ELSEVIER

Aquatic Toxicology 69 (2004) 149–163

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Gastrointestinal uptake and fate of cadmium in rainbow trout acclimated to sublethal dietary cadmium

M.J. Chowdhury*, D.G. McDonald[1], C.M. Wood

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ont., Canada L8S 4K1

Received 21 November 2003; received in revised form 12 May 2004; accepted 13 May 2004

Abstract

Adult rainbow trout were pre-exposed to a sublethal concentration of dietary Cd (500 mg/kg dry wt.) for 30 days to induce acclimation. A gastrointestinal dose of radiolabeled Cd (276 μg/kg wet wt.) was infused into the stomach of non-acclimated and Cd-acclimated trout through a stomach catheter. Repetitive blood samples over 24 h and terminal tissue samples were taken to investigate the gastrointestinal uptake, plasma clearance kinetics, and tissue distribution of Cd. Only a small fraction of the infused dose (non-acclimated: 2.4%; Cd-acclimated: 6.6%) was internalized across the gut wall, while most was bound in the gut tissues (10–24%) or remained in the lumen (16–33%) or lost from the fish (∼50%) over 24 h. Cadmium loading during pre-exposure produced a profound increase of total Cd in the blood plasma (∼28-fold) and red blood cells (RBC; ∼20-fold). The plasma Cd-time profiles consisted of an apparent rising (uptake) phase and a declining (clearance) phase with a maximum value of uptake in 4 h, suggesting that uptake of gastrointestinally infused Cd was very rapid. Acclimation to dietary Cd did not affect plasma Cd clearance (∼0.5 ml/min), but enhanced new Cd levels in the plasma (but not in the RBC), and resulted in a longer half-life for plasma Cd. Tissue total and new Cd levels varied in different regions of the gastrointestinal tract, and overall levels in gut tissues were much greater than in non-gut tissues, reflecting the Cd exposure route. Dietary Cd, but not the infused Cd, greatly increased total Cd levels of all gut tissues in the order posterior-intestine (640-fold) > cecae (180-fold) > mid-intestine (94-fold) > stomach (53-fold) in Cd-acclimated fish relative to naïve fish. Among non-gut tissues in the Cd-acclimated fish, the great increases of total Cd levels were observed in the liver (73-fold), kidney (39-fold), carcass (35-fold), and gills (30-fold). The results provide some clear conclusions that may be useful for environmental risk assessment of dietary Cd exposure in fish.

Keywords: Dietary; Cadmium; Acclimation; Chronic; Kinetics; Trout; Plasma; RBC; Clearance

1. Introduction

Cadmium (Cd) is a ubiquitous metal that can be found in the aquatic environment from anthropogenic sources such as metal mining and processing, and agricultural use of pesticides. Cadmium is toxic to fish even at low concentrations (Sorensen, 1991; Wood, 2001), the freshwater quality criteria values for the protection of aquatic life in North America are less

* Corresponding author. Present address: USEPA - MED, 6201 Congdon Boulevard, Duluth, MN 55804, USA.
Tel.: +1 218 529 5221.
E-mail addresses: chowdhury.mohammed@epamail.epa.gov (M.J. Chowdhury), gomcdona@uoguelph.ca (D.G. McDonald).
[1] Present address: Department of Zoology, University of Guelph, Guelph, Ont., Canada.

0166-445X/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2004.05.002

than 1 μg/L at low hardness (USEPA, 2001; CCME, 2002).

Freshwater fish can take up waterborne Cd via the gills and dietary Cd via the gut. The mechanism of uptake for both routes is considered to be the nonselective uptake of Cd through calcium uptake systems (Handy, 1996; Wood, 2001). After uptake across gill or gut epithelia, Cd is probably bound to transport proteins in the blood plasma as in higher vertebrates (Scott and Bradwell, 1984; Zalups and Ahmad, 2003) and distributed via the circulatory system to various internal organs where toxicological responses may be elicited (Roesijadi and Robinson, 1994). Cadmium accumulates in organs such as the gills, liver, kidney, and gastrointestinal tract of fish in an unregulated manner (Harrison and Klaverkamp, 1989; McGeer et al., 2000; Chowdhury et al., 2003). In mammals, accumulated Cd is excreted in the bile, urine, and faeces with the loss of adsorbed Cd due to sloughing of mucosal cells (Andersen, 1989; Zalups and Ahmad, 2003). In fish, a large proportion of dietary Cd excretion occurs as a result of intestinal sloughing and a small quantity is probably excreted via the bile and gills (Handy, 1996).

Currently most of our knowledge on the bioavailability and toxicity of Cd to fish is based on waterborne exposure experiments and as a result, the guidelines and criteria for Cd in aquatic systems are primarily based on the same (Sorensen, 1991; Wood, 2001; USEPA, 2001; CCME, 2002). Similarly, most of the studies on the effects of pre-exposure or acclimation focused only on waterborne metals (Hollis et al., 1999, 2001; Stubblefield et al., 1999; McGeer et al., 2000; Grosell et al., 2001; Wu and Hwang, 2003; Chowdhury et al., 2003). In contrast, the effects of dietary metals in fish are not well known (Handy, 1996), and as a result regulatory guidelines do not directly consider the potential toxic effects of chronic dietary loading. However, the transfer of Cd through food chains can be high enough to bring about toxic levels of Cd accumulation in fish tissues (Dallinger and Kautzky, 1985; Farag et al., 1994). Indeed, Cd-contaminated live food or prepared diet has been found to contribute greatly to metal accumulation in trout (Harrison and Klaverkamp, 1989; Harrison and Curtis, 1992; Woodward et al., 1995; Farag et al., 1999) and in carp (Kraal et al., 1995). Szebedinszky et al. (2001) showed that fish acclimated to dietary Cd via chronic exposure accumulated a much greater overall Cd body burden relative to fish acclimated to waterborne Cd. Furthermore, these "dietary acclimated" fish exhibited altered uptake dynamics for waterborne Cd at the gills, and increased tolerance (expressed as 96 h LC50) to waterborne Cd.

To date, nothing is known in fish about the influence of dietary Cd acclimation on the subsequent handling, uptake, and tissue-specific disposition of metal dosed into the gastrointestinal tract. Therefore, the primary goal of the present study was to compare the responses of rainbow trout to a gastrointestinal dose of radiolabelled Cd after 30 days pre-exposure to either a sublethal level of "cold" Cd in the diet (500 mg/kg diet, Cd acclimated group) or to a control, nominally Cd-free diet (non-acclimated group). The study allowed an analysis of the kinetics of gastrointestinal uptake and plasma clearance in the two groups, as well as a quantitative analysis of tissue-specific Cd disposition. This served as a follow-up of our previous investigation on the effects of waterborne Cd acclimation on plasma clearance and tissue uptake of intra-arterially injected "new" Cd (Chowdhury et al., 2003).

2. Materials and methods

2.1. Experimental animals

Rainbow trout (*Oncorhynchus mykiss*; range 190–280 g) were obtained from Humber Springs Trout Hatchery (Mono Mills, Ont., Canada) and held under laboratory conditions for at least two weeks before experimental use. Fish were held in 500-L tanks, each supplied with a minimum of 2.5 L/min flow-through of moderately hard dechlorinated Hamilton City tapwater (0.5 nM Cd; 47 nM Cu; 270 nM Zn; 1 mM Ca; 0.6 mM Na; 0.7 mM Cl). The temperature and pH of water were kept at 12 ± 1 °C and 8.0 ± 0.2, respectively, throughout the experiment. The fish were fed commercial, floating trout chow (Martin's Feed Mills, Ont., Canada) at a ration of 1.5% of their body weight three times per week.

2.2. Acclimation to dietary cadmium

For acclimation to dietary Cd, 40 fish in one holding tank (Cd-acclimation group) were fed a 1.5% daily ration (dry feed/wet body wt.) of a prepared Cd-diet containing nominally 500 mg Cd/kg dry feed for 30

days (actual measured concentration: 419.5 ± 2.3 mg Cd/kg food or 6.29 mg Cd/kg fish/day). Another holding tank was used simultaneously as a control tank in which 40 fish (non-acclimation group) were fed a prepared control diet with no added Cd (actual measured concentration: 0.1617 ± 0.0035 mg Cd/kg) at the same ration. Water samples from both tanks were taken every second day, acidified with HNO_3 (1%), and analyzed for Cd by graphite atomic absorption spectroscopy (graphite-AAS; SpectrAA-220, Varian, Mississauga, Ont., Canada). The measured Cd concentrations in water filtered through a 0.45 μm filter were 0.14 ± 0.01 μg/L (1.21 ± 0.07 nmol/L) and 0.22 ± 0.01 μg/L (1.96 ± 0.11 nmol/L) in non-acclimation and Cd-acclimation tanks, respectively. The fish were not fed for 48 h prior to surgery.

Specific growth rates of fish (SGRs in % per day) during the 30 days acclimation were determined from the natural log (ln) of fish weights:

$$\mathrm{SGR} = \left[\frac{1}{30}(\ln w_{30} - \ln w_0)\right] \times 100 \quad (1)$$

where w_0 is the average initial weight (g) determined at day 0 from the bulk weight of 20 fish selected randomly from both tanks and w_{30} is the individual final weight (g) of each fish determined at day 30 before cannulation.

2.3. Diet preparation

Cadmium diet was prepared by mixing a concentrated stock of $Cd(NO_3)_2$ $4H_2O$ (6.86 g/L; Fisher Scientific, Ont., Canada) into the trout chow (Martin's Feed Mills) containing 42% crude protein, 16% crude fat, 40% crude carbohydrate, 0.35% sodium, 1% calcium, and 0.65% phosphorus. Trout chow (∼500 g) was ground in a blender and mixed with an appropriate amount of the $Cd(NO_3)_2$ solution (∼100 ml) in a commercial pastamaker (Popiel Ronco, Compton, CA, USA) for at least 30 min. Subsequently, 200 ml of deionized water was added while mixing for another 30 min and the paste was extruded from the pastamaker into long strings. The hydrated feed strings were dried at 60 °C for 10 h and then broken into small pellets (∼6–10 mm in length and ∼6 mm in diameter). The control diet was prepared following the same method but without the addition of Cd.

2.4. Cannulation

While under MS-222 anesthesia (0.1 g/L) on an operating table, fish (non-acclimated: 344 ± 17 g; Cd-acclimated: 324 ± 10 g) were cannulated by surgically implanting two catheters (PE-50 polyethylene tubing, Intramedic, Becton Dickinson, Sparks, MD, USA; internal diameter 0.58 mm, external diameter 0.97 mm, 30 cm long). One was inserted down the oesophagus for dosing of radiolabelled Cd solution into the stomach (stomach catheter, Clearwater et al., 2000) and the other into the dorsal aorta for sampling blood (arterial catheter, Soivio et al., 1972). After cannulation each fish was placed in an aerated experimental chamber (2.5 L) supplied with flowing water (250 ml/min).

2.5. Experimental procedures

After 36 h recovery from surgery, an initial control blood sample (200 μL) was obtained via the arterial catheter and replaced with Cortland saline (Wolf, 1963). Immediately after this sampling, fish were given a single dose (276 μg/kg or 2455.3 nmol/kg) of radioactive Cd (^{109}Cd) in glucose solution into the stomach via stomach catheter. The solution was prepared by adding ^{109}Cd stock ($^{109}CdCl_2$; specific activity: 102.2 kBq/μg; NEN Life Science Products, Boston, MA, USA) to 0.1 M glucose solution containing an appropriate concentration of stable Cd to obtain a final radioactivity of 1081 kBq/ml and a total Cd concentration of 165.5 μg/ml (1472.3 nmol/ml).

After infusion of radiolabelled Cd into the stomach, blood samples were taken following the method used previously for measuring intra-arterially injected Cu and Cd in trout plasma (Grosell et al., 2001; Chowdhury et al., 2003). Approximately 300 μl of blood was removed via the catheter at 0.5, 1, 1.5, 2, 4, 6, 9, 12, 14, 21, and 24 h after gastrointestinal infusion and replaced with an equal volume of Cortland saline (Wolf, 1963). A preliminary experiment indicated that an apparent uptake phase and a clearance (distribution) phase of internalized plasma Cd existed over this time course. All blood samples were centrifuged (3 min at 14,000 × *g*) to separate plasma from blood cells and analyzed for radioactivity and total Cd concentration in both compartments of the blood.

After the final blood sampling (24 h), the fish were killed immediately by an overdose of MS-222 (0.6 g/L) and dissected to obtain tissue samples. The liver, gall bladder including bile, gill filaments, kidney, and brain were obtained as whole organs. For muscle, the skin was removed from one side of the fish and a sample of white muscle (6–10 g) was collected. For gut tissues, the whole gastrointestinal tract was removed from the body cavity and cut into four pieces: whole stomach, anterior intestine including pyloric cecae (hereafter referred to as cecae), mid-intestine, and posterior intestine. The gut tissues were rinsed with distilled water to clear mucus, food fragments, and non-absorbed Cd from the lumen, and analyzed individually. The rinsing wash was saved in plastic vials and measured for radioactivity to determine the amount of the radiolabelled Cd dose still remaining in the gut cavity (lumen). The proportion of Cd dose that might be bound too strongly to the mucosal surface of the gut to be recovered by rinsing (cecae = 5.6%, mid-intestine = 3.6%, posterior intestine = 0.9%, whole gut = 3%; Ojo and Wood, unpublished data), was not quantified in the analysis and thus was assigned to the gut tissue (not in the gut lumen). In order to determine background concentrations of "cold" Cd in the tissues before dosing, six non-acclimated and six Cd-acclimated fish were sampled directly from the holding tanks, and tissue samples were obtained as above. All radioactive tissue samples were counted for gamma radioactivity, and all tissue samples were thereafter digested for the determination of total Cd concentrations.

After removal of the above tissue samples, the fish carcass was homogenized in a blender with 500 ml of 1N HNO_3 (trace metal grade, Fisher Scientific). Three aliquots of approximately 15 ml of the suspension for each fish were collected in plastic vials and counted for gamma radioactivity. Subsequently, the suspension was digested by placing the vials in an oven at 60 °C for 48 h to determine total Cd in the carcass.

2.6. Measurements and calculations

All radioactive samples were counted in a Minaxi-γ Auto-gamma 5530 counter (Canberra Packard, Mississauga, Ont., Canada) to determine ^{109}Cd radioactivity. After counting, RBC and tissue samples were digested in approximately three to five volumes of 1N HNO_3 at 60 °C for 48 h. Concentrations of total Cd were measured by atomic absorption spectrophotometry (SpectrAA-220, Varian, Mississauga, Ont., Canada) with graphite furnace atomization. Absorbance of the appropriately diluted unknown samples (duplicate or triplicate) was related to the absorbance of a series of known standards made from a certified stock solution of Cd (Fisher Scientific) to obtain Cd concentrations expressed on the basis of per-gram wet tissue and RBC, per-milliliter plasma, or per-liter water.

Concentrations of radioactive ^{109}Cd in samples were converted to absolute values (ng Cd per unit wet weight or volume) to obtain newly accumulated Cd (ng/g or ng/ml):

$$\text{New Cd} = \frac{R}{M \times \text{SA}} \quad (2)$$

where R is the ^{109}Cd radioactivity in the sample after correction for background and radiodecay (cpm), M the tissue wet mass (g) or plasma volume (ml), and SA is the measured specific activity of radioactive Cd in the infusion solution (cpm/ng total Cd).

To calculate the relative distribution of the infused radiolabelled Cd, the total burdens of new Cd (ng) in the plasma, RBC, sampled tissues, and carcass were converted into the percentage of infused dose. To calculate the total burden in blood compartments, the relative mass of blood in rainbow trout was assumed to be 6.0% (Bushnell et al., 1998), and the RBCs were assumed to represent 30% of the mass of whole blood, the rest being plasma (Gallaugher and Farrell, 1998). Muscle mass was assumed to be 60% of body mass (Giblin and Massaro, 1973). Internalized Cd (%) was the proportion of radiolabelled Cd dose internalized beyond the gut wall and calculated by adding the percentages of new Cd found in non-gut internal tissues, carcass, plasma, and RBCs. Cadmium lost from the fish (%) was calculated by subtracting the percentage of internalized Cd plus that found in gut tissue and gut lumen, from the infused dose (100%).

2.7. Modeling and determination of plasma clearance rate

The plasma clearance of new Cd was determined by fitting the plasma concentration data to a three-compartment pharmacokinetic model (Barron et al., 1990). This is an extension of the

two-compartment model used to analyze the plasma clearance of a Cd bolus injected in the artery of trout (Chowdhury et al., 2003), in which it was assumed that Cd was injected into a central compartment (compartment-1) from which the metal is exchanged with the peripheral compartment (compartment-2). The present model assumes that Cd is absorbed to the central compartment from the gut (compartment-3) and unabsorbed Cd is lost from the system via this compartment. A preliminary analysis of data suggests that the delay time for absorption of Cd from gut lumen was negligible (∼10 min). Assuming that transfer of Cd follows first-order kinetics, Cd fluxes (ng/min/kg) between two compartments are:

$$F_{ij} = k_{ij} q_i \tag{3}$$

where F_{ij} is the flux of new Cd from ith to jth compartment with transfer rate constant k_{ij}, and q_i is the amount of Cd (ng/kg) in ith compartment at time t. Thus the differential equations are

$$\frac{dq_1}{dt} = k_{21}q_2 - k_{12}q_1 - k_{13}q_1 \tag{4}$$

$$\frac{dq_2}{dt} = k_{12}q_1 - k_{21}q_2 \tag{5}$$

$$\frac{dq_3}{dt} = k_{21}q_2 - k_{31}q_3 - k_{30}q_3 + M_{gi} \tag{6}$$

where M_{gi} is the radiolabelled Cd infused into the gut (ng/kg) and k_{30} is the rate constant for Cd lost from the system. The model fitted the plasma concentration data (C_p in ng/ml) using the following equation:

$$C_p = \frac{q_1}{V_p} \tag{7}$$

where V_p is the apparent volume of the central compartment (ml/kg). V_p refers to the distribution volume of new Cd in extracellular fluid in fish and was estimated simultaneously with rate constants as unknown parameters during model fitting. Apparent volumes (ml/kg) of distribution for gut tissues (V_g) and the other peripheral compartment (V_t), which characterize size or storage capacity of the metal in the tissue, were calculated from Barron et al. (1990):

$$V_g = V_p\left(\frac{k_{31}}{k_{13}}\right) \tag{8}$$

$$V_t = V_p\left(\frac{k_{12}}{k_{21}}\right) \tag{9}$$

The clearance rate of new Cd (CL_p), interpreted as the volume of plasma cleared of the metal per unit time per unit body weight (ml/min/kg) and distributed to different tissues, was calculated using the equation:

$$CL_P = V_p(k_{12} + k_{13}) \tag{10}$$

The half-life of new Cd in the plasma ($t_{1/2}$ in min) is proportional to V_p and inversely proportional to clearance (Barron et al., 1990):

$$t_{1/2} = \ln 2 \frac{V_p}{CL_p} \tag{11}$$

A software program for kinetic processes (SAAM II, Washington, USA) was used to formulate the model, and to fit it to the experimental data. A Bayesian approach was used for the estimation of some parameters by assigning the known values from our previous study (Chowdhury et al., 2003).

2.8. Statistical analysis

Data are expressed as mean ±1 S.E. (n) where n is the number of fish. Parameter estimates of the pharmacokinetic model are presented with their 95% confidence intervals obtained during model fitting to experimental data. To analyze differences between plasma and RBC concentration-time profiles in non-acclimated and Cd-acclimated groups, a two-factor ANOVA, with acclimation and time as the main factors, was used. Student's unpaired t-test or Newman–Keuls test was applied to compare tissue levels of total and newly accumulated Cd between acclimated and non-acclimated fish, or to compare them with their controls. Percentages and proportions were subjected to arcsine transformation before statistical analysis. All statistical tests were performed using the computer software Statistica (StatSoft, Tulsa, OK, USA) or InStat (GraphPad, San Diego, CA, USA). A fiducial limit of $P \leq 0.05$ was used throughout.

3. Results

3.1. Growth and mortality

Exposure of trout to 500 mg/kg Cd in the diet resulted in no mortality during the 30 days exposure for acclimation, and there were no obvious changes in ap-

petite or swimming activity. The specific growth rate of Cd-exposed fish (0.68 ± 0.08% per day) was not significantly lower than in non-exposed fish (0.85 ± 0.16% per day). However, the Cd-diet induced some gross morphological changes in the gastrointestinal tract such as epithelial edema, hemorrhage, and increased mucus secretion. Dietary Cd stimulated gut evacuation based on the observation that Cd-exposed fish but not non-exposed fish had empty guts after starvation for the same length of time. No grossly visible changes were observed in the gills of Cd-exposed fish.

3.2. Distribution of the infused Cd dose

Only a small fraction of the radiolabelled Cd dose (276 μg/kg) was absorbed across the gut tissues by 24 h and partitioned amongst internal organs (internalized fraction, Fig. 1A). However, the internalized

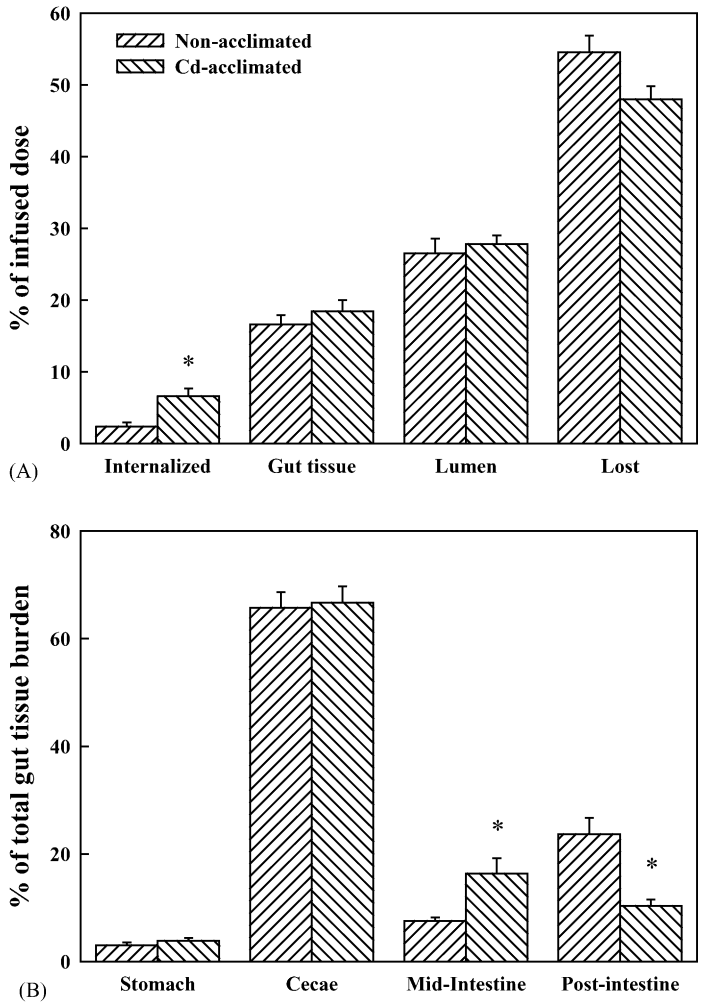

Fig. 1. (A) The percentage of the radiolabelled Cd dose (276 μg Cd/kg wet wt.) distributed to internal non-gut tissues (internalized) and gut tissues, and the percentage of the dose recovered in the lumen or lost, 24 h after radiolabelled Cd infusion into the stomach of non-acclimated and Cd-acclimated trout via a stomach catheter. (B) Relative distribution of infused radiolabelled Cd into gut tissues as percentage of the total radiolabelled Cd burden in gut tissues. The acclimated fish were pre-exposed to dietary stable Cd (500 mg/kg diet) for 30 days. Results are presented as mean ± S.E. ($n = 6$–9). *Significant difference between non-acclimated and Cd-acclimated fish for the same tissue ($P < 0.05$). Post-intestine: posterior-intestine.

fraction in Cd-acclimated fish (6.6 ± 1.1%, $n = 6$) was significantly higher than in non-acclimated fish (2.4 ± 0.6%, $n = 9$). Approximately 17% and 27% of the dose were found in gut tissues and gut lumen, respectively. The fractions bound to gut tissues and found in the lumen were similar in non-acclimated and Cd-acclimated fish. Approximately 50% of the dose was lost from the fish of both groups by 24 h (Fig. 1A).

The distribution of Cd in the gut tissues indicated that most of the infused Cd moved past the stomach by 24 h and distributed along the rest of the gut (Fig. 1B). Stomach contained only a small fraction (non-acclimated: 3.0%, and Cd-acclimated: 3.9%) but the pyloric cecae, mid-intestine, and posterior intestine contained major fractions (66 and 67%, 7 and 16%, and 24 and 10%, respectively) of the total new Cd burden (50.8 ± 4.3 μg/kg fish, $n = 6$ or 9) found in the gut tissues of both groups. Only in the mid-intestine and posterior-intestine were the differences in the new Cd fractions between non-acclimated and Cd-acclimated fish significant, though they were opposite in the two sections (Fig. 1B).

3.3. Plasma Cd kinetics

The background concentrations (mean ± S.E.) of total Cd in plasma were 0.45 ± 0.06 ng/ml ($n = 6$) and 12.47 ± 0.55 ng/ml ($n = 6$) in non-acclimated and Cd-acclimated fish, respectively. Upon a gastrointestinal dose of 276 μg/kg radiolabelled Cd, the total and newly accumulated Cd in plasma increased gradually in time with an apparent maximum at 4 h (non-acclimated total: 4.3 ± 0.5, $n = 8$; non-acclimated new: 4.7 ± 0.7, $n = 7$; Cd-acclimated total: 17.13 ± 1.9, $n = 5$; Cd-acclimated new: 8.3 ± 0.6, $n = 5$ ng Cd/ml), then deceased to approximately background levels by 24 h in both treatment groups (Fig. 2). Thus, plasma Cd kinetics consisted of two phases: one uptake phase and one clearance phase. The mean concentrations of total Cd were 7–15 fold greater in Cd-acclimated fish than in non-acclimated fish (Fig. 2A). The mean concentrations of new Cd were 1.4–2.6 fold greater in Cd-acclimated fish than in non-acclimated fish (both significant overall by ANOVA; Fig. 2B).

The pharmacokinetic model well described the plasma uptake and clearance of Cd infused into the gut through a stomach catheter (Fig. 2B). The parameter

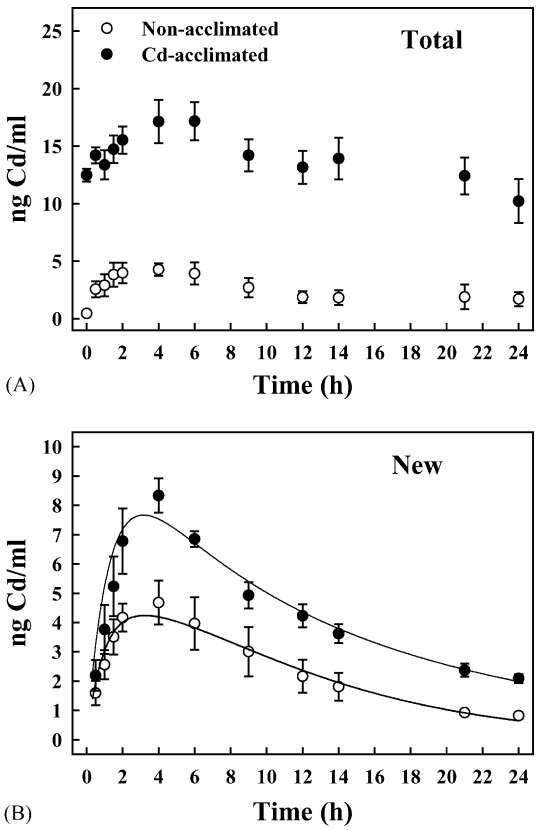

Fig. 2. Plasma concentration–time profile of total Cd (A) and newly accumulated Cd (B) in non-acclimated and Cd-acclimated rainbow trout after a gastrointestinal dose of radiolabelled Cd (276 μg/kg wet wt.) infused into the stomach. The acclimated fish were pre-exposed to dietary stable Cd (500 mg/kg diet) for 30 days. Symbols represent experimental values (mean ± SE, $n = 5$–9). The solid lines in panel (B) are the fit of the pharmacokinetic model (Eqs. (3)–(11)) to the data values. ANOVA revealed significant differences between non-acclimated and Cd-acclimated fish both for total Cd ($P < 0.001$; A) and newly accumulated Cd ($P < 0.05$; B).

estimates from model fitting are presented in Table 1. The plasma clearance rates (Cl_p) of new Cd were similar (~0.50 ml/min/kg) in both groups (Table 1), indicating that acclimation to dietary Cd has no effect on plasma clearance of Cd taken up via the gut. However, the estimated half-life of plasma Cd ($t_{1/2}$) was longer in Cd-acclimated fish (58 min) than in non-acclimated fish (44 min). In Cd-acclimated fish, apparent distribution volumes of the central (V_p) and peripheral (V_t) compartments were larger in compar-

Table 1
Parameter estimates and 95% confidence intervals (in parentheses) for clearance kinetics of newly accumulated Cd from the plasma of non-acclimated and Cd-acclimated trout

Parameters[a]	Non-acclimated	Cd-acclimated
k_{30} (min^{-1})	0.0028 (0.0027–0.0029)	0.0027 (0.0021–0.0033)
k_{31} (min^{-1})	<0.0001	0.0001
k_{13} (min^{-1})	0.008 (0.007–0.009)	0.006 (0.004–0.007)
k_{12} (min^{-1})	0.007 (0.006–0.009)	0.006 (0.005–0.007)
k_{21} (min^{-1})	0.005 (0.004–0.007)	0.002 (0.001–0.003)
V_p (ml/kg)	31 (29–33)	42 (40–44)
V_g (ml/kg)	3295 (3038–3552)	3048 (2458–3637)
V_t (ml/kg)	42 (35–48)	113 (77–149)
Cl_p (ml/min/kg)	0.489 (0.455–0.523)	0.504 (0.449–0.559)
$t_{1/2}$ (min)	44 (39–49)	58 (52–63)

[a] Parameters were estimated by fitting the compartment model (Eqs. (3)–(11)) to data presented in Fig. 2B. k_{ij}: transfer rate constant between compartments where i and j represent compartment numbers; V_p: apparent volume of the central compartment, V_g: apparent volume of the gut compartment, V_t: apparent volume of the peripheral compartment excluding gut; Cl_p: clearance rate of new Cd from the blood plasma; $t_{1/2}$: half-life of new Cd in the blood plasma.

ison to non-acclimated fish. The distribution volume of Cd in the gut compartment (V_g) was many-fold larger than that of the central and peripheral compartments, and indeed larger than the fish itself probably due to extensive binding of Cd in the gut tissues. The distribution volumes of the gut compartment were more or less similar in both treatment groups (Table 1).

3.4. RBC Cd kinetics

Background concentrations (mean ± S.E.) of total Cd in RBCs were 19-fold greater in Cd-acclimated fish relative to control (62.0 ± 3.5 versus 3.2 ± 0.7 ng/g; $n = 6$). The RBC Cd-time profiles (Fig. 3) were different from the plasma Cd-time profiles (Fig. 2). With an infused dose of 276 μg/kg radiolabelled Cd, neither the total nor newly accumulated Cd in RBCs showed any consistent trend of increase or decrease over time, in contrast to plasma. The concentrations of total Cd in RBCs were 7–19 fold greater in Cd-acclimated fish (49–68 ng/g) than in non-acclimated fish (3.2–7.9 ng/g; Fig. 3A). Compared with plasma total Cd (Fig. 2A), the total Cd in RBCs were 1.8–9.0 and 2.7–5.8 fold greater in non-acclimated and Cd-acclimated trout, respectively. In contrast, the concentrations of RBC new Cd in Cd-acclimated fish (0.3–1.2 ng/g) were not significantly different from those in non-acclimated fish (1.4–2.8 ng/g, Fig. 3B). While total Cd levels in RBCs (Fig. 3A) were greater than those in plasma (Fig. 2A), the RBC levels for new Cd (Fig. 3B) were similar to plasma levels in non-acclimated fish and only 7–50% of plasma levels in Cd-acclimated fish (Fig. 2B).

3.5. Concentrations of Cd in gut tissues

The background concentrations of total Cd in non-acclimated (naïve) trout were 22.7 ± 4.7, 94.2 ± 14.7, 63.3 ± 8.0, and 192.0 ± 28.8 ng/g ($n = 6$) in the stomach, pyloric cecae, mid-intestine, and posterior-intestine, respectively (Fig. 4A). In non-acclimated fish, the gastrointestinal infusion of Cd resulted in large significant increases of total Cd in all of these tissues except stomach (cecae: 43-fold, mid-intestine: 30-fold, posterior intestine: 13-fold, and stomach: 5-fold).

Due to loading of dietary Cd during the pre-exposure of 30 days, gut total Cd levels in Cd-acclimated trout were much greater in every tissue in comparison to naïve trout (Fig. 4A). The greatest relative increases were found in the order posterior-intestine (∼640-fold) > cecae (∼180-fold) > mid-intestine (∼94-fold) > stomach (∼53-fold) with observed concentrations of 123.2 ± 14.1, 16.9 ± 3.0, 5.9 ± 0.7, and 1.2 ± 0.1 μg/g Cd ($n = 6$), respectively. However, the infusion of Cd to the Cd-acclimated fish did not result in a significant increase of total Cd in any gut tissue except the mid-intestine. Indeed, the Cd burden in the posterior-intestine of Cd-acclimated fish

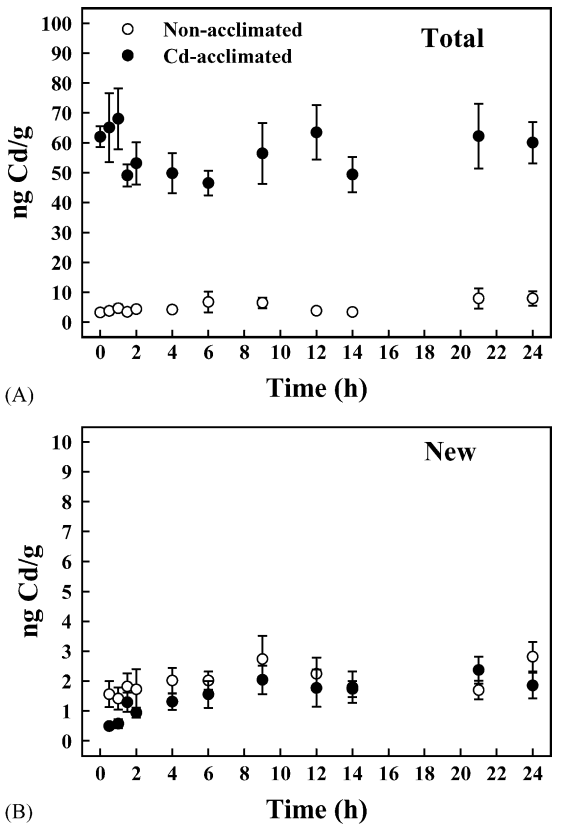

Fig. 3. Red blood cell concentration – time profile of total Cd (A) and newly accumulated Cd (B) in non-acclimated and Cd-acclimated rainbow trout after a gastrointestinal dose of radiolabelled Cd (276 μg/kg wet wt.) infused into the stomach. The acclimated fish were pre-exposed to dietary stable Cd (500 mg/kg diet) for 30 days. Symbols represent experimental values (mean ± SE, $n = 5$–9). ANOVA revealed significant differences between non-acclimated and Cd-acclimated fish for total Cd ($P < 0.001$; A), but not for newly accumulated Cd ($P > 0.05$; B).

was reduced by approximately 78% over the period of 24 h after infusion (Fig. 4A).

The pattern of new Cd accumulation was more or less similar to that of total Cd in all gut tissues except posterior-intestine (Fig. 4B). The higher concentrations of new Cd were observed in the stomach, cecae, and mid-intestine of Cd-acclimated fish in comparison to non-acclimated fish, although a significant difference was only seen in the latter two tissues. Exceptionally, the new Cd burden of posterior-intestine was smaller in Cd-acclimated fish than in non-acclimated fish (Fig. 4B).

3.6. Concentrations of Cd in non-gut, internal tissues

Total Cd levels in every (non-gut) tissue except muscle and bile were higher in Cd-acclimated fish in comparison to the non-acclimated control group (Fig. 5A). In Cd-acclimated fish, the greatest relative increases of tissue total Cd were in the order liver (73-fold) > kidney (39-fold) > carcass (35-fold) > gills (30-fold), with observed concentrations of 1.3 ± 0.1, 2.0 ± 0.4, 0.08 ± 0.01, and 0.82 ± 0.04 μg/g Cd ($n = 6$), respectively. However, in neither non-acclimated fish nor Cd-acclimated fish, did total Cd levels of internal tissues show any significant increases attributable to Cd infusion (Fig. 5A), probably because the internalized fractions of the infused dose in both groups were very small (2.4 and 6.6%, respectively).

The Cd infusion resulted in significantly greater new Cd burdens in the liver, bile, carcass, and brain of Cd-acclimated relative to non-acclimated fish, but no difference was found in the gills and muscle (Fig. 5B). Unlike total Cd, the highest concentrations of new Cd of non-gut tissues were observed in the order liver (67.0 ± 8.6 ng/g) > kidney & bile (∼45 ng/g) > carcass (18.0 ± 4.5 ng/g) > gills (10.2 ± 1.2) ($n = 6$–9) of Cd-acclimated fish (Fig. 5B), each of which except carcass (∼5%) contained only <0.5% of the infused Cd dose.

4. Discussion

4.1. Mortality and growth

The lack of mortality or growth retardation in fish chronically exposed to dietary Cd (500 mg/kg) for 30 days is in agreement with a number of studies (Mount et al., 1994; Berntseen and Lundebye, 2001; Szebedinszky et al., 2001). However, in a few studies with rainbow trout, brown trout, and cutthroat trout fed diets of benthic macroinvertebrates from contaminated rivers and containing Cd and other metals, even at lower concentrations, there was evidence of mortality and reduced growth in the laboratory (Woodward et al., 1995; Farag et al., 1999). This discrepancy may be related to diet types, metal forms in the diet, the presence of other metals in the diet, or differences in the nutritional values of metal-contaminated macroinvertebrates.

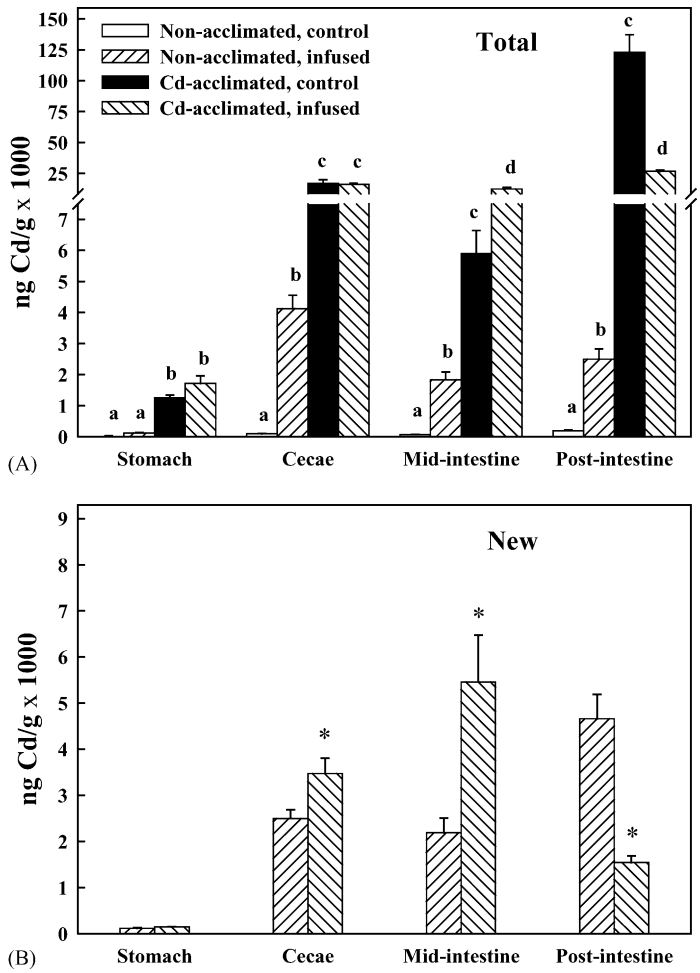

Fig. 4. (A) Concentrations of total Cd in the gut tissues (stomach, pyloric cecae, mid-intestine, and posterior-intestine) of non-infused, non-acclimated control fish, in non-infused, Cd-acclimated control fish, and in both non-acclimated and Cd-acclimated rainbow trout 24 h after a gastrointestinal dose of radiolabelled Cd (276 μg/kg wet wt.) infused into the stomach. (B) Concentrations of newly accumulated Cd in the same tissue samples. The acclimated fish were pre-exposed to dietary stable Cd (500 mg/kg diet) for 30 days. Results are presented as mean ± S.E. ($n = 6$–9). For total Cd (A), bars with different letters are significantly different ($P < 0.05$) for the same tissue. For new Cd (B), *represents a significant difference between non-acclimated and Cd-acclimated fish for the same tissue ($P < 0.05$).

4.2. Uptake of Cd across the gastrointestinal tract

The observed distribution of the gastrointestinally infused radioactive Cd (276 μg/kg) indicates that only a small fraction of the dose (non-acclimated: 2.4%; Cd-acclimated: 6.6%) was internalized across the gut wall, while most of the Cd was either bound in the gut tissues (10–24%), or remained in the lumen (16–33%), or was lost from the fish (∼50%) over 24 h after infusion (Fig. 1). Clearly, the gut serves as a barrier in transporting Cd from the lumen to blood by buffering ingoing Cd in its tissues, because gut tissues that were only a small part of trout body mass (2.96 ± 0.12%) contained almost 1/5th of the infused Cd in both non-acclimated (16.6 ± 1.3%) and Cd-acclimated trout (18.5 ± 1.6%). This, together with high concentrations of Cd in gut tissues (Fig. 4) and much larger distribution volumes of Cd in gut tissues ($V_{g\text{-non-acclimated}} = 3295$ ml/kg; $V_{g\text{-Cd-acclimated}} = 3048$ ml/kg; Table 1)

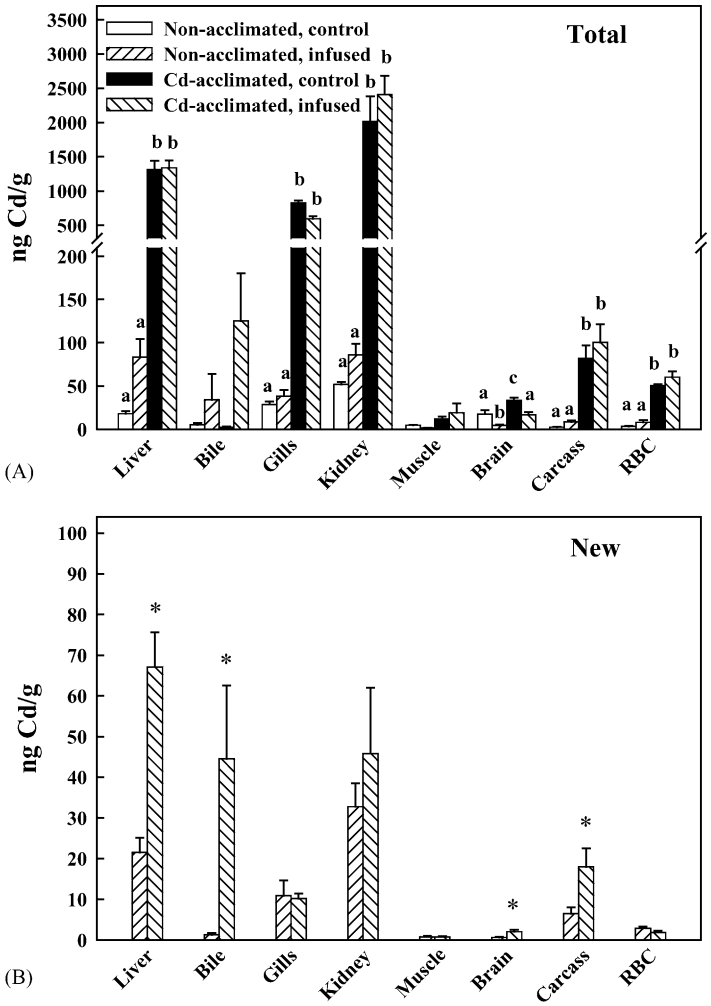

Fig. 5. (A) Concentrations of total Cd in the non-gut tissues of non-infused, non-acclimated control fish, in non-infused, Cd-acclimated control fish, and in both non-acclimated and Cd-acclimated rainbow trout 24 h after a gastrointestinal dose of radiolabelled Cd (276 μg/kg wet wt.) infused into the stomach. (B) Concentrations of newly accumulated Cd in the same tissue samples. The acclimated fish were pre-exposed to dietary stable Cd (500 mg/kg diet) for 30 days. Results are presented as mean ± SE ($n = 6$–9). For total Cd (A), bars with different letters are significantly different ($P < 0.05$) for the same tissue. For new Cd (B), *represents a significant difference between non-acclimated and Cd-acclimated fish for the same tissue ($P < 0.05$). RBC: red blood cell.

than in peripheral tissues ($V_{t\text{-non-acclimated}} = 42$ ml/kg; $V_{t\text{-Cd-acclimated}} = 113$ ml/kg; Table 1) indicates that the gut wall has a high storage capacity for Cd and thus contributes to protection against uptake of dietary Cd.

The small but significantly greater (>2-fold) internalized fraction of infused Cd in Cd-acclimated fish than in non-acclimated fish (Fig. 1A) suggests that pre-exposure to dietary Cd enhanced the uptake of new Cd across the gut. In mammals, Cd binds to the luminal surface of the mucosal cells and is taken up by an energy-independent process across the apical membrane (Zalups and Ahmad, 2003). Once inside the cells, Cd is thought to form a complex with cytosolic metallothionein (MT). Cadmium derived from the MT complex eventually crosses the basolateral membrane into the blood, perhaps by a calcium dependent

active process (Andersen, 1989; Zalups and Ahmad, 2003). In a related study, we have found that MT concentrations in gut tissues of Cd-acclimated trout were significantly greater than in non-acclimated trout, but the calculated ratios of tissue total metal (cadmium + copper + zinc) to theoretical maximum metal–MT binding sites (Hollis et al., 2001) were greater than 1.0 (Chowdhury and Wood, unpublished results), meaning that total metal outnumbered the available MT binding sites in Cd-acclimated fish. This probably facilitated an increased uptake of infused Cd in Cd-acclimated fish, because there was more new Cd available in the ionic form to traverse to blood.

4.3. Kinetic analysis of gastrointestinal Cd uptake

Cadmium absorption from gastrointestinal solution is a rapid process, as radiolabelled new Cd started appearing in the plasma by 0.5 h after infusion and increased almost linearly until the fourth hour (Fig. 2B). The rapid uptake probably means that Cd absorption started in the stomach, as is the case for Cu (Wapnir, 1998; Kamunde et al., 2002), and that the anterior part of the intestinal tract plays an important role in Cd absorption (discussed below). In the real world, however, the delay time for Cd absorption from a particulate diet is probably longer to allow for enzymatic action after ingestion.

A plasma Cd-time profile consisting of an apparent rising (uptake) phase and a declining (clearance) phase, as in Fig. 2, is typically observed for drug kinetics of oral suspension (Curry, 1974; Hollinger, 2003). During the rising phase, drug absorption predominates and during the declining phase, drug elimination predominates. It should be realized, however, that both processes occur simultaneously after initial uptake of Cd into the blood circulation.

In the present study, the plasma Cd-time profile of infused Cd was significantly greater in Cd-acclimated fish than in non-acclimated fish (Fig. 2). This is probably attributable to a greater uptake of new Cd in Cd-acclimated fish ($k_{31} = 0.0001\,\text{min}^{-1}$; Table 1) relative to non-acclimated fish ($k_{31} < 0.0001\,\text{min}^{-1}$; Table 1), but a similar clearance rate of new Cd from blood plasma (Cl_p: 0.489 and 0.504 ml/min/kg; Table 1), resulting in a longer half-life of plasma new Cd in Cd acclimated trout ($t_{1/2}$: 44 min and 58 min; Table 1). In contrast, after 30 days pre-exposure to waterborne Cd, a significantly enhanced clearance rate of new Cd from blood plasma (0.477 ml/min/kg versus 0.394 ml/min/kg) and identical half-lives of plasma new Cd (43.3 min versus 40.8 min) were reported in our previous study, in which radioactive Cd was directly injected into the blood (Chowdhury et al., 2003). Thus, it appears that the internal handling of Cd by trout depends on Cd exposure routes.

4.4. Plasma versus RBC Cd handling

Pre-exposure to dietary Cd (500 mg/kg) for 30 days produced a profound increase of total Cd (~28-fold) in the blood plasma (12.5 ng/ml) in comparison to naïve fish (Fig. 2A). This dietary Cd loading was double that of the plasma total Cd level (6.7 ng/ml) obtained from a 30 days exposure of trout to waterborne Cd (3 μg/L) under similar experimental conditions (Chowdhury et al., 2003). Pre-exposure to dietary Cd also produced a 19-fold increase in RBC total Cd concentrations. However, new Cd levels were not elevated in the RBCs of dietary acclimated fish (Fig. 3B), in contrast to the 1.4–2.6 fold increase seen in plasma new Cd levels in this treatment (Fig 2B). This is again in contrast to our previous study in which there were approximately 1.2–2.2 fold greater levels of new Cd in the RBCs of trout acclimated to waterborne Cd (Chowdhury et al., 2003). Compared with plasma total Cd (Fig. 2A), the total Cd in RBCs was 1.8–9.0 fold greater in Cd-acclimated trout (Fig. 3A). Similarly, the concentration of RBC total Cd (12.8 ng/g) was almost twice as much as plasma total Cd (6.7 ng/ml) in trout exposed to waterborne Cd for 30 days (Chowdhury et al., 2003). A greater loading of Cd in RBCs (89 ng/ml) in comparison to plasma (~13 ng/ml) was also observed in rat chronically exposed to Cd in drinking water (Crowe and Morgan, 1997). Therefore, red blood cells play an important role in binding Cd in fish as well as in mammals regardless of exposure routes.

4.5. Conclusions relevant for risk assessment

Our study provides some clear conclusions about the consequences of dietary Cd exposure in trout which may be useful for environmental risk assessment.

First, collective Cd levels in gut tissues (Fig. 4) were much greater than in non-gut tissues (Fig. 5),

which was clearly a reflection of Cd exposure route. This is consistent with previous studies (Harrison and Klaverkamp, 1989; Szebedinszky et al., 2001) showing that fish exposed to Cd via diet versus via water took up high Cd burdens in the gut versus gills, respectively, in addition to other target tissues such as kidney and liver.

Second, a substantial gill Cd burden resulted from dietary Cd exposure (Fig. 5A), consistent with the findings of Szebedinszky et al. (2001) who showed that similar gill Cd burdens could result from either of dietary or waterborne routes. The gill is thought to be an important excretory organ for Cd (Handy, 1996). Thus gill burdens alone are not diagnostic of the exposure route, but rather the ratio of gill to gut accumulation (non-acclimated $= 0.26 \pm 0.08$; Cd-acclimated $= 0.03 \pm 0.005$; $n = 6$), calculated based on overall gut tissue burdens (non-acclimated $= 0.14 \pm 0.03\,\mu g/g$; Cd-acclimated $= 35.0 \pm 5.6\,\mu g/g$; $n = 6$).

Third, the Cd burdens amongst different gut regions were not the same. The highest accumulation per gram tissue was found in the posterior intestine, followed by the pyloric cecae and mid-intestine, and the lowest in the stomach (Fig. 4A), which was in accord with the regional distribution of MT concentrations in these tissues (Chowdhury and Wood, unpublished results). To our knowledge, there are no published data showing Cd uptake in different gut regions. However, Clearwater et al. (2000) showed a similar pattern of Cu binding in the gut tissues of rainbow trout. Bury et al. (2001) and Kamunde et al. (2002) demonstrated the highest binding of Fe and Cu in the posterior intestine, respectively, and suggested that this is the most important region for metal absorption. [it should be noted that the higher proportion of new Cd found in the anterior intestine with pyloric cecae than in the posterior intestine (Fig. 1B) was due to its higher tissue mass contribution (cecae: $40.4 \pm 1.2\%$, posterior intestine: $10.5 \pm 0.7\%$; $n = 15$) to the total gut mass (8.7 ± 0.6 g; $n = 15$)]. However, in Cd-acclimated trout, Cd infusion not only caused a decrease in Cd burden (Fig. 4), but also in Zn, Cu and MT levels (Chowdhury and Wood, unpublished results) in the posterior intestine, probably due to an enhanced sloughing of mucosal tissue, as indicated by electron microscopy (Kamunde, Chowdhury, and Wood, unpublished result). Cadmium loss by sloughing of dead cellular materials and mucus from the gills and/or gut to accelerate excretion of Cd has been reported for mammals (Andersen, 1989) and fish (Sorensen, 1991; Handy, 1996).

Fourth, among the non-gut, internal tissues, Cd accumulation in the kidney and liver from the dietary source were much greater than in other tissues on a per gram basis (Fig. 5A). Preferential accumulation of Cd in kidney and liver from both waterborne and dietary sources has been typically found in other studies and these two organs are considered to be most significant for Cd metabolism and detoxification (Harrison and Klaverkamp, 1989; Farag et al., 1994; Kraal et al., 1995; Szebedinszky et al., 2001). However, greater uptake of new Cd was found only in the liver and bile of Cd-acclimated fish in the present study (Fig. 5B) and neither in the liver and kidney in our waterborne Cd exposure study (Chowdhury et al., 2003). This suggests that acclimation to dietary Cd, but not to waterborne Cd, has an accelerating effect on both hepatic clearance and biliary excretion of internalized Cd, while neither dietary nor waterborne acclimation affects the kidney clearance of internalized Cd.

Fifth, the negligible levels of total and new Cd in the muscle and brain (Fig. 5) agree with the previous findings (Szebedinszky et al., 2001; Chowdhury et al., 2003), indicating that these are protected tissues during both dietary and waterborne exposures. As muscle is the tissue that is most likely to be consumed by humans, the risks to human health of consumption of fish subject to either dietary or waterborne Cd exposure are likely to be negligible.

Acknowledgements

This research was supported by a Strategic Research Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada and by the International Lead Zinc Research Organization (ILZRO), the International Copper Association (ICA), the Copper Development Association (CDA), the Nickel Producers Environmental Research Association (NiPERA), Falconbridge, Cominco, and Noranda. C.M. Wood is supported by the Canada Research Chair Program. The authors sincerely thank Dr. C. Kamunde for his assistance with tissue sampling and Dr. P. Chapman for his constructive comments on the draft manuscript.

References

Andersen, O., 1989. Oral cadmium exposure in mice: toxicokinetics and efficiency of chelating agents. Crit. Rev. Toxicol. 20, 83–112.

Barron, M.G., Stehly, G.R., Hayton, W.L., 1990. Pharmacokinetic modeling in aquatic animals I. Models and concepts. Aquat. Toxicol. 18, 61–86.

Berntseen, M.H.G., Lundebye, A.K., 2001. Energetics in Atlantic salmon (*Salmo salar* L.) parr fed elevated dietary cadmium. Comp. Biochem. Physiol. 128C, 311–323.

Bury, N.R., Grosell, M., Wood, C.M., Hogstrand, C., Wilson, R.W., Rankin, J.C., Busk, M., Lecklin, T., Jensen, F.B., 2001. Intestinal iron uptake in the European flounder (*Platichthys flesus*). J. Exp. Biol. 204, 3779–3787.

Bushnell, P.G., Conklin, D.J., Duff, D.W., Olson, K.R., 1998. Tissue and whole-body extracellular, red blood cell, and albumin spaces in the rainbow trout as a function of time: A reappraisal of the volume of secondary circulation. J. Exp. Biol. 201, 1381–1391.

CCME (Canadian Council of Ministers of the Environment), 2002. Canadian Environmental Quality Guidelines (update 2002).

Chowdhury, M.J., Grosell, M., McDonald, D.G., Wood, C.M., 2003. Plasma clearance of cadmium and zinc in non-acclimated and metal-acclimated trout. Aquat. Toxicol. 64, 259–275.

Clearwater, S.J., Baskin, S., Wood, C.M., McDonald, D.G., 2000. Gastrointestinal uptake and distribution of copper in rainbow trout. J. Exp. Biol. 203, 2455–2466.

Crowe, A., Morgan, E.H., 1997. Effect of dietary cadmium on iron metabolism in growing rats. Toxicol. Appl. Pharmacol. 145, 136–146.

Curry, S.H., 1974. Drug Disposition and Pharmacokinetics with a Consideration of Pharmacological and Clinical Relationships. Blackwell Scientific Publications, Oxford, UK. 213 p.

Dallinger, R., Kautzky, H., 1985. The importance of contaminated food for the uptake of heavy metals by rainbow trout (*Salmo gairdneri*): a field study. Oecologia 67, 82–89.

Farag, A.M., Boese, C.J., Woodward, D.F., Bergman, H., 1994. Physiological changes and tissue metal accumulation in rainbow trout exposed to foodborne and waterborne metals. Environ. Toxicol. Chem. 13, 2021–2029.

Farag, A.M., Woodward, D.F., Brumbaugh, W., Goldstein, J.N., MacConnell, E., Hogstrand, C., Barrows, F.T., 1999. Dietary effects of metals-contaminated invertebrates from the Coeur d'Alene River, Idaho, on cutthroat trout. Trans. Am. Fish. Soc. 128, 578–592.

Gallaugher, P., Farrell, A.P., 1998. Hematocrit and blood oxygen-carrying capacity. In: Perry, S.F., Tufts, B. (Eds.), Fish Physiology (Vol. 17): Fish Respiration. Academic Press, California, pp. 185–227.

Giblin, F.J., Massaro, E.J., 1973. Pharmacodynamics of methyl mercury in the rainbow trout (*Salmo giardneri*): tissue uptake, distribution and excretion. Toxicol. Appl. Pharmacol. 24, 81–91.

Grosell, M., McGeer, J.C., Wood, C.M., 2001. Plasma copper clearance and biliary copper excretion are stimulated in copper-acclimated trout. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280, R796–R806.

Handy, R.D., 1996. Dietary exposure to toxic metals in fish. In: Taylor, E.W. (Ed.), Toxicology of Aquatic Pollution: Physiological, Molecular, and Cellular Approaches. Society of Experimental Biology Seminar Series 57. Cambridge University Press, Cambridge, pp. 29–60.

Harrison, S.E., Klaverkamp, J.F., 1989. Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Coregonus clupeaformis* Mitchill). Environ. Toxicol. Chem. 8, 87–97.

Harrison, S.E., Curtis, P.J., 1992. Comparative accumulation efficiency of cadmium from natural food (*Hyalella azteca*) and artificial diet by rainbow trout (*Oncorhynchus mykiss*). Bull. Environ. Contam. Toxicol. 49, 757–764.

Hollinger, M.A., 2003. Introduction to Pharmacology, 2nd ed., Taylor and Francis, London, UK.

Hollis, L., McGeer, J.C., McDonald, D.G., Wood, C.M., 1999. Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long term sublethal Cd exposure in rainbow trout. Aquat. Toxicol. 46, 101–119.

Hollis, L., Hogstrand, C., Wood, C.M., 2001. Tissue-specific cadmium accumulation, metallothionein induction, and tissue zinc and copper levels during chronic sublethal cadmium exposure in juvenile rainbow trout. Arch. Environ. Contam. Toxicol. 41, 468–474.

Kamunde, C., Clayton, C., Wood, C.M., 2002. Waterborne vs. dietary copper uptake in rainbow trout and the effects of previous waterborne copper exposure. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283, R69–R78.

Kraal, M.H., Kraak, M.H.S., De Groot, C.J., Davids, C., 1995. Uptake and tissue distribution of dietary and aqueous cadmium by carp (*Cyprinus carpio*). Ecotox. Environ. Saf. 31, 179–183.

McGeer, J.C., Szebedinszky, C., McDonald, D.G., Wood, C.M., 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd, or Zn in rainbow trout 2: tissue specific metal accumulation. Aquat. Toxicol. 50, 245–256.

Mount, D.R., Barth, A.K., Garrison, T.D., Barten, K.A., Hockett, J.R., 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.

Roesijadi, G., Robinson, W.E., 1994. Metal regulation in aquatic animals: mechanisms of uptake, accumulation, and release. In: Mullins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives. Lewis Publishers, Florida, pp. 387–419.

Scott, B.J., Bradwell, A.R., 1984. Metal binding to serum proteins. In: Peters, N. (Ed.), Protides of the Biological Fluids. Pergamon Press, Oxford, pp. 15–18.

Soivio, A., Westmann, K., Nyholm, K., 1972. Improved methods of dorsal aorta catheterization: hematological effects followed for three weeks in rainbow trout. Finn. Fish Res. 1, 11–22.

Sorensen, E.M., 1991. Metal Poisoning in Fish. CRC Press, Florida.

Stubblefield, W.A., Steadman, B.L., La Point, T.W., Bergman, H.L., 1999. Acclimation-induced changes in the toxicity of

zinc and cadmium to rainbow trout. Environ. Toxicol. Chem. 18, 2875–2881.

Szebedinszky, C., McGeer, J.C., McDonald, D.G., Wood, C.M., 2001. Effects of chronic Cd exposure via the diet or water on internal organ-specific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 20, 597–607.

USEPA (US Environmental Protection Agency), 2001. 2001 Update of Ambient Water Quality Criteria for Cadmium. EPA-822-R-01-001, Washington, DC.

Wapnir, R.A., 1998. Copper absorption and bioavailability. Am. J. Clin. Nutr. 67, 1054s–1068s.

Wolf, K., 1963. Physiological salines for freshwater teleosts. Progr. Fish Cult. 25, 140–153.

Wood, C.M., 2001. Toxic responses of the gill. In: Schlenk, D., Benson, W.H. (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts. Taylor and Francis, London, pp. 1–87.

Woodward, D.F., Farag, A.M., Bergman, H.L., DeLonay, A.J., Little, E.E., Smith, C.E., Barrows, F.T., 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.

Wu, S.M., Hwang, P.P., 2003. Copper or cadmium pretreatment increases the protection against cadmium toxicity in tilapia larvae (*Oreochromis mossambicus*). Zool. Stud. 42, 179–185.

Zalups, R.K., Ahmad, S., 2003. Molecular handling of cadmium in transporting epithelia. Toxicol. Appl. Pharmacol. 186, 163–188.