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# Effects of dietary calcium and cadmium on cadmium accumulation, calcium and cadmium uptake from the water, and their interactions in juvenile rainbow trout

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**Abstract**

The objective of this study was to examine the effects of chronically elevated dietary $Ca^{2+}$ (as $CaCO_3$), alone and in combination with elevated dietary Cd, on survival, growth, and Cd and $Ca^{2+}$ accumulation in several internal compartments in juvenile rainbow trout (*Oncorhynchus mykiss*). In addition, effects on short-term branchial uptake and internal distribution of newly accumulated waterborne $Ca^{2+}$ and Cd during acute waterborne Cd exposure (50 μg/L as $CdNO_3$ for 3 h) were monitored using radiotracers ($^{45}Ca$, $^{65}Cd$). Fish were fed with four diets: 20 mg $Ca^{2+}$/g food (control), 50 mg $Ca^{2+}$/g food, 300 μg Cd/g food, and 50 mg $Ca^{2+}$/g + 300 μg Cd/g food for 30 days. There were no significant effects on growth, mortality, or total body $Ca^{2+}$ accumulation. The presence of elevated $Ca^{2+}$, Cd, or $Ca^{2+}$ + Cd in the diet all reduced waterborne $Ca^{2+}$ uptake in a short-term experiment (3 h), though the inhibitory mechanisms appeared to differ. The effects were marked after 15 days of feeding, but attenuated by 30 days, except when the diet was elevated in both $Ca^{2+}$ and Cd. The presence of elevated $Ca^{2+}$ in the diet had only modest influence on Cd uptake from the water during acute Cd challenges but greatly depressed Cd uptake from the diet and accumulation in most internal tissues. None of the treatment diets prevented the decreases in waterborne $Ca^{2+}$ uptake and new $Ca^{2+}$ accumulation in internal tissues caused by acute exposure to waterborne Cd. In conclusion, there are complex interactions between waterborne and dietary effects of $Ca^{2+}$ and Cd. Elevated dietary $Ca^{2+}$ protects against both dietary and waterborne Cd uptake, whereas both waterborne and dietary Cd elevations cause reduced waterborne $Ca^{2+}$ uptake.


*Keywords:* Dietary cadmium; Dietary calcium; Cadmium uptake; Calcium uptake; Fish

## 1. Introduction

Waterborne Cd is usually found in surface waters that receive discharges of metal smelting operations and other industrial processes. Long-term exposures

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(20 days or more) to waterborne Cd at sub-lethal concentrations led to a decrease in growth in juvenile and adult rainbow trout (*Oncorhynchus mykiss*; Ricard et al., 1998), as well as to mortality and reduced growth in juvenile bull trout (*Salvelinus confluentus*; Hansen et al., 2002) and guppy (*Poecilia reticulate*; Miliou et al., 1998). Chronic mortality due to waterborne Cd probably occurs because this metal can affect hepatic enzymes (De Smet and Blust, 2001), liver size, and glycogen content (Ricard et al., 1998) and can induce hypocalcemia (Larsson et al., 1981; Giles, 1984; Pratap et al., 1989; McGeer et al., 2000) by inhibiting the branchial gill $Ca^{2+}$-ATPase (Verbost et al., 1987; Wong and Wong, 2000).

However, in freshwater ecosystems, contaminated food may sometimes be a more important source of toxic metals than the water itself. Chronic discharges of low levels of waterborne metals over long periods of time may lead to metal accumulation in sediments. From the sediments, the metals can be transferred to plants, then to grazers, or directly to benthic invertebrates, and finally to fish that feed on these contaminated animals (Dallinger and Kautzky, 1985). Accumulation of Cd from food was proportionally higher than from water for rainbow trout and lake whitefish (*Coregonus clupeaformis*) in a 72-day exposure (Harrison and Klaverkamp, 1989). Dietary Cd caused ionic disturbances in the plasma and changes in the structure of gill cells of tilapia (*Oreochromis mossambicus*) comparable to those provoked by waterborne Cd (Pratap et al., 1989; Pratap and Wendelaar Bonga, 1993). However, prolonged exposure of juvenile rainbow trout to elevated dietary Cd caused an apparent reduction of waterborne Cd uptake, a reduction in acute waterborne Cd toxicity, as well as changes in the binding affinity and capacity of the gills for Cd (Szebedinszky et al., 2001).

$Ca^{2+}$ and Cd share the same transport pathway in the gills (Verbost et al., 1989; Playle et al., 1993; Niyogi and Wood, 2004), and consequently, an increase in waterborne $Ca^{2+}$ reduces waterborne Cd uptake (Hollis et al., 2000) and decreases Cd toxicity in several fish species (Pratap et al., 1989; Pratap and Wendelaar Bonga, 1993; Hansen et al., 2002). When Cd-contaminated food was fed to tilapia, Cd uptake occurred by the gastrointestinal tract and caused hypermagnesemia, hypocalcemia, and degeneration of pavement and chloride cells in the gills (Pratap et al., 1989; Pratap and Wendelaar Bonga, 1993). Waterborne $Ca^{2+}$ had an ameliorating effect on calcium and magnesium metabolism of fish fed food with dietary Cd, but gill ultrastructure was not affected.

In addition to the influence of water chemistry, metal toxicity can also be affected by diet. Normal growth of fish (trout, catfish, tilapia) can be obtained with as little as 4.5–7.0 mg $Ca^{2+}$/g food, even in low-$Ca^{2+}$ water (Robinson et al., 1986; O'Connell and Gatlin, 1994). Elevated dietary $Ca^{2+}$ (30–60 mg $Ca^{2+}$/g food) inhibited waterborne Cd uptake and accumulation in both acutely and chronically Cd-exposed rainbow trout (Zohouri et al., 2001; Baldisserotto et al., 2004b). Sherwood et al., 2000 reported that yellow perch (*Perca flavescens*) in metal-contaminated (Cd, Zn, Cu) lakes tended to eat more invertebrates and less fish than perch from non-polluted areas. The reason(s) behind this change of diet (changes in abundance of prey or efficiency of predation) were not studied by the authors, but this strategy would likely increase the dietary $Ca^{2+}$ intake of the perch. However, even in places with low water contamination, Cd content in aquatic isopods and snails can be high (Dallinger and Kautzky, 1985), and therefore higher dietary $Ca^{2+}$ might also lead to higher dietary Cd.

Consequently, the objective of this study was to examine the effects of elevated dietary $Ca^{2+}$ and Cd (alone and in combination) on survival and growth, on Cd and $Ca^{2+}$ accumulation in several internal compartments, and on short-term branchial uptake and internal distribution of radio-labeled waterborne $Ca^{2+}$ and Cd uptake in juvenile rainbow trout. Acute exposures to radio-labeled waterborne Cd were performed 15 and 30 days after the fish started their experimental diets to determine whether effects were consistent over time, or were modulated by acclimation or sensitization.

## 2. Material and methods

### 2.1. Experimental animals

Juvenile rainbow trout (12–15 g) were purchased from Humber Springs Fish Hatchery (Orangeville, ON). Fish were maintained for 1 week in an aerated 200 L polypropylene tank supplied with approximately 1 L/min dechlorinated Hamilton tap water (mmol): [Na] = 0.6, [Ca] = 1.0, [Cl] – 0.7,