainbow trout fed different Pb diets

become saturated with Pb. Manton and Cook (1984) showed that human RBCs have the capacity to fully bind Pb, at blood Pb levels up to about 2.4 µmol Pb/L (0.5 µg Pb/g). However, when blood Pb concentrations are above this level there is an increase in serum Pb levels. The data in the present study suggest that the RBCs in trout have the capacity to bind about 4.8 µmol Pb/L (1.0 µgPb/g) (Figure 6), as there was a significant increase in plasma Pb beyond this level (after 21 days in trout fed the high dietary Pb treatment). The ability of trout to potentially bind more Pb in the RBCs, may be related to the high oxygen carrying capacity (higher hematocrit), and longer life span of the erythrocytes (150 days), when compared to humans (Bushnell *et al*. 1985; Fange 1992).

Hematocrit was not affected in this study in contrast to the waterborne Pb study of Hodson *et al*. (1978). This suggests that the under the conditions tested in the current experiment, anemia was not induced. In future studies, it will be of interest to directly assess possible dietary Pb effects on ALAD, since waterborne Pb has been shown to effect ALAD activity (Hodson *et al*. 1978).

The physiological mechanism of acute waterborne Pb toxicity has been characterized as an inhibition of $Ca^{2+}$ uptake and an inhibition of the $Na^+$, $K^+$-ATPase and carbonic anhydrase enzymes at the gill epithelium, which result in disruptions in $Ca^{2+}$ homeostasis and in $Na^+$ and $Cl^-$ regulation (Rogers *et al*. 2003, 2005; Rogers and Wood 2004). In contrast, dietary Pb did not inhibit $Ca^{2+}$ or $Na^+$ influx rates. Rogers *et al*. (2003) found that $Ca^{2+}$ and $Na^+$ influx rates of juvenile rainbow trout were inhibited by 65% and 50%, respectively, during waterborne Pb exposures of 890 to 1200 µg/L. The gills accumulated close to 200 µg Pb/g at 96 h LC50 waterborne Pb concentrations of 1004 µg/L in the same study. Based on the above studies, it seems likely that $Ca^{2+}$ or $Na^+$ influx rates would not be expected to change during the present dietary exposure, since the gill Pb burden was only about 2.1 µg/g at day 21 in the highest dose treatment.

Nevertheless, waterborne Pb studies showing major impacts on $Ca^{2+}$ and $Na^{+}$ influx rates at the gill suggest that future experiments that involve dietary Pb exposure in fish should consider measuring $Ca^{2+}$ and $Na^{+}$ influx rates at the intestine, as well as intestinal $Na^{+}$, $K^{+}$ ATPase activity, since the intestine in this study had the greatest Pb burden, which could potentially interfere with $Ca^{2+}$ and $Na^{+}$ uptake at the intestine.

In summary, the present study provides evidence that dietary Pb, at levels representative of those in naturally contaminated diets, does accumulate in the internal tissues of rainbow trout. Nevertheless, physiological disturbances were minimal, and feeding, growth, and food conversion efficiency were relatively unaffected over the 21 days of the experiment. Effects of this level of accumulation over a longer timeframe, and the possibility of neurological and reproductive effects have not been evaluated. This present study has provided a basis to explore chronic dietary Pb effects on membrane transport in the intestine, and, given the high accumulation in RBCs, its potential effect on the heme synthesis pathway in future studies.

*Acknowledgments.* This work was supported by the Natural Sciences and Engineering Research Council of Canada CRD Program, the International Lead Zinc Research Organization, the Nickel Producers Environmental Research Association, the International Copper Association, the Copper Development Association, Teck-Cominco, Noranda-Falconbridge, and Inco. We thank Dr. Peter Chapman (Golder Associates) and Dr. Walter Kuit (Teck-Cominco) for helpful comments on the manuscript, and two anonymous reviewers for constructive criticisms. C.M.W. is supported by the Canada Research Chair Program.

## References

Alexander JB, Ingram GA (1980) A comparison of five of the methods commonly used to measure protein concentration in fish sera. J Fish Biol 16:115–122

Bijvelds MJ, Van der Velden JA, Kolar ZI, Flik G (1998) Magnesium transport in freshwater teleosts. J Exp Biol 201:1981–1990

Bressler JP, Olivi L, Cheong JH, Kim Y, Bannon D (2004) Divalent metal transporter 1 in lead and cadmium transport. Ann NY Acad Sci 1012:141–152

Bushnell PG, Nikinmaa M, Oikari A (1985) Metabolic effects of dehydroabietic acid on rainbow trout erythrocytes. Comp Biochem Physiol C 81:391–394

Campana O, Sarasquete C, Blasco J (2003) Effect of lead on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish *Halobatrachus didactylus*. Ecotox Environ Safety 55:116–125

Crespo S, Nonnotte G, Colin DA, Leray L, Nonnotte L, Aubree A (1986) Morphological and functional alterations induced in trout intestine by dietary cadmium and lead. J Fish Biol 28:69–80

Davies PH, Goettl JR Jr, Sinley JR, Smith NF (1976) Acute and chronic toxicity of lead to rainbow trout, *Salmo gairdneri*, in hard and soft water. Water Res 10:199–206

Demayo A, Taylor MC, Taylor KW, Hodson PV (1982) Toxic effects of lead and lead compounds on human health, aquatic life, wildlife, plants, and livestock. CRC Crit Rev Environ Cont 12:257–305

Fange R (1992) Fish Blood Cells. In: Hoar WS, Randall DJ, Farrell AP, (eds) Fish Physiology, Vol. XII, part B: The cardiovascular system. Academic Press, San Diego, pp 1–54

Farag AM, Woodward DF, Brumbaugh W, Goldstein JN, MacConnell E, Hogstrand C, Barrows FT (1999) Dietary effects of metals-contaminated invertebrates from the Coeur d' Alene River, Idaho on cutthroat trout. Trans Am Fish Soc 128:578–592

Farag AM, Boese CJ, Woodward DF, Bergman HL (1994) Physiological changes and metal tissue accumulation in rainbow trout exposed to foodborne and waterborne metals. Environ Toxicol Chem 13:2021–2029

Flik G, Verbost PM (1993) Calcium transport in fish gills and intestine. J Exp Biol 184:17–29

Flegal AR, Rosman KJK, Stephenson MD (1987) Isotope systematics of contaminant leads in Monterey Bay. Environ Sci Technol 21:1075–1079

Franklin NM, Glover CN, Nicol JA, Wood CM (2005) Calcium/cadmium interactions at uptake surfaces in rainbow trout: waterborne versus dietary routes of exposure. Environ Toxicol Chem 24:2954–2964

Glover CN, Hogstrand C (2002) *In vivo* characterisation of intestinal zinc uptake in freshwater rainbow trout. J Exp Biol 205:141–150

Hodson PV, Blunt BR, Spry DJ (1978) Chronic toxicity of waterborne and dietary lead to rainbow trout (*Salmo gairdneri*) in Lake Ontario water. Water Res 12:869–878

Holcombe GW, Benoit DA, Leonard EN, McKim JM (1976) Long term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). J Fish Res Bd Can 33:1731–1741

Manton WI, Cook JD (1984) High accuracy (stable isotope dilution) measurements of lead in serum and cerebrospinal fluid. Br J Ind Med 41:313–319

Mount DR, Barth AK, Garrison TD, Barten KA, Hockett JR (1994) Dietary and waterborne exposure of rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, lead and zinc using a live diet. Environ Toxicol Chem 13:2031–2041

Olson KR (1992) Blood and extracellular fluid volume regulation: role of the renin-angiotensin system, kallikrein-kinin system, and atrial natriuretic peptides. In: Hoar WS, Randall DJ, Farrell AP, (eds) Fish Physiology, Vol. XII, part B: The cardiovascular system. Academic Press, San Diego, pp 136–232

Reichert WL, Federighi DA, Malins DC (1979) Uptake and metabolism of lead and cadmium in coho salmon (*Oncorhynchus kisutch*). Comp Biochem Physiol 63C:229–234

Ricker WE (1979) Growth rates and models. In: Hoar WS, Randall DJ, Bretts, (eds) Fish Physiology, Vol. VIII: Bioenergetics and growth. Academic Press, Florida, pp 677–743

Rogers JT, Wood CM (2004) Characterization of branchial lead-calcium interactions in freshwater rainbow trout (*Oncorhynchus mykiss*). J Fish Biol 207:813–825

Rogers JT, Richards JG, Wood CM (2003) Ionoregulatory disruption as the acute toxic mechanism for lead in rainbow trout (*Oncorhynchus mykiss*). Aquat Tox 64:215–234

Rogers JT, Patel M, Gilmour KM, Wood CM (2005) Mechanisms behind Pb-induced disruption of $Na^{+}$ and $Cl^{-}$ balance in the rainbow trout (*Oncorhynchus mykiss*). Am J Physiol R 289:R463–472

Smith LS, Bell GR (1976) A practical guide to the anatomy and physiology of Pacific salmon. Fish Mar Serv Misc Spec Publ 27:14

Sorensen EM (1991) Lead. In: Sorensen EM (ed) Metal poisoning in fish. CRC Press, Boca Raton, pp 95–118

Van Ginneken L, Blust R (1995) Sequential determination of a combined $\gamma/\beta$ and pure $\beta$-emitter by gamma and liquid scintillation counting: application to the transport of metal across fish gills. Anal Biochem 224:92–99

Varanasi U, Gmur DJ (1978) Influence of water-borne and dietary calcium on uptake and retention of lead by coho salmon (*Oncorhynchus kisutch*). Tox Appl Pharm 46:65–75

Woodward DF, Brumbaugh WG, DeLonay AJ, Little EE, Smith CE (1994) Effects on rainbow trout fry of metals-contaminated diet

of benthic invertebrates in the Clark Fork River, Montana. Trans Am Fish Soc 123:51–62

Woodward DF, Farag AM, Bergman HL, DeLonay AJ, Little EE, Smith CE, Barrows FT (1995) Metals-contaminated benthic invertebrates in the Clark Fork River, Montana: effects on age-0 brown trout and rainbow trout. Can J Fish Aquat Sci 52:1994–2004

World Health Organization (1995) Environmental Health Criteria 165. International Programme on Chemical Safety. Geneva