

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*)CHERYL SZEDEDINSZKY,* JAMES C. MCGEER, D. GORDON McDONALD, and CHRIS M. WOOD
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Abstract—New regulatory approaches to metal toxicity (e.g., biotic ligand model [BLM]) focus on gill metal binding and tissue-specific accumulation of waterborne metals; the dietary route of exposure and dietary/waterborne interactions are not considered, nor are the consequences of chronic exposure by either route. Therefore, we studied the effect of the same gill Cd load (~2.5 µg/g), achieved by a chronic, 30-d exposure to Cd either via the diet (1,500 mg/kg) or the water (2 µg/L), on tissue-specific Cd distribution and subsequent uptake of waterborne Cd in juvenile rainbow trout (*Oncorhynchus mykiss*). These two exposure regimes resulted in a branchial Cd load that had been taken up across either apical gill membranes (waterborne Cd) or basolateral gill membranes (through the bloodstream for dietary Cd). The BLM characteristics of the gills (i.e., short-term Cd uptake kinetics) were altered: affinity ($\log K_{Cd-Gill}$ [95% confidence level]) decreased from 7.05 (6.75–8.76) for control to 6.54 (6.32–7.03) for waterborne Cd and 5.92 (5.83–6.51) for dietary Cd, whereas binding capacity (B_{max}) increased from 3.12 (2.14–4.09) to 4.80 (3.16–6.43) and 5.50 (2.86–8.17) nmol·g⁻¹ for control, waterborne, and dietary Cd, respectively. Fish exposed to dietary Cd accumulated a much greater overall chronic Cd body burden relative to fish exposed to waterborne Cd or control fish. The carcass accumulated the greatest percentage of total body Cd in control and waterborne-exposed fish, whereas the intestinal tissue accumulated the greatest percentage in dietary-exposed fish. Tissue-specific Cd burdens were highest in the kidney in both dietary and waterborne treatments. We conclude that chronic Cd exposure alters Cd uptake dynamics, and that the route of Cd exposure, whether waterborne or dietary, results in differences of internal Cd accumulation and branchial Cd uptake characteristics. These factors should be considered in future BLM development.

Keywords—Cadmium Rainbow trout Bioaccumulation Dietary/waterborne exposure Biotic ligand model

INTRODUCTION

Cadmium is a toxic metal that can be found at elevated levels in the water, sediment, and benthos as a result of mining, industrial processes, forestry, waste disposal, and fuel combustion; it has no essential function in physiological processes [1–3]. Depending on the route of exposure (i.e., water vs diet), the two most important sites for Cd absorption in freshwater fishes are the gills and the gastrointestinal tract. Initial uptake from water into the gills is followed by subsequent transfer to the blood for distribution throughout the body [4]. Uptake of Cd via the alimentary canal occurs with an initial transfer from food to the gut tissue, followed by movement into the blood and subsequent internal distribution via the circulation [4]. Whether absorbed across the gills (i.e., waterborne Cd exposure) or the intestinal tract (i.e., dietary Cd exposure), Cd is bound to transport proteins in the blood plasma and distributed via the circulation to the various tissues [3].

The specific internal organs and tissues in fish that accumulate Cd appear to vary with the route of uptake and species [3,5]. For example, in rainbow trout (*Oncorhynchus mykiss*) during waterborne exposure, the organs with the highest Cd accumulation (on a per-gram basis) are the gills and kidney [6,7], whereas dietary Cd accumulates mostly in the gastrointestinal tract and kidney [8–10]. Although differential organ-specific distribution has been studied for both dietary and wa-

terborne Cd exposures, the effect of tissue-specific Cd burdens on subsequent Cd uptake and deposition is unknown.

As reviewed by McDonald and Wood [11], chronic sublethal exposure to waterborne Cd can produce acclimation (i.e., an increased resistance to acute Cd challenges). Recent work by Hollis et al. [6] has suggested that the gills function as a barrier in Cd-acclimated trout and, thereby, minimize internal Cd loading. In contrast to waterborne Cd toxicity, the effects of oral doses of metals in fish are less well known [5], and environmental regulations do not directly consider the potentially toxic effects that may result from chronic dietary loading or from the interactions of dietary and waterborne metals. In polluted aquatic ecosystems, sediment Cd content is usually higher than waterborne Cd levels, and trophic transfer from sediment to fish has the potential to result in deleterious accumulations of Cd [2,12–14]. Indeed, it has been suggested that invertebrate prey of fish, if contaminated with metals, contribute greatly to metal accumulation in the tissues [12,15,16].

Recently, there has been much interest in high-affinity gill surface-binding models (or biotic ligand models [BLM]), such as those introduced by Playle et al. [17]. These may be used for predicting metal toxicity as a function of ambient water chemistry and, ultimately, for generating site-specific ambient water-quality criteria [18–20]. These models characterize the gill surface as a metal-binding ligand for which affinity ($\log K_{Cd-Gill}$) and capacity (B_{max}) values can be experimentally determined and then inserted into aquatic geochemical modeling programs such as MINEQL⁺ [21]. The programs are then used to predict whether toxic saturation of the gill surface with metal

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will occur at a given water quality. It is important to consider if such approaches will work when fish are chronically exposed to sublethal metal concentrations for long periods of time or via a route other than waterborne [18].

Hollis et al. [6] showed that the changes in gill Cd-binding kinetics that occurred as a result of chronic sublethal waterborne exposure involved both a decrease in affinity and an increase in capacity of the gill surface for Cd, which were probably related to the large burden of Cd carried in the gills of chronically exposed fish. Dietary exposure has also been reported to result in Cd accumulation in the gills [5,9]. In this case, the uptake pathway into the branchial tissue must be fundamentally different, involving transfer across the basolateral cell membranes from the bloodstream rather than direct uptake from the water, which is thought to involve Cd²⁺ entry through apical Ca²⁺ channels [22,23]. However, to our knowledge, the effects of gill Cd loading via the diet on subsequent waterborne Cd uptake dynamics at the gill have never been studied. These observations highlight the need for comparative gill-binding studies in fish that have been chronically exposed by each of the two routes.

The primary objectives of our work were to compare the effects of chronic sublethal Cd loading (one-month exposures) via dietary versus waterborne routes on internal organ-specific accumulation patterns and on subsequent gill Cd binding and uptake kinetics. To ensure a valid comparison, it was critical that the gill Cd loading via the two different routes be similar. Recent work by McGeer et al. [7] provided a guideline for waterborne exposure: trout exhibited a gill Cd concentration of approximately 2.5 µg·g⁻¹ when exposed to 3 µg·L⁻¹ for 60 d in the same, moderately hard Hamilton (ON, Canada) tap water (from Lake Ontario). However, little quantitative information is available regarding gill loading via the dietary route. Therefore, a first series of experiments was performed to establish dietary Cd exposure levels that produce gill Cd concentrations matching those that occur during chronic sublethal waterborne Cd exposure. A second series focused on direct comparisons: gill Cd binding via short (3 h) and longer (2 d) ¹⁰⁹Cd uptake tests, and acclimation responses via acute toxicity tests. Both series characterized the relationships between dietary Cd exposure level, tissue-specific bioaccumulation, growth, and mortality of rainbow trout in freshwater.

MATERIALS AND METHODS

Experimental outline

Series 1 was a dietary rangefinder experiment in which fish were exposed to five different levels of dietary Cd for 36 d. Series 2 was a waterborne Cd versus dietary Cd study in which fish were either exposed to one of three different levels of dietary Cd or to waterborne Cd at a level of 2 µg·L⁻¹ for 30 d. Immediately thereafter, various physiological and toxicological tests were performed. The experimental protocols described in the following sections apply to both series 1 and series 2, unless otherwise specified.

Experimental animals

Juvenile rainbow trout (2–7 g) were held at a density of approximately 1 fish per liter in 500-L tanks for at least two to three weeks before exposures. All fish were held and subsequently exposed to dietary or waterborne Cd in flowing, aerated, dechlorinated Hamilton tap water (Ca = 40 mg·L⁻¹ or 1 mmol·L⁻¹, Na = 14 mg·L⁻¹ or 0.6 mmol·L⁻¹, Cl = 25

Table 1. Measured concentration (mg·kg⁻¹) of Cd in the feeds used for series 1 dietary exposures^a

Treatment	Cd concentration (mg·kg ⁻¹)		
	Nominal	Measured	% Mortality
Control	0 (controls)	1.47 ± 0.5	0
Diet 1	1.5	3.9 ± 0.1	0
Diet 2	15	21.8 ± 0.2	0
Diet 3	150	117.0 ± 1.3	2.8
Diet 4	1,500	1,419 ± 207	8.3

^a The mean ± standard error of the mean (*n* = 4) are shown, and the mortalities associated with 36 d of feeding the diets included.

mg·L⁻¹ or 0.7 mmol·L⁻¹, dissolved organic matter = 3 mg⁻¹ as carbon, hardness = 140 ppm as CaCO₃, alkalinity = 95 ppm as CaCO₃, pH 8.0, and temperature = 14.0–17.0°C); Fish were fed once daily at 2% of body mass. Daily maintenance of tanks involved siphoning of residual waste from the bottom and checking for mortalities. In all experiments, groups of fish were nonselectively removed from the tank, then euthanized with an excess of MS222 (200 mg·L⁻¹) and a blow to the head. This method of removing fish was nonselective but did involve a possibility that systematic capture biases, though unavoidable, could have been introduced as a result.

Diet preparation

Diets containing Cd were prepared by mixing Cd(NO₃)₂·4H₂O into commercial trout food (Martin's Trout Feed: 42% crude protein, 16% crude fat, 40% crude carbohydrate, 0.35% sodium, 1% calcium, and 0.65% phosphorus; Martin Feed Mill, Elmira, ON, Canada). Trout food was ground in a blender, followed by hydration with approximately 50% (w/v) of deionized water and mixing. Dependent on the desired dietary Cd content, a measured amount of Cd salt was dissolved in deionized water, added to the food paste, and mixed for at least 1 h. The paste was then extruded using a retail pastemaker (Popiel Ronco, Compton, CA, USA) into long strings, which were dried at 60°C for 2 h and then broken into small pellets (~5 mm in length and 2 mm in diameter). The control diets were prepared by the same method but without the addition of Cd. The final Cd concentration of all diets was analyzed as outlined later.

Exposure conditions and sampling

Series 1. Trout (average weight = 5.0 ± 0.7 g, *n* = 155) from Rainbow Springs Hatchery (Thamesford, ON, Canada) were nonselectively placed in five separate, 50-L polyethylene tanks (*n* = 31 in each). Tanks were supplied with flowing water at a rate of 2.0 L·min⁻¹, with continuous aeration at 17°C. Fish were exposed for 36 d to one of the four dietary Cd treatments or a control diet, with nominal Cd levels of either 0 (control), 1.5, 15, 150, or 1,500 mg·kg⁻¹. Measured values for the Cd content of treatment food are given in Table 1.

Water samples (15 ml) were collected daily from all tanks and immediately acidified with 50 µl of concentrated HNO₃ (trace metal grade; Fisher Scientific, Nepean, ON, Canada). To ensure that any Cd burdens, particularly in the gills, were a direct result of dietary contamination, water samples were taken on four separate days. During each 24 h period, water samples were taken seven times at 0.25, 0.75, 1, 3, 7, 10, and 24 h after feeding of Cd-contaminated diets.

On days 5, 9, 19, and 36 of exposure, eight fish were non-

Table 2. Measured concentration in feed for series 2 exposure as well as mortality and specific growth rate (SGR) over 30 d of exposure^a

Treatment	CD in food (mg·kg ⁻¹)	Mortality (%)	SGR (%·d ⁻¹)
Control Water	1.47 ± 0.05	0	3.07 ± 0.22
2 µg·L ⁻¹ Diet	1.47 ± 0.05	0.7	2.80 ± 0.04
800 mg·kg ⁻¹	786 ± 47	1.5	2.64 ± 0.07
1,500 mg·kg ⁻¹	1,395 ± 185	43.2	2.81 ± 0.4
2,200 mg·kg ⁻¹	2,265 ± 426	71.8	1.42 ± 0.2 ^b

^a For Cd content of food and SGR, mean ± standard error of the mean ($n = 4-5$) are shown.

^b Significant difference from control SGR ($p < 0.05$).

selectively removed from each tank and euthanized, and then tissue samples were taken for subsequent metal analysis. Gills were removed, rinsed for 10 s in deionized water, blotted dry, weighed, and saved. Following removal of the entire gastrointestinal tract (esophagus to rectum, which was discarded on days 5, 9, and 19), the liver and remaining carcass (mainly muscle, skin, and bone) were weighed separately and saved. Additionally, on day 36, kidney, brain, and anterior and posterior intestinal tissues were collected, as was a sample of fecal material from the posterior rectum. Intestinal segments were separated from the gastrointestinal tract, rinsed vigorously with deionized water, and gently blotted dry before weighing. This ensured that any Cd remaining in the gut from residual food or loosely bound to the gut wall was washed away in the intestinal samples measured.

Series 2. Fish (average weight = 2.0 ± 0.3 g, $n = 1,500$) from Humber Springs Hatchery (Orangeville, ON, Canada) were nonselectively placed in one of five 200-L tanks ($n = 300$ in each), which were aerated and supplied with flowing water (14°C) at a rate of 1.6 L·min⁻¹. One tank served as a control treatment (unexposed), and these fish were fed a diet that was nominally Cd-free and held in water without added Cd (control). Of the four groups of fish exposed to Cd, one was a waterborne exposure only (fed a control diet), and the other three were fed Cd-contaminated diets only (held in control water). The waterborne Cd exposure level was set at 2 µg·L⁻¹ (nominal), and the actual measured water Cd concentration was 2.37 ± 0.30 µg·L⁻¹ ($n = 15$). Waterborne Cd levels were maintained by adding 0.05% acidified Cd (as Cd[NO₃]₂·4H₂O) stock solution via a mariotte bottle to a mixing head-tank served with flowing, fresh water. At the start of the exposure, sufficient stock solution was added directly to the tank to immediately achieve the desired Cd concentration. The nominal dietary Cd treatments used in the study were 800, 1,500, or 2,200 mg·kg⁻¹, with actual measured values reported in Table 2. The dietary Cd levels were chosen based on the results of the series 1 dietary exposure. These indicated that a dietary Cd level close to 1,500 mg·kg⁻¹ would produce an accumulation of Cd in the gills that would be close to the level anticipated for fish exposed to waterborne Cd at 2 µg·L⁻¹. The experimental period of Cd exposure was 30 d.

On days 0, 9, 17, and 30, six fish from each of the five treatments were nonselectively sampled for gills, liver, kidney, carcass, and posterior and anterior sections of the intestine as previously described for series 1. Water samples were collected (methods as described above) every fifth day until day 30. Bulk weights (i.e., total tank biomass) were measured on fish sampling days to monitor growth and adjust the feeding rate

to maintain the 2% daily ration. All fish from a tank were removed and placed in a tared sieve within a bucket of water from the exposure tank. The bucket was weighed, the fish were returned to the tank (using the sieve), and the empty bucket and sieve were reweighed. Total tank biomass was calculated from the difference between the mass of the bucket and sieve with and without fish.

Metal uptake and acclimation tests (series 2)

On completion of the 30-d exposure, two different waterborne Cd uptake tests and a 96-h acute toxicity test were performed. The two metal uptake tests (i.e., acute, 3-h gill Cd binding and long-term, 46-h Cd uptake and distribution) were conducted on three groups: control, waterborne exposed, and 1,500 mg·kg⁻¹ dietary exposed. The fish from the 1,500 mg·kg⁻¹ diet were chosen because gill Cd accumulation in this group was closest to that of the waterborne Cd-exposed fish.

The acute gill Cd-binding test measured new Cd uptake into the gills during 3-h exposures at a range of measured waterborne Cd concentrations. Fish from each treatment, in groups of six, were placed nonselectively in 4 L of water containing one of the five Cd concentrations. To quantify new Cd uptake against the elevated background levels in gills of Cd-exposed trout, ¹⁰⁹Cd (as CdCl₂, specific activity = 2.75 mCi·mg⁻¹; NEN, Boston, MA, USA) was added to the water to achieve an activity of approximately 2 µCi·L⁻¹. Water in each container was aerated, and the temperature (13°C) was maintained by immersion in a constant-temperature bath. After 3 h, fish were euthanized, and the gills were removed (as described for series 1) and saved for subsequent analysis. Water samples (10 ml) were taken at the beginning and end of the 3-h static exposure for measurement of total Cd concentration and radioactivity.

The 46-h, long-term uptake and distribution measurements also employed ¹⁰⁹Cd to track new Cd uptake, but in this test, a single waterborne concentration of 2 µg·L⁻¹ was used throughout. Trout, in groups of 24, were removed from each of the control, waterborne, and dietary (1,500 mg·kg⁻¹) treatments and nonselectively distributed among one of five 15-L tanks that were part of a 150-L recirculating system with thorough aeration and temperature control (13°C). Sufficient ¹⁰⁹Cd was added to the system to achieve approximately 1 µCi·L⁻¹ before fish were added. Six fish from each treatment were nonselectively removed from the tank and euthanized at 0, 12, 22, and 46 h of exposure, and gills, liver, kidney, and gut samples were excised, weighed, and saved.

At the end of the 30-d exposure, 96-h median lethal concentration (LC50) tests were performed using fish previously exposed to dietary Cd at both 800 and 1,500 mg·kg⁻¹ as well as waterborne-exposed and control fish. Groups of 7 to 10 trout were placed in 15-L tanks and exposed to Cd via a flow-through system. The Cd challenge concentrations (measured) were 1.0 ± 0.6, 12.9 ± 0.49, 32.7 ± 0.90, 55.1 ± 2.4, 107.3 ± 14.6, and 242.04 ± 22.5 µg·L⁻¹ ($n = 8$). Dead fish were removed, and times of mortality were recorded.

Measurements and calculations

Gill, liver, gut, brain, posterior and anterior intestine, carcass, feces, and kidney samples were digested in approximately five volumes of 1 N HNO₃ (trace metal grade; Fisher Scientific) for 24 h at 70°C [24]. The Cd content of tissues and water samples was measured by atomic absorption spectrophotometry (AA-1275; Varian, Walnut Creek, CA, USA) with graphite

Table 3. Cd concentrations ($\mu\text{g}\cdot\text{g}^{-1}$) in gills, livers, and carcasses (mainly muscle, skin, and bone) of rainbow trout exposed to dietary Cd for up to 36 d (series 1 exposure)^a. Mean \pm SEM ($n = 8$) are shown and * indicates a significant difference between controls and exposed fish at each sampling day ($p < 0.05$)

Tissue	Treatment	Day of exposure			
		5	9	19	36
Gills	0 (Control)	0.22 \pm 0.01	0.27 \pm 0.04	0.13 \pm 0.01	0.17 \pm 0.03
	1.5 mg·kg ⁻¹	0.20 \pm 0.015	0.26 \pm 0.04	0.07 \pm 0.01	0.30 \pm 0.10
	15 mg·kg ⁻¹	0.18 \pm 0.01	0.18 \pm 0.01	0.10 \pm 0.01	0.20 \pm 0.03
	150 mg·kg ⁻¹	0.24 \pm 0.025	0.22 \pm 0.01	0.28 \pm 0.01 ^b	0.32 \pm 0.03
	1,500 mg·kg ⁻¹	0.54 \pm 0.06 ^b	1.08 \pm 0.05 ^b	1.37 \pm 0.07 ^b	1.77 \pm 0.05 ^b
Liver	0 (Control)	0.63 \pm 0.11	0.55 \pm 0.08	0.38 \pm 0.04	0.35 \pm 0.02
	1.5 mg·kg ⁻¹	0.37 \pm 0.05	0.35 \pm 0.07	0.31 \pm 0.02	0.29 \pm 0.01
	15 mg·kg ⁻¹	0.36 \pm 0.03	0.40 \pm 0.19	0.34 \pm 0.02	0.20 \pm 0.06
	150 mg·kg ⁻¹	0.49 \pm 0.07	0.71 \pm 0.07	0.88 \pm 0.07 ^b	0.93 \pm 0.19
	1,500 mg·kg ⁻¹	1.20 \pm 0.06 ^b	3.53 \pm 0.27 ^b	1.35 \pm 0.16 ^b	6.47 \pm 0.87 ^b
Carcass	0 (Control)	0.38 \pm 0.18	0.25 \pm 0.03	0.34 \pm 0.07	0.46 \pm 0.17
	1.5 mg·kg ⁻¹	0.32 \pm 0.02	0.27 \pm 0.06	0.23 \pm 0.01	0.19 \pm 0.03
	15 mg·kg ⁻¹	1.18 \pm 0.54	1.73 \pm 0.43	0.31 \pm 0.03	0.28 \pm 0.20
	150 mg·kg ⁻¹	0.37 \pm 0.02	1.67 \pm 0.74	0.34 \pm 0.03	0.66 \pm 0.15
	1,500 mg·kg ⁻¹	1.03 \pm 0.30 ^b	0.71 \pm 0.03 ^b	1.82 \pm 0.45 ^b	1.80 \pm 0.42 ^b

^a Mean \pm standard error of the mean ($n = 8$) are shown.

^b Significant difference between controls and exposed fish at each sampling day ($p = 0.05$).

furnace (GTA-95) atomization after appropriate dilution against Fisher-certified standards using 10- μL injection volumes and N₂ gas. Standards, unknowns, and certified reference material were run in the same matrices, and all unknowns were read above the lowest standard on the curve. Operating conditions were as described by Varian, with 30 s of drying time at 90°C, 12 s at 120°C, and 4 s at 1,800°C, during which Cd was read for 2.9 s. Cadmium concentrations in tissues were expressed on a per-gram wet tissue basis.

Tissue and water ¹⁰⁹Cd activity was measured on a Minaxi Auto-Gamma 5000 Series gamma counter (Canberra Packard Instrument Company, Meriden, CT, USA). Tissue ¹⁰⁹Cd concentrations (ng·g⁻¹) were converted to absolute values (new Cd) using the measured specific activity and total Cd content of the water via the following formula:

$$\text{New Cd} = a(bc^{-1})^{-1} = \text{ng Cd}\cdot\text{g}^{-1} \quad (1)$$

where a is ¹⁰⁹Cd (cpm/g tissue [wet wt]), b is ¹⁰⁹Cd counts in the water (cpm·L⁻¹), and c is the total Cd concentration in the water ($\mu\text{g}\cdot\text{L}^{-1}$).

Using the gill 3-h ¹⁰⁹Cd uptake data and the water ionic Cd²⁺ concentration (as calculated by MINEQL⁺ [21] from measured water chemistry), gill-Cd association constants ($K_{\text{Cd-gill}}$) and gill maximum Cd-binding capacities (B_{max}), both with the 95% confidence level (CL), were estimated by nonlinear regression analysis (Sigma Plot; Jandel, San Rafael, CA, USA). This approach yielded mean values of $K_{\text{Cd-gill}}$ and B_{max} that were virtually identical to those obtained by Scatchard adsorption isotherm analysis as outlined by Reid and McDonald [25]. Ionic Cd²⁺ represented 53 to 83% of the total water Cd, depending on the measured total Cd concentration.

The LC50 values \pm 95% CL were determined by log-probit analysis in SPSS Version 8 for Windows (SPSS, Chicago, IL, USA) using measured waterborne Cd concentrations.

The specific growth rate (SGR), as a percentage per day, of rainbow trout in the series 2 exposure was calculated using average individual fish weights and the formula

$$\text{SGR} = 100 \times ([\ln(wt_2) - \ln(wt_1)]/t) \quad (2)$$

where wt refers to the average weight of individuals at the

start (wt_1) and end (wt_2) of the interval and t is the length of the time interval in days. Average individual fish weight was calculated from the tank biomass measurements, and SGR was calculated by linear regression of ln weight versus time using SPSS Version 8 for Windows, which yields mean \pm one standard error for SGR. For tissue measurements, individual fish served as replicates in each treatment; however, LC50 and SGR experiments were not replicated.

Data are expressed as the mean \pm one standard error of the mean, except in the case of LC50 and gill-binding constants, for which the mean \pm 95% CL is reported; the use of 95% CL is specifically indicated. Data for control and experimental groups were analyzed for statistical significance by analysis of variance, and Duncan's multiple range test was used when appropriate. The LC50 values were compared by means of a Bonferroni test. A confidence level of $p < 0.05$ was used throughout.

RESULTS

Series 1 (dietary rangefinder study)

Exposure of trout to dietary Cd at levels less than 150 mg·kg⁻¹ for 36 d resulted in no mortalities (Table 1). However, a 2.8% mortality rate was found in the 150 mg·kg⁻¹ treatment group, and fish fed a diet with 1,500 mg·kg⁻¹ exhibited a mortality rate of 8.3% (Table 1).

Exposure of trout to a dietary Cd concentration of 1,500 mg·kg⁻¹ resulted in significant accumulation of Cd in the gills, liver, kidney, intestine, and carcass, which clearly increased with exposure duration in the gills, liver, and carcass (Tables 3 and 4). Only fish exposed to the highest dietary Cd level (1,500 mg·kg⁻¹) acquired a gill Cd burden significantly different than that of controls (Table 3), with a burden of approximately 1.8 $\mu\text{g}\cdot\text{g}^{-1}$ on day 36. This burden was accumulated via the diet, because the mean water Cd concentration for tanks exposed to Cd via this diet was 0.29 \pm 0.16 $\mu\text{g}\cdot\text{L}^{-1}$ ($n = 20$), which was similar to those samples (0.24 \pm 0.06 $\mu\text{g}\cdot\text{L}^{-1}$, $n = 30$) taken from the control tanks. These results established that a dietary concentration close to 1,500 mg/kg was appropriate for achieving a gill Cd burden comparable to

Table 4. Cd concentration ($\mu\text{g}\cdot\text{g}^{-1}$) in the anterior and posterior intestine, kidney, brain, and fecal material of rainbow trout exposed to different dietary Cd levels for 36 d (series 1 exposure)^a

Treatment ($\text{mg}\cdot\text{kg}^{-1}$)	Anterior intestine	Posterior intestine	Kidney	Brain	Feces
0 (Control)	3.4 ± 1.4	8.5 ± 0.80	1.5 ± 0.38	0.03 ± 0.04	8.5 ± 0.8
1.5	6.5 ± 2.4	19.2 ± 9.3	1.2 ± 0.5	0.06 ± 0.01	8.6 ± 9.3
15	9.0 ± 1.8^b	5.6 ± 4.6	2.4 ± 0.2^b	0.07 ± 0.00	182 ± 45.1^b
150	36.2 ± 10.8^b	124 ± 83	6.9 ± 0.7^b	0.06 ± 0.00	184 ± 99.8^b
1,500	39.3 ± 2.9^b	531 ± 66^b	10.9 ± 3.1^b	0.10 ± 0.02	244 ± 20.6^b

^a Mean \pm standard error of the mean are shown, with $n \geq 7$ except for the groups exposed to 1.5 and 150 $\text{mg}\cdot\text{kg}^{-1}$, in which $n = 5$.

^b Significant difference between controls and exposed fish ($p < 0.05$).

that predicted from a 2.0 $\mu\text{g}/\text{L}$ waterborne exposure based on the results of McGeer et al. [7].

A similar pattern, with overall levels similar to those in the gills, was seen in the carcass (Table 3). Consistent accumulation of Cd in the liver of exposed fish was only evident at the highest dietary exposure concentration (Table 3). Aside from levels on day 19, no significant difference was observed between liver burdens in the control group and the 1.5, 15, and 150 $\text{mg}\cdot\text{kg}^{-1}$ treatment groups. In the liver, Cd levels were two- to threefold greater than those in the gills (Table 3).

The 15, 150, and 1,500 $\text{mg}\cdot\text{kg}^{-1}$ treatment groups all accumulated significant levels of Cd in the kidney by day 36 (Table 4), and these levels were consistently higher than either the gill or liver Cd content (Table 3). Interestingly, the Cd content of the brain was the same as that in controls (Table 4) for all dietary treatments.

For trout fed Cd, a tendency for greater accumulation of Cd was observed in the posterior than in the anterior portion of the intestine (Table 4), with significant accumulations occurring at the higher exposure levels. Fecal Cd levels (Table 4) were similar ($\sim 200 \mu\text{g}/\text{g}$) in fish fed 15, 150, and 1,500 $\text{mg}\cdot\text{kg}^{-1}$ diets.

Series 2 (waterborne vs dietary exposure)

Based on the results of Series 1, fish were fed nominal dietary Cd levels of either 800, 1,500, or 2,200 $\text{mg}\cdot\text{kg}^{-1}$. Fish exposed to 1,500 $\text{mg}\cdot\text{kg}^{-1}$ (dietary) suffered 43% mortality

(Table 2), whereas fish exposed to 2,200 $\text{mg}\cdot\text{kg}^{-1}$ had a much greater mortality of 72%. Mortality was negligible in the 800 $\text{mg}\cdot\text{kg}^{-1}$ dietary and 2 $\mu\text{g}\cdot\text{L}^{-1}$ waterborne exposure groups, as in the controls. The SGR was significantly lower for the fish exposed to a dietary Cd concentration of 2,200 $\text{mg}\cdot\text{kg}^{-1}$ (Table 2) than for the controls, for the fish exposed to 2 $\mu\text{g}\cdot\text{L}^{-1}$ in the water, or for the fish exposed to 800 and 1,500 $\text{mg}\cdot\text{kg}^{-1}$ in the diet, which were all similar.

All three Cd diets caused significant accumulation of Cd in the gills (Fig. 1). As with series 1, these accumulations originated from the dietary Cd, because the average water Cd concentrations were $0.11 \pm 0.02 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 9$) for tanks exposed to Cd via the diet and $0.09 \pm 0.03 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 5$) for the control tank. The average water Cd concentration in the nominal 2.0 $\mu\text{g}\cdot\text{L}^{-1}$ waterborne exposure was $2.37 \pm 0.3 \mu\text{g}\cdot\text{L}^{-1}$ ($n = 14$). Fish exposed to 2 $\mu\text{g}\cdot\text{L}^{-1}$ waterborne Cd had significant accumulations in gill tissue, and at day 30, these accumulations were not significantly different from those of the 1,500 and the 2,200 $\text{mg}\cdot\text{kg}^{-1}$ dietary concentration exposures (Fig. 1). Thus, the goal of accumulating similar gill Cd burdens by the two different routes of exposure was achieved.

All three dietary groups showed significant and continuous accumulation of Cd in the liver (Fig. 2) and kidney (Fig. 3). Waterborne Cd exposure at 2 $\mu\text{g}\cdot\text{L}^{-1}$ did not result in accumulation of Cd in the liver (Fig. 2), but the kidney did accumulate significant though lower amounts than those in the dietary treatments (Fig. 3).

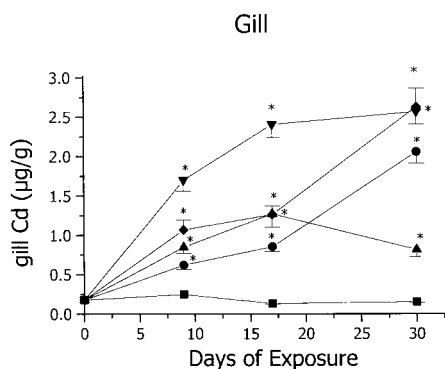


Fig. 1. Accumulation of Cd in the gills of juvenile rainbow trout exposed for 30 d to either waterborne Cd at 2 $\mu\text{g}\cdot\text{L}^{-1}$ (●) or one of three dietary Cd concentrations: 800 $\text{mg}\cdot\text{kg}^{-1}$ (▲), 1,500 $\text{mg}\cdot\text{kg}^{-1}$ (◆), or 2,200 $\text{mg}\cdot\text{kg}^{-1}$ (▼). Data are presented as mean \pm standard error of the mean (error bars; $n = 6$); an asterisk indicates a significant difference from unexposed controls (■) on that day ($p < 0.05$). Note that on day 30, no significant difference was observed between the two highest dietary Cd concentrations (2,200 and 1,500 $\text{mg}\cdot\text{kg}^{-1}$) and fish exposed to waterborne Cd (2 $\mu\text{g}\cdot\text{L}^{-1}$).

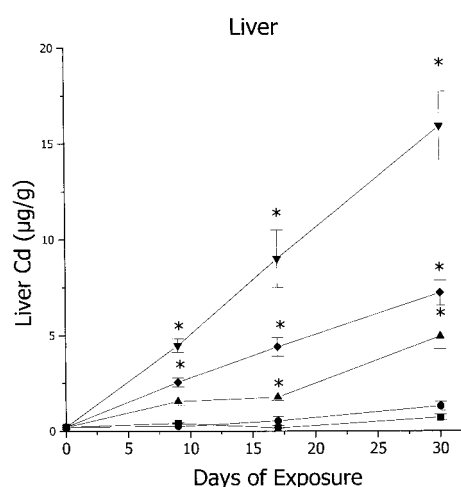


Fig. 2. Accumulation of Cd in the livers of juvenile rainbow trout exposed for 30 d to either waterborne Cd at 2 $\mu\text{g}\cdot\text{L}^{-1}$ (●) or one of three dietary Cd concentrations: 800 $\text{mg}\cdot\text{kg}^{-1}$ (▲), 1,500 $\text{mg}\cdot\text{kg}^{-1}$ (◆), or 2,200 $\text{mg}\cdot\text{kg}^{-1}$ (▼). Other details are as described in Figure 1.

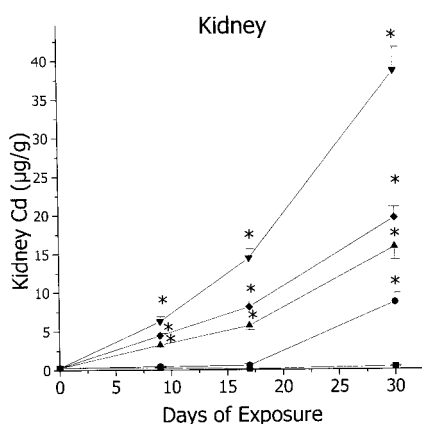


Fig. 3. Accumulation of Cd by the kidneys of juvenile rainbow trout exposed for 30 d to either waterborne Cd at $2 \mu\text{g}\cdot\text{L}^{-1}$ (●) or one of three dietary Cd concentrations: $800 \text{ mg}\cdot\text{kg}^{-1}$ (▲), $1,500 \text{ mg}\cdot\text{kg}^{-1}$ (◆), or $2,200 \text{ mg}\cdot\text{kg}^{-1}$ (▼). Other details are as described in Figure 1.

Although the levels of Cd in the anterior and posterior intestine (Table 5) varied over time, all three dietary groups showed significant accumulation of Cd in both portions of the gut on day 30. In contrast to series 1, no consistent difference was observed between anterior and posterior portions. Exposure to Cd via the water did not produce significant Cd accumulation in either the anterior or posterior portion of the intestine (Table 5). The 1,500 and $2,200 \text{ mg}\cdot\text{kg}^{-1}$ dietary-exposed fish accumulated low but significant amounts of Cd in the carcass throughout all sampling days, but the $800 \text{ mg}\cdot\text{kg}^{-1}$ dietary-exposed and the waterborne-exposed fish did not (Table 5). Thus, a chronic waterborne exposure producing a similar gill Cd burden to chronic dietary exposure produced internal accumulation only in the kidney, whereas the dietary exposure produced internal accumulations in all measured compartments of this study. Even in the kidney, the levels

from waterborne exposure were significantly lower than those from dietary exposure (Fig. 3).

Fish exposed to Cd via the diet accumulated a much greater overall amount of total body Cd in comparison with unexposed (i.e., control) and waterborne-exposed fish (Fig. 4A). The majority of total Cd accumulated in the intestinal tissues of dietary-exposed fish, whereas the carcass held the greatest Cd burden in waterborne-exposed fish, comparable to the native Cd burden in control fish (Fig. 4A). When organ-specific Cd accumulation was expressed relative to total body load, intestinal tissue represented the greatest percentage of total body Cd in dietary-exposed fish (Fig. 4B) and the carcass slightly less. However, the carcass accumulated the largest percentage of total body Cd for control (i.e., unexposed) and, to a lesser degree, waterborne-exposed fish (Fig. 4B). On a relative basis, the gill and kidney also contributed notably to the total body Cd burden in fish exposed to Cd via the water (Fig. 4B).

Short-term gill concentration kinetics test

Following 30 d of exposure to Cd, control, waterborne-exposed, and $1,500 \text{ mg}\cdot\text{kg}^{-1}$ dietary-exposed groups were chosen for short-term gill Cd-binding tests (Fig. 5). Note that before these kinetics tests, the total gill Cd content was similar for both the dietary- and waterborne-exposed groups and far higher than that in the controls (Fig. 1). The fish previously exposed to dietary Cd accumulated significantly lower levels of new Cd in the gill compared to controls and waterborne-exposed fish at all comparable concentrations (Fig. 5). Fish that were previously exposed to waterborne Cd tended to have higher accumulation of new Cd at most comparable concentrations, but none of the differences were significant.

Nonlinear regression analysis of Cd binding for the 3-h exposures to radioactive ^{109}Cd (Fig. 5) yielded the values of $\log K_{\text{Cd-gill}}$ (i.e., logarithm of the association constants, expressed as ionic Cd^{2+}) and B_{max} (i.e., binding capacity) reported

Table 5. Cd concentrations ($\mu\text{g}\cdot\text{g}^{-1}$) in the anterior and posterior intestine and carcasses of rainbow trout sampled on days 9, 17, and 30 of exposure to either waterborne Cd or one of three dietary Cd levels (series 2)^a

Tissue	Cd exposure treatment	Day of exposure		
		9	17	30
Anterior intestine	Control	0.39 ± 0.2	0.35 ± 0.03	0.06 ± 0.005
	Water			
	$2 \mu\text{g}\cdot\text{L}^{-1}$	0.27 ± 0.1	1.13 ± 0.59	0.19 ± 0.02
	Diet			
	$800 \text{ mg}\cdot\text{kg}^{-1}$	25.9 ± 5.1	33.1 ± 4.84^b	159 ± 17.7^b
	$1,500 \text{ mg}\cdot\text{kg}^{-1}$	150 ± 27^b	54.1 ± 9.72^b	191 ± 39.6^b
	$2,200 \text{ mg}\cdot\text{kg}^{-1}$	190 ± 69^b	32.3 ± 5.69^b	274 ± 48.4^b
Posterior intestine	Control	0.05 ± 0.05	0.13 ± 0.04	0.22 ± 0.04
	Water			
	$2 \mu\text{g}\cdot\text{L}^{-1}$	0.14 ± 0.09	1.26 ± 0.31	1.20 ± 0.47
	Diet			
	$800 \text{ mg}\cdot\text{kg}^{-1}$	54.9 ± 14.4	44.8 ± 8.60^b	198 ± 34.7^b
	$1,500 \text{ mg}\cdot\text{kg}^{-1}$	58.5 ± 12.7	33.2 ± 6.10^b	218 ± 16.8^b
	$2,200 \text{ mg}\cdot\text{kg}^{-1}$	637 ± 220^b	22.2 ± 2.10^b	221 ± 15.3^b
Carcass	Control	0.15 ± 0.03	0.11 ± 0.01	0.18 ± 0.03
	Water			
	$2 \mu\text{g}\cdot\text{L}^{-1}$	0.12 ± 0.03	0.09 ± 0.02	0.21 ± 0.05
	Diet			
	$800 \text{ mg}\cdot\text{kg}^{-1}$	0.95 ± 0.38^b	0.03 ± 0.01	0.76 ± 0.12
	$1,500 \text{ mg}\cdot\text{kg}^{-1}$	1.38 ± 0.37^b	0.82 ± 0.27^b	2.37 ± 0.65^b
	$2,200 \text{ mg}\cdot\text{kg}^{-1}$	1.51 ± 0.58^b	1.6 ± 0.36^b	2.54 ± 0.40^b

^a Mean \pm standard error of the mean ($n = 6$) are shown.

^b Significant difference between unexposed (control) and exposed fish ($p < 0.05$).

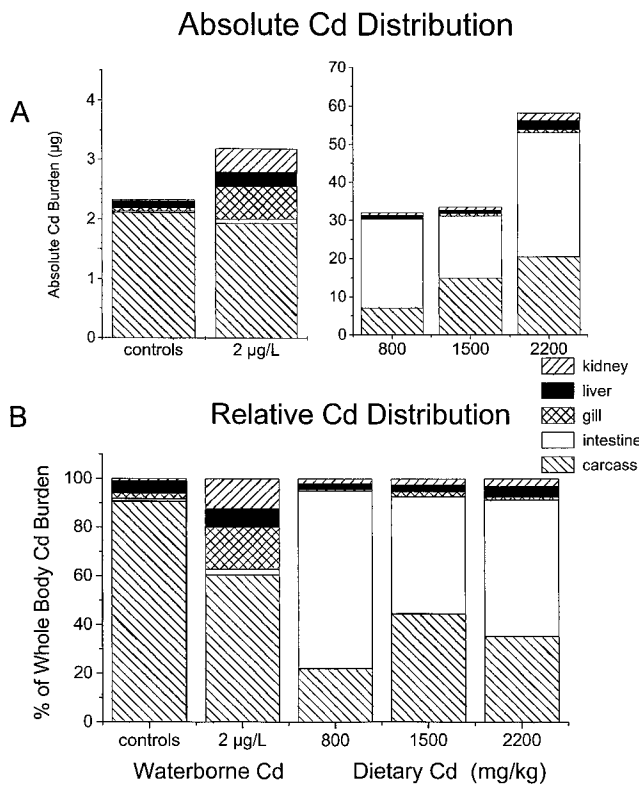


Fig. 4. Partitioning of whole-body Cd accumulation expressed either as absolute amounts per organ (A) or as the relative contribution of each organ (B) after 30 d of exposure to either waterborne Cd at 2 µg·L⁻¹ or one of three dietary Cd concentrations: 800, 1,500, or 2,200 mg·kg⁻¹. Note the different scales used in the two parts of A.

in Table 6 for the three treatment groups. Note that these values are expressed with 95% CL. Dietary-exposed fish exhibited a significant fall in gill Cd affinity (decreased $K_{Cd-gill}$) relative to controls, whereas the waterborne-exposed fish exhibited an intermediate $K_{Cd-gill}$. The opposite trend was seen with binding capacity, though none of the B_{max} values were significantly different.

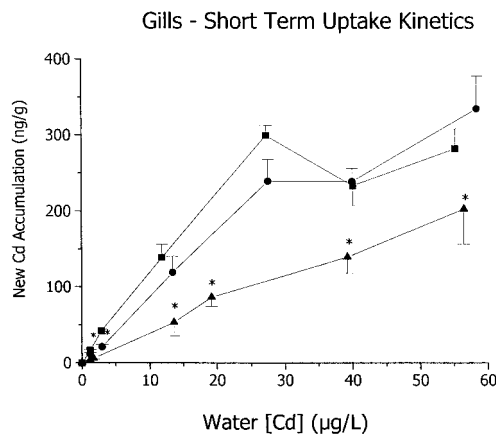


Fig. 5. Accumulation of new Cd (ng·g⁻¹), as measured by the uptake of ¹⁰⁹Cd, in the gills of juvenile rainbow trout exposed to waterborne Cd concentrations ranging from 1.2 to 58 µg·L⁻¹ for 3 h. Fish were previously exposed for 30 d to either waterborne Cd at 2 µg·L⁻¹ (●) or a dietary Cd concentration of 1,500 mg·kg⁻¹ (▲). Data are presented as mean ± standard error of the mean (error bars; n = 6); an asterisk indicates a significant difference from unexposed controls (■) at a comparable concentration of Cd (p < 0.05).

Table 6. Log $K_{Cd-gill}$ (logarithm of the apparent dissociation constants, expressed as ionic Cd²⁺) and B_{max} (binding capacity, nmol·g⁻¹) values calculated for rainbow trout gills during short-term (3-h) ¹⁰⁹Cd-binding tests following 30-d exposures

Treatment	Log $K_{Cd-gill}$	B_{max} (nmol·g ⁻¹)
Controls	7.05 (6.75–8.76) ^a	3.12 (2.14–4.09)
2 µg·L ⁻¹ b	6.54 (6.32–7.03)	4.80 (3.16–6.43)
1,500 mg Cd·kg ⁻¹ c	5.92 (5.83–6.51)	5.50 (2.86–8.17)

^a 95% Confidence limits.
^b Waterborne.
^c Dietary.

Long-term uptake test

At the end of the 30-d exposure, a long-term (46-h) ¹⁰⁹Cd uptake test was performed at a steady concentration of 2 µg·L⁻¹ using the same treatment groups as those for the short-term gill kinetics tests. The fish previously exposed to either waterborne or dietary Cd exhibited progressive accumulation of new Cd over time (Fig. 6A). However, the control group reached steady-state branchial accumulation by 8 h, and no further branchial accumulation of new Cd was observed up to 46 h (Fig. 6A). Even so, in the liver (Fig. 6B) and kidney (Fig. 6C), the control fish showed steady accumulation of new Cd.

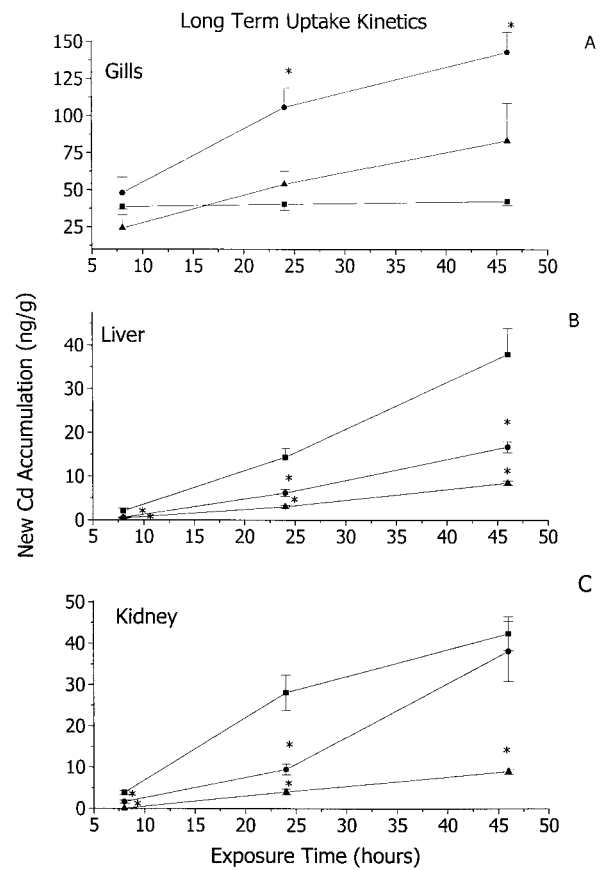


Fig. 6. Accumulation of new Cd, as measured by the uptake of ¹⁰⁹Cd, in the gills (A), livers (B), and kidneys (C) of juvenile rainbow trout at 8, 24, and 46 h of exposure to waterborne Cd at a concentration of 2 µg·L⁻¹. Fish were previously exposed for 30 d to either waterborne Cd at 2 µg·L⁻¹ (●) or a dietary Cd concentration of 1,500 mg·kg⁻¹ (▲). Data are presented as mean ± standard error of the mean (error bars; n = 6); an asterisk indicates a significant difference from unexposed controls (■) at that time (p < 0.05).

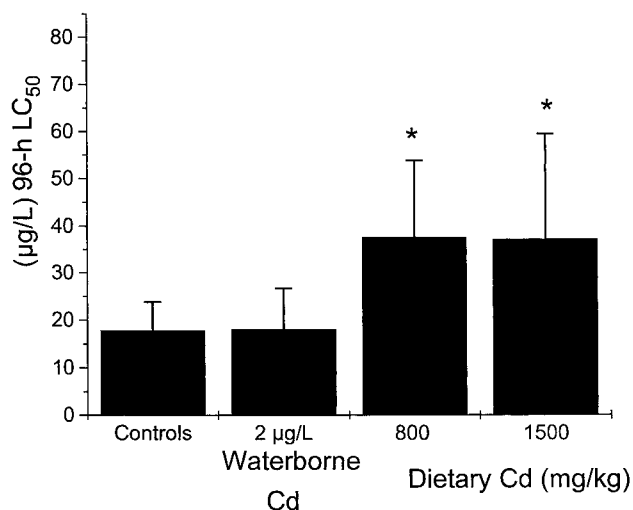


Fig. 7. Acute toxicity (96-h LC₅₀ + 95% CL) of Cd in juvenile rainbow trout. Fish were previously exposed for 30 d to either 0 µg·L⁻¹ (controls), 2 µg·L⁻¹ (waterborne Cd), 800 mg·kg⁻¹ (dietary Cd), or 1,500 mg·kg⁻¹ (dietary Cd). An asterisk indicates a significant difference from unexposed controls at that concentration of Cd ($p < 0.05$); error bars represent the standard error of the mean.

Fish exposed to dietary Cd (1,500 mg·kg⁻¹) accumulated the least amount of new Cd over time in both the liver and kidney, whereas gill loading was not significantly different from that in controls. The waterborne-exposure group accumulated the most new Cd in the gills (Fig. 6A) and intermediate levels in the liver (Fig. 6B) and kidney (Fig. 6C).

Acute toxicity tests

Acclimation to Cd was observed for dietary-exposed trout, because these groups showed a significantly increased tolerance to waterborne Cd (Fig. 7). The 96-h LC₅₀ values (shown with 95% CL) were twofold greater for fish previously exposed to a dietary Cd concentration of either 800 or 1,500 mg·kg⁻¹ in comparison to fish exposed to Cd via the water and to unexposed control fish. The LC₅₀ value for the waterborne-exposed group was not different than that for the controls (Fig. 7).

DISCUSSION

Overview

Our results show that chronic sublethal Cd exposure via the diet can alter uptake of Cd from the water into the gill, independent of a previous waterborne exposure, and that this, in turn, can alter acute toxicity. When compared to trout chronically exposed to waterborne Cd, gill metal uptake characteristics differed in spite of similar gill Cd burdens. These differences in gill Cd uptake were observed in both the short-term (3-h; Fig. 5) and long-term (46-h; Fig. 6) experiments. Therefore, the route of gill Cd loading, whether basolateral (i.e., dietary exposure) or apical (i.e., waterborne exposure), results in differences in Cd uptake dynamics from the water. Our work indicates that the physiological mechanisms of and responses to chronic Cd exposure, whether via the diet or the water, seem to be more complex than the processes currently incorporated into the models (e.g., BLM) being developed to predict acute toxicity based on short-term tissue loading [18–20].

Chronic exposure to dietary or waterborne Cd also results in different tissue-specific accumulations. The greatest Cd concentration was measured in the intestinal tissues (Table 5) for dietary-exposed fish, whereas Cd was measured in the highest concentration (µg·g⁻¹) in the kidney (Fig. 3) and gills (Fig. 1) for fish exposed to Cd via the water. In terms of total Cd accumulation, both the absolute and relative organ-specific internal distributions of Cd varied depending on the route of exposure (Fig. 4).

Gill uptake dynamics

Rainbow trout exposed to a dietary Cd concentration of 1,500 mg·kg⁻¹ showed significantly less new metal uptake at the gills during the short-term Cd loading experiment compared to both controls and 2 µg·L⁻¹ waterborne-exposed fish (Fig. 5). Nonlinear regression analysis of these data showed that both dietary and waterborne Cd exposure produced a decrease in the affinity (lower log $K_{Cd-gill}$) and an increase in the capacity (higher B_{max}) of the gills for Cd, although only the former was significant. The binding characteristics for trout exposed to 2 µg·L⁻¹ of waterborne Cd (Table 6) followed the same trends as those reported by Hollis et al. [6] for similar fish chronically exposed to 3 µg·L⁻¹ in similar water. Hollis et al. showed a decrease in log $K_{Cd-gill}$ from 7.6 for controls to 7.2 for exposed fish, whereas the number of binding sites (B_{max}) increased from 1.61 to 2.59 nmol·g⁻¹ (control and exposed, respectively), changes that are qualitatively similar to the present observations.

The very similar binding-site numbers, in conjunction with similar Cd loads, among dietary- and waterborne-exposed fish suggest that gill Cd burden, either via water or dietary exposure, regulates the subsequent short-term exchange capacity of the gill. However, log $K_{Cd-gill}$ values differed among dietary- and waterborne-exposure groups, indicating that the affinity of the gill for Cd is influenced both by the gill Cd burden and by changes that occur on the apical membrane during waterborne exposure. These conclusions are based on the assumption that the apical surface of the gill was not exposed to Cd in trout fed Cd-contaminated diets. Measurements of water Cd confirm this on a bulk water basis, but we cannot rule out the possibility that small amounts of internally accumulated Cd leaked across the gills into the water/mucous boundary layer surrounding the gill, thereby mimicking waterborne exposure.

The BLM is a mechanistically based approach to the prediction of acute toxicity [17–20,25]. Specifically, it is based on applying geochemical principles to short-term gill metal binding to predict acute toxicity and, therefore, depends on a consistent relationship between these two variables. In this study, both waterborne and dietary exposure to Cd altered subsequent short-term gill Cd binding. Furthermore, the acute toxic threshold was changed in response to chronic dietary exposure to Cd, which is in agreement with the findings of Hollis et al. [6], but not in response to chronic waterborne Cd exposure, which is in disagreement with the findings of Hollis et al. This difference may indicate that the threshold for acclimation [11] in Hamilton water lies between 2 µg·L⁻¹ (present study) and 3 µg·L⁻¹ [6]. Regardless, the key point is that the relationships between water geochemistry, gill Cd binding, and acute toxicity changed as a result of chronic exposure. Therefore, BLM predictions may require special interpretation when fish have been chronically exposed to Cd via dietary or waterborne routes. Until now, this point has not been considered in the BLM approach. Although the dietary concentrations

used in this study are generally greater than those found in the natural environment, this experiment illustrates that the BLM may require further development before it can predict acute toxicity for rainbow trout previously exposed to either waterborne or dietary Cd.

When time-dependent uptake dynamics at a waterborne Cd concentration of $2 \mu\text{g}\cdot\text{L}^{-1}$ were measured during long-term exposure, fish exposed to dietary Cd accumulated the same amount of, or slightly more, new metal on the gills than control fish (Fig. 6A) but far less in internal organs such as the liver (Fig. 6B) and kidney (Fig. 6C). This trend was similar, but less distinct, for trout that were chronically exposed to waterborne Cd, which accumulated the most new Cd on the gills and rather less in the internal organs. These results support those of the short-term Cd loading experiment (Fig. 5) and agree with those of Hollis et al. [6]. Therefore, the burden of Cd on the gills of Cd-exposed rainbow trout functions, in some manner, to reduce accumulation in the kidney and liver. At least in part, this may contribute to the acclimation response. Interpretation of these results may be complicated by isotopic exchange, whereby radiolabeled Cd (the tracer for new Cd) is exchanged with already-accumulated cold Cd in the chronically exposed fish. Nevertheless, it remains interesting that much lower accumulation of new Cd from the water was found in the liver (Fig. 6B) and kidney (Fig. 6C) of dietary-exposed compared to waterborne-exposed trout, despite similar gill cold Cd burdens (Fig. 1). This could have resulted from apical changes present only in waterborne-exposed fish, from basolateral changes only present in dietary-exposed fish, and/or from the very elevated internal tissue burdens already present as a result of chronic dietary exposure (Figs. 2 and 3).

Environmental relevance of Cd exposure

The water Cd concentration of $2 \mu\text{g}\cdot\text{L}^{-1}$ used in this study was within the range of environmentally realistic concentrations ($\leq 5 \mu\text{g}\cdot\text{L}^{-1}$ in North American surface waters as reviewed by Spry and Wiener [26] and the Canadian Council of Ministers of the Environment [27]). The guideline Cd concentration in Canada for a water hardness of $140 \text{ mg}\cdot\text{L}^{-1}$ is $1.3 \mu\text{g}\cdot\text{L}^{-1}$ [27], and the acute and chronic ambient water-quality criteria are 5.7 and $1.5 \mu\text{g}\cdot\text{L}^{-1}$, respectively [28]. The present dietary Cd concentrations of 1.5 to $2,200 \text{ mg}\cdot\text{kg}^{-1}$ started within the natural range but progressed to much higher levels than those found in freshwater environments. Dallinger and Kautzky [12] measured Cd concentrations of $1.8 \text{ mg}\cdot\text{kg}^{-1}$ in benthic invertebrates from polluted waters, and levels ranging from 0.13 to $28 \text{ mg}\cdot\text{kg}^{-1}$ were observed in benthic invertebrates in the highly polluted Clark Fork River area (MT, USA) [16,29,30]. Interestingly, the measured Cd concentration of the control feed (i.e., without added Cd) was $1.47 \pm 0.05 \text{ mg}\cdot\text{kg}^{-1}$, which is within the normal range [31]. Therefore, small but significant amounts of Cd were already present in trout feed, and farmed fish likely are exposed to this level of Cd before experimentation. However, the control fish in this study did not accumulate additional amounts of Cd in any of the measured tissues during the 30- to 36-d exposures.

Dietary Cd toxicity

Although not evaluated in detail, no decrease was observed in the appetite of any fish exposed to dietary Cd concentrations in series 2, but higher levels of dietary exposure resulted in mortalities and a lower SGR (Table 2). In the absence of data on the weights of fish that died, it is impossible to conclude

whether the lower SGR resulted simply from a selection against larger fish or from a true reduction in the growth rate. However, if the latter was the case, impaired growth in trout fed elevated levels of Cd may have resulted from decreased nutrient absorption and/or increased costs associated with physiological stress, with the latter diverting energetic inputs that would normally be used for growth.

The physiological reasons behind the death of fish exposed to dietary Cd are currently unclear. The majority of mortalities occurred within the first two weeks of exposure. From the deaths recorded during series 2, a 14-d dietary LC50 would be between $1,500$ and $2,200 \text{ mg}\cdot\text{kg}^{-1}$ (Table 2), which is somewhat lower than the apparent chronic dietary toxicity value of approximately $10,000 \text{ mg}\cdot\text{kg}^{-1}$ as suggested by Handy [32]. This difference may be due to a size difference between the fish used in this study and those used in Handy's experiment, which were approximately 2 and 131 g , respectively. It is also possible that the fish in series 2 had a greater sensitivity for dietary Cd, because the dietary Cd exposure level of $1,500 \text{ mg}\cdot\text{kg}^{-1}$ in series 1 resulted in much lower mortality rates. This mortality difference between series 1 and 2 was not related to differences in tissue-specific Cd burdens (Tables 3 and 4 and Figs. 1–3) but could have resulted from differences in sample size, fish size, water temperature, season, and/or sources of fish used.

Uptake and internal distribution of Cd

Uptake of Cd across the gut during dietary exposures resulted in substantial accumulation of Cd in internal organs, especially the gastrointestinal tract (Tables 4 and 5), kidney (Table 3 and Fig. 3), and liver (Table 3 and Fig. 2), which is similar to observations in other studies [2,9,10,12]. In our work, the highest tissue Cd accumulations for dietary-exposed fish were at the site of uptake, the posterior and anterior intestine (Tables 4 and 5), which are results similar to those of Handy [10]. Although accumulation of Cd in internal organs was directly related to dietary-exposure concentrations, it was not related to the total accumulation of Cd in intestinal tissue (Tables 4 and 5). For example, in series 2, the fish exposed to 800 , $1,500$, and $2,200 \text{ mg}\cdot\text{kg}^{-1}$ accumulated similar Cd levels in both anterior and posterior intestinal tissues, suggesting that saturation had occurred. Fecal material was only assayed in series 1 (Table 4); nevertheless, it is interesting that fecal Cd tended to be relatively constant over a wide dietary range. The explanation for this is unclear.

Cadmium accumulation in the kidney was significant for fish exposed to dietary Cd concentrations greater than $15 \text{ mg}\cdot\text{kg}^{-1}$, and levels were higher than those found in the liver (Tables 3 and 4 and Figs. 2 and 3). In other studies, elevated Cd levels have similarly been found in the kidney as a result of both dietary and waterborne Cd exposure, and in dietary exposures, the kidney burden typically exceeds that of the liver [2,3,6,8–10,13]. Cadmium is often described as a nephrotoxicant due to its selective accumulation in kidney tissue; Kumada et al. [8] suggested that the kidney has a central role in the elimination of Cd during chronic sublethal exposure. Although significant Cd accumulation also occurred in the kidney of trout exposed to waterborne Cd in the present study (Fig. 3), these levels were less than those in dietary-exposed fish with equal gill burdens. Furthermore, waterborne Cd exposure did not result in liver accumulation of Cd, whereas dietary exposure did. This may reflect that Cd absorbed into the blood from the gut is transported directly to the liver via the hepatic

portal system, whereas the Cd transferred into the blood from the gills is distributed via the arterial system throughout the internal organs, which (especially the kidney) may scavenge this Cd before it reaches the liver.

In spite of dramatic accumulation in most internal organs as a result of dietary exposure, the brain did not show increased Cd content (Table 4), thus illustrating the protective effect of the blood–brain barrier. Dietary-exposed trout exhibited carcass accumulations of Cd that were only approximately 5- to 10-fold greater than the level found as a result of the waterborne Cd exposure (Table 5). In comparison with other tissues, the carcass, which is composed mainly of white muscle, skin, and bone, accumulated a very small Cd burden on a per-gram basis. However, due to its large mass, the carcass represents a large portion of the total body burden of Cd in unexposed, dietary-exposed, and waterborne-exposed fish (Fig. 4A).

Establishing similar gill Cd burdens via dietary (i.e., basolateral) and waterborne (i.e., apical) routes proved to be possible, but the dietary concentration necessary to achieve a similar gill burden was approximately 1 million-fold greater in comparison to the waterborne concentration (1,500,000 $\mu\text{g}\cdot\text{kg}^{-1}$ in diet vs 2 $\mu\text{g}\cdot\text{L}^{-1}$ in water). Uptake of Cd^{2+} from the water occurs via Ca^{2+} -specific transport channels in the apical membranes of the ion-transporting cells of the gills, the chloride cells [22,23]. Once in the gill cell, movement across the basolateral membrane to the blood may be by facilitated diffusion, because Ca-ATPase is inhibited by Cd [23]. The mechanism of Cd uptake from the diet is not understood as well, but Handy [5] suggests that Cd binds to the luminal surface of the mucosal cells by electrostatic attraction and, subsequently, is taken up into the blood. It is possible that Cd^{2+} is taken up from the gut via Ca^{2+} uptake and transport mechanisms, because Pratap et al. [1] reported that dietary Cd caused hypermagnesemia and hypocalcemia in tilapia adapted to water with low Ca^{2+} levels. Schoenmakers et al. [33] suggested that dietary Cd not only accumulates in the tissues but likely is transported into the blood via a Na^+/Ca^+ exchanger in the basolateral membrane of intestinal cells.

Nonetheless, once in the blood, how Cd enters other tissues, particularly across the basolateral surface of the gills, is not known. It would seem to be unlikely that the facilitated diffusion mechanism on the basolateral membrane could transport Cd from blood to gill cells. Because gill Ca^{2+} movement and, therefore, transporter orientation in the gills of freshwater fish is directed inwardly (i.e., gill cell \rightarrow blood), no efficient way may exist to move Cd from blood to gill cells. Perhaps this explains why such greatly elevated Cd levels in the diet were required compared to waterborne-exposure levels to achieve the same gill burden. Clearly, the gut tissue provides a much better barrier than the gill to internal accumulation. The mechanisms of uptake and accumulation of Cd in gut tissue as well as of transfer to internal organs are worthy of further research.

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