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Comparative Accumulation Efficiency of ^{109}Cadmium from Natural Food (*Hyalella azteca*) and Artificial Diet by Rainbow Trout (*Oncorhynchus mykiss*)

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Bioaccumulation models have been proposed to predict the accumulation of chemicals throughout aquatic food-chains (Aoyama et al. 1978; Thomann and Connolly 1984). The efficiency with which organisms at each trophic level absorb these substances from their diet is an important component of such models.

Metal absorption from food is typically estimated by applying metal salts surficially to commercial fish diets. However, this method may not provide an accurate estimate of in situ accumulation because the efficiency of absorption of metals applied surficially to commercial diets may differ from that of metals absorbed through the food chain. For example, both sunfish (Lepomis gibbosus) (Merlini et al. 1976) and plaice (Pleuronectes platessa) (Pentreath 1973) accumulated ^{65}zinc applied surficially to foods more efficiently than ^{65}zinc incorporated into live foods. There have apparently been no similar comparisons made for cadmium using fish. Furthermore, few studies have fitted data to exponential equations to provide estimates of the absorption efficiency coefficient (E) and the excretion coefficient (k_2) for cadmium.

We report here the results of direct comparisons of cadmium absorption from commercial trout diet surficially-contaminated with ^{109}Cd and from natural food (the amphipod Hyalella azteca) cultured in ^{109}Cd-contaminated microcosms. We calculated absorption efficiency and excretion coefficients for cadmium by monitoring ^{109}Cd accumulation and excretion in rainbow trout fingerlings fed on the two diets.

MATERIALS AND METHODS

The rainbow trout fingerlings were Nisqually strain, from Washington state, U.S.A. At the beginning of the experiment, the fish were about 3 months old and those which were fed trout diet weighed 2.06 g (SD 0.52), while those fed amphipods weighed 1.75 g (SD 0.69).

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The amphipods were from a laboratory stock which originated in the La Salle River at Winnipeg, Manitoba. Mean weight of a sample of amphipods was 4 mg (n=23).

Each fish was maintained in a separate polystyrene container, 16.5 cm l x 11 cm w x 9 cm d. A 3.2 cm hole in each end, 3.5 cm above the bottom, was covered with 1000-micron Nylon screen attached with silicone sealant. Polyethylene lids prevented fish from escaping. The containers were placed in a water bath 170 cm l x 66 cm w. Water depth was 5 cm. The inflow rate of dechlorinated water to the water bath was 2.3 L/min, for a 99% replacement time of 1.8 hr.

Winnipeg city water was dechlorinated by activated charcoal to < 3 μg/l. Major water chemistry parameters were: Na^+, 0.09 mM; K^+, 0.03 mM; Ca^{2+}, 0.60 mM; Mg^{2+}, 0.23 mM; Cl^-, 0.16 mM; dissolved organic carbon, 1.39 mM; $CaCO_3$ hardness, 0.8 mM (80 mg/L), pH 7.8. Mean water temperature in the bath during the experiment was 10.5°C (SD 0.3°C). Aeration was supplied constantly to the water in the bath. Dissolved oxygen within the containers averaged 97% of saturation (SD 2%).

The trout diet, Martin Mills #3, contained 0.21 μg Cd/g, 19.7 mg Ca/g, and 1.57 mg Mg/g (dry wt). Tabachek (1984) reported that this diet contained 9.5% water, 47.9% protein (dry wt), and 3.60 Kcal/g of metabolizable energy (dry wt). The amphipods contained 75.9% water, <0.08 μg Cd/g, 40.41 mg Ca/g, and 0.62 mg Mg/g (dry wt). Driver et al. (1974) reported that H. azteca contained 45.3% to 49.8% protein (dry wt), and 3.40 to 4.19 Kcal/g (dry wt).

Sufficient ^{109}Cd as $CdCl_2$ (specific activity 1.4 x 10^5 cpm/ng) was mixed into the trout diet to produce a concentration of 15 cpm/mg (wet wt) of food. The amphipods were placed in two aquaria 77 cm l x 32 cm w x 31 cm h, containing 14 cm of water (34 L). Sufficient ^{109}Cd as $CdCl_2$ was added to the water to provide a concentration of 200,000 cpm/L, which produced a mean concentration of 99 cpm/mg (wet wt SD 34) of amphipod within one week. The sole source of food for the amphipods was algae growing in the aquaria.

A low feeding rate of 0.8 % of the mean weight of the fish was used to minimize growth during the experiment. Fish were fed daily, Monday to Friday. Four amphipods, consisting of one ^{109}Cd-contaminated animal and three uncontaminated animals, were fed daily to each fish in one group, while 16 mg of ^{109}Cd-contaminated trout diet was fed daily to each of the five fish in the other group. Gamma emissions were counted in each daily feeding before the food was offered to the fish. At the end of week 1, the feeding rate was increased to 1% of the mean fish weight because several of the fish had lost weight. For the duration of the experiment, the fish were fed 25% more food daily, consisting of 20 mg of trout diet, or one ^{109}Cd-contaminated amphipod with four uncontaminated amphipods. Both amphipods and trout diet were consumed immediately upon presentation to the fish. The mean quantities of ^{109}Cd provided at each feeding

of the two diets were: amphipods, 398 cpm (SD 137); trout diet, 297 cpm (SD 59).

Accumulation of ^{109}Cd was monitored for 7 weeks, then excretion was monitored for an additional 3 weeks. During the excretion phase, fish were fed the same quantity of uncontaminated food daily, Monday to Friday, as they had received during the accumulation phase.

On Monday of each week, ^{109}Cd content of each fish was estimated by counting gamma emissions. A tube for live counting of individual fish was made by covering a polystyrene culture tube 16 mm in diameter and 100 mm tall with black bookbinding tape to restrict light. Each fish was lightly anaesthetized in 0.2 mg/L of MS-222 (tricaine methanesulfonate), then the fish was placed in fresh water in the counting tube and the tube was stoppered. Gamma emissions were counted in a gamma counter for one minute; the fish was then removed from the counting tube, lightly blotted, transferred to a tared beaker of water on a balance, weighed, and returned to its container.

The excretion coefficient (k_2) was calculated for each fish by fitting the ^{109}Cd-elimination data to the equation:

$$Cd_t = Cd_0 \exp(-k_2 t) \qquad (1)$$

Cd_t is the ^{109}Cd concentration in the fish at time t and Cd_0 is the ^{109}Cd concentration at the beginning of the excretion phase.

The absorption efficiency coefficient (E) was then calculated for each fish by fitting the ^{109}Cd-accumulation data to the equation:

$$Cd_t = [(E \cdot f)/k_2]\ Cd_{fd}\ (1-\exp(-k_2 t)) \qquad (2)$$

Cd_t is the ^{109}Cd concentration in each fish at time t, f is the feeding rate in g/g of fish, Cd_{fd} is the ^{109}Cd concentration in the food and k_2 is the excretion coefficient for the same fish as calculated from equation (1) above (Bruggeman et al. 1981).

Growth rates were estimated using linear regression of fish weights vs time, and were used with equations (1) and (2) to correct for growth (Neely 1980).

T-tests were used to test for differences in k_2 and E between the two groups of fish.

RESULTS AND DISCUSSION

Absorption efficiency coefficients for cadmium (E) were, on average, over 5 times greater (significant at p=0.0141) for fish which were fed amphipods than for fish which were fed trout diet (Figure 1, Table 1). Excretion coefficients (k_2) were not significantly different (p=0.615) between the two groups (Figure 2, Table 1), indicating that differences in accumulation cannot be attributed to dif-

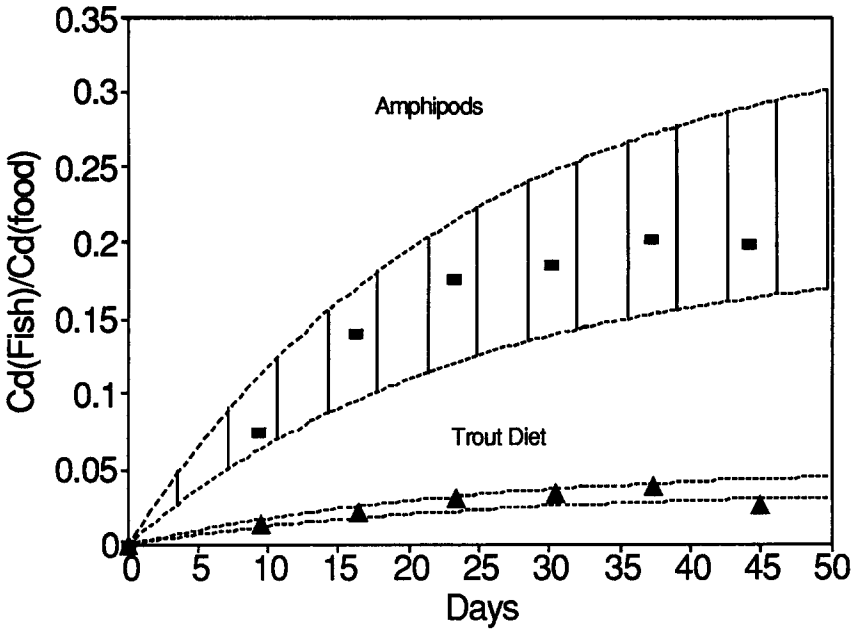

Figure 1. Accumulation of ^{109}Cd by rainbow trout fingerlings, plotted as the ratio of the ^{109}Cd concentration in fish to the average ^{109}Cd concentration in food during the uptake phase. The range was calculated using the mean estimated absorption efficiency coefficient (E) +/-SD. Squares represent means for fish fed amphipods (N=5) and triangles represent means for fish fed trout diet (N=4).

ferences in excretion rates. These data suggest that environmentally-contaminated natural foods should be used in future studies of cadmium absorption efficiency. However, surficially-contaminated artificial diets seem to be acceptable for estimating cadmium excretion coefficients.

Fish which were fed amphipods did not grow during the experiment (mean rate -0.8 mg/d, SD 1.6), whereas fish which were fed trout diet grew linearly (mean r^2=0.99) at a mean rate of 16 mg/d (SD 3), and at the end of the exposure, weighed on average 86% more than fish which were fed amphipods. Accumulation and excretion data were corrected for growth (see Materials and Methods).

It is unlikely that ^{109}Cd leached from trout diet before it was consumed because fish consumed the trout diet immediately upon its presentation. To verify that ^{109}Cd did not readily leach from trout diet, we added trout diet containing 8271 cpm of ^{109}Cd to one liter of water; after 16 hours, we could not detect ^{109}Cd in 10 ml samples of water but recovered 63% of the ^{109}Cd in food particles aspirated from the bottom of the vessel.

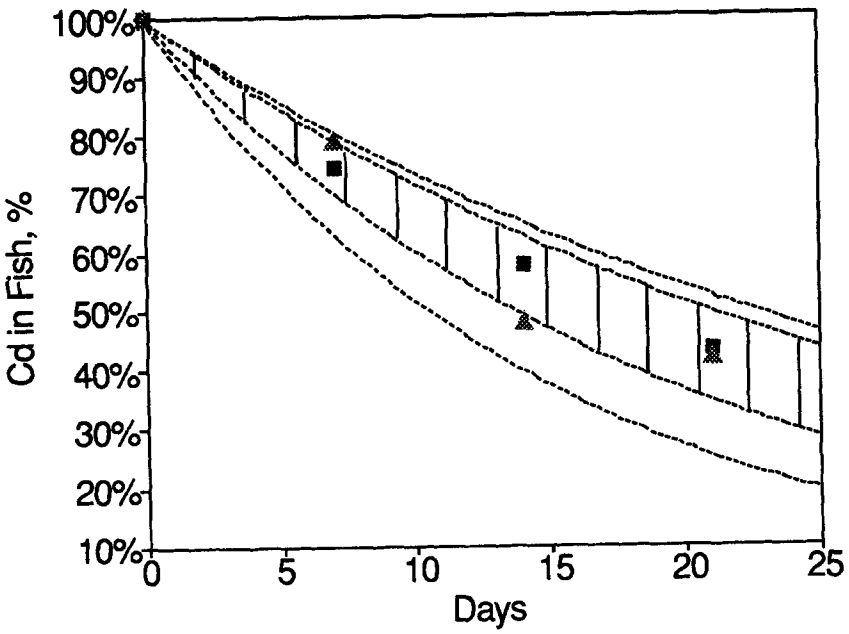

Figure 2. Excretion of ^{109}Cd by rainbow trout fingerlings, as a percentage of the ^{109}Cd concentration in the fish at the beginning of the elimination phase. The range was calculated using the mean excretion coefficient (k_2) +/- SD. Squares represent means for fish fed amphipods (N=5) and triangles represent means for fish fed trout diet (N=4).

The differing calcium content of the two diets should not have influenced ^{109}Cd accumulation. At each feeding, the fish which were fed trout granules received approximately twice as much calcium as the fish which were fed amphipods (17.8 mg/g vs 9.74 mg/g wet wt, respectively). A low-calcium diet has been shown to promote an increased uptake of cadmium by rats (Washko and Cousins 1977). Amphipods, however, are not calcium deficient. The calcium concentration (dry wt) in amphipods is about 200 times more than the calcium requirement of 0.02% per unit weight of food or 0.2 mg/g rainbow trout (Lall 1989).

Cadmium in amphipods appears to have crossed the gut wall of the fish more readily than cadmium surficially-applied to artificial diet, but the reasons are not clear. Cadmium has a strong affinity for sulfydryl groups as found in the amino acid cysteine (McAuliffe and Murray 1972). Therefore, ^{109}Cd in amphipods may have been attached to protein by strong sulfydryl bonds, whereas surficially-applied ^{109}Cd may have been attached to trout diet by less powerful surface adsorption forces. Fish easily digest proteins to their constituent amino acids, which are in turn rapidly absorbed intact

Table 1. Coefficients for cadmium absorption (E) and excretion (k_2) for rainbow trout fed amphipods and trout diet.

Treatment	Fish No.	k_2	E
Amphipods	1	0.055	1.302
	2	0.046	1.045
	3	0.039	0.542
	4	0.036	0.446
	5	0.034	0.732
	Mean	0.042	0.813
	SD	0.009	0.356
Trout Diet[a]	6	0.040	0.135
	7	0.046	0.136
	8	0.074	0.203
	9	0.039	0.143
	Mean	0.050	0.154
	SD	0.016	0.033

[a] n=4 because one fish was killed acidentally during the exposure phase.

by the intestine (Fänge and Grove 1979). In our study, ^{109}Cd may have crossed the intestinal membrane while bound to cysteine or to the metal-binding protein, metallothionein, which has a high cysteine content. This hypothesis is supported by data acquired with rats. Revis and Osborne (1984) reported that cadmium accumulation by rats increased when a low-protein diet was supplemented with cysteine. Cadmium metallothionein from rats is extremely resistant to proteolysis (Cousins 1979); Cherian (1979) suggested that rats absorb oral cadmium metallothionein intact from the gastrointestinal tract. By contrast, surficially-applied ^{109}Cd may have crossed the gut wall of the fish less easily because it was not bound to protein and was competing with essential ions such as calcium for binding sites on ion-transport systems. Washko and Cousins (1977) suggested that in rats, a calcium-binding protein which binds cadmium almost as readily as calcium (Bredderman and Wasserman 1974) was involved in the transport of cadmium across the gut wall. A similar mechanism may exist in fish, although teleost fish reportedly derive most of their calcium from water (Taylor 1985).

Cadmium seems to differ from zinc in that fish reportedly accumulate more ^{65}Zn from artificial diet than from natural food (Pentreath 1973; Merlini et al. 1976). However, zinc is an essential element whereas cadmium is not. Animals have mechanisms to regulate accumulation of essential elements; therefore, unbound zinc from artificial diet may be actively absorbed across the gut wall. Without a

similar active uptake mechanism, cadmium may more easily cross the gut wall when combined with an amino acid or protein as described earlier.

Absorption efficiency of cadmium seems to increase with metabolic rate. The absorption efficiency coefficients reported here for fish which were fed trout diet averaged 4.5 times higher than the coefficient of 0.034 reported for rainbow trout 25 times larger (Harrison and Klaverkamp 1989). The metabolic rate per unit weight of the small (1.75 to 2.0 g) rainbow trout used in this experiment was probably higher than that of the larger (46 g) rainbow trout because the metabolic rate of rainbow trout decreases with increased body weight (Rao 1968). In contrast, excretion coefficients seem less sensitive to size differences. Excretion coefficients reported here for fish fed trout diet averaged only 1.4 times the coefficient of 0.037 reported for 25 times larger fish (Harrison and Klaverkamp 1989).

Our data show that ^{109}Cd accumulated by natural foods from their environment is absorbed by rainbow trout 5 times more efficiently than ^{109}Cd applied surficially to artificial diet. These results should be considered in future studies of absorption efficiency with other food-chain species and metals. Furthermore, in long-term studies, sublethal effects may be observed at lower cadmium doses when environmentally-contaminated natural food is provided.

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