Transactions of the American Fisheries Society 123:51–62, 1994

Effects on Rainbow Trout Fry of a Metals-Contaminated Diet of Benthic Invertebrates from the Clark Fork River, Montana

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Abstract.—The upper Clark Fork River in northwestern Montana has received mining wastes from the Butte and Anaconda areas since 1880. These wastes have contaminated areas of the river bed and floodplain with tailings and heavy metal sludge, resulting in elevated concentration of metals in surface water, sediments, and biota. Rainbow trout *Oncorhynchus mykiss* were exposed immediately after hatching for 91 d to cadmium, copper, lead, and zinc in water at concentrations simulating those in Clark Fork River. From exogenous feeding (21 d posthatch) through 91 d, fry were also fed benthic invertebrates from the Clark Fork River that contained elevated concentrations of arsenic, cadmium, copper, and lead. Evaluations of different combinations of diet and water exposure indicated diet-borne metals were more important than water-borne metals—at the concentrations we tested—in reducing survival and growth of rainbow trout. Whole-body metal concentrations (μg/g, wet weight) at 91 d in fish fed Clark Fork invertebrates without exposure to Clark Fork water were arsenic, 1.4; cadmium, 0.16; and copper, 6.7. These were similar to concentrations found in Clark Fork River fishes. Livers from fish on the high-metals diets exhibited degenerative changes and generally lacked glycogen vacuolation. Indigenous Clark Fork River invertebrates provide a concentrated source of metals for accumulation into young fishes, and probably were the cause of decreased survival and growth of age-0 rainbow trout in our laboratory exposures.

The Clark Fork River (Figure 1) in northwestern Montana is the largest tributary of the Columbia River and provides an important recreational fishery for trout (Johnson and Schmidt 1988). The watershed of the upper Clark Fork River in western Montana also contains one of the world's largest ore deposits of copper (Cu), cadmium (Cd), lead (Pb), manganese (Mn), and zinc (Zn) (Miller 1973). More than 400 million metric tons of these metals were extracted and smelted from sulfide ore deposits between 1880 and 1972 (Meyer et al. 1968). Before 1950, untreated wastes and tailings from the Butte and Anaconda areas were discarded directly into the Clark Fork River (MDHES and USEPA 1989). Although waste treatment has greatly improved over the last 40 years, about 15 million cubic meters of metal tailings and heavy metal sludge remain in river bed, floodplain, and reservoir sediments (Brooks and Moore 1989; Moore et al. 1989; Moore and Luoma 1990). The sport fishery in the Clark Fork River above Milltown Reservoir depends almost totally on brown trout *Salmo trutta* (Johnson and Schmidt 1988). Although the habitat should support 1,250 fish/km, populations vary between 21 and 125/km. Rainbow trout *Oncorhychus mykiss* inhabit the tributaries but are nearly absent from the main stem of the Clark Fork River above Milltown Reservoir. Although the condition of brown trout over 18 cm in the Clark Fork River seems good, age-0 trout are relatively scarce. Recruitment seems to be limiting trout populations (Johnson and Schmidt 1988), and poor water quality associated with elevated metal concentrations is suggested as one source of the problem.

Metals most frequently elevated in water, sediment, and biota of the Clark Fork basin include As (arsenic), Cd, Cu, Pb, and Zn (Moore and Lu-

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oma 1990; Lambing 1991). Metals concentrations in aquatic insects in the Clark Fork are 2–14 times greater than concentrations in the same taxa from less-contaminated tributaries (Luoma et al. 1989). Metals are available to benthic organisms and fish through uptake of dissolved forms across the gills and by assimilation through the food chain (Lee and Jones 1984; Swartz et al. 1985). Benthic invertebrates are important food sources for fish and waterfowl and occupy an essential niche in trophic energy transfer and nutrient cycling. Thus, fish and other vertebrates feeding at higher trophic levels may be chronically exposed to metals via both food chain and water.

Although dietary uptake of Cd, Cu, Pb, and Zn is an important pathway of metal accumulation in fishes (Willis and Sunda 1984; Dallinger and Kautzky 1985; Crespo et al. 1986; Dallinger et al. 1987; Pratap et al. 1989), aqueous exposure may also contribute to metals contamination of organisms in the Clark Fork River. The objective of this study was to determine if the biology and metal concentrations of age-0 rainbow trout would be affected by exposure to water and a diet of aquatic invertebrates representative of conditions in the upper Clark Fork River.

Methods

Water exposure.—Test water simulated water chemistry conditions existing during the spring in the Clark Fork River when water exposure would be most toxic: hardness, 100 mg/L; alkalinity, 100 mg/L; pH, 7.2–7.8. Water was prepared by diluting spring water with water produced by reverse osmosis and deionization. Water was analyzed daily to verify that hardness, alkalinity, and conductivity were within 5% of the target values.

An aqueous mixture of 1.1 μg Cd/L ($CdCl_2$), 12 μg Cu/L ($CuCl_2$), 3.2 μg Pb/L ($PbCl_2$), and 50 μg Zn/L ($ZnCl_2$) was used as one treatment (denoted the 1× treatment). This treatment was representative of ambient concentrations of metals measured in the Clark Fork River (Lambing 1991) and also represented the maximum concentrations considered acceptable by USEPA (1987) in a water hardness of 100 mg/L. Additional treatments included an exposure at twice this concentration (2×) and a control (0×) to which no metals were added.

Dietary exposure.—One diet consisted of Clark Fork River invertebrates (CFI) collected just below Warm Springs Creek (Figure 1). Monitoring at this location indicated these invertebrates had high concentrations of metals (Cain et al. 1992). One reference invertebrate diet (SRI) was collected from the Snake River near Wilson, Wyoming. This location had no history of mining effects and was close to our laboratory. A second reference diet was collected at Turah Bridge (TBI), 180 km downstream from the CFI location. A control diet was obtained from a commercial source, Biodiet (BIO).

Invertebrates from all three locations were collected by raking the river bottom upstream from a small-mesh seine. Invertebrates were picked from the seine with plastic forceps and frozen immediately on dry ice. One week before the start of the exposure, the CFI and SRI diets were added to an equal weight of water, homogenized in a blender, and refrozen in 1- × 10-cm plastic petri dishes. The frozen diets were removed daily from the petri dishes, broken up, and apportioned by weight for feeding. Feeding rate was calculated on a dry-weight basis (Piper et al. 1982).

Experimental design.—Two experiments were performed. In the first experiment, there were three water exposures: 0×, 1×, and 2×. Each water treatment was split into two equal fractions and delivered to two 4.2-L aquariums. Fish in the two aquariums in a water treatment were randomly assigned SRI or CFI diets. There were four replications of each combination of water and diet, or 24 experimental units (3 water × 2 diets × 4 replicates = 24). Each aquarium received 10 volume additions per day. The concentration of metals in each exposure unit was maintained by an automated pipetting system (Micromedic), which metered the metals mixture from prepared stock solutions.

Eyed embryos of rainbow trout were obtained from the Ennis National Fish Hatchery, Montana, and held in Heath incubators at 10 ± 1°C until hatching. Each experimental unit received 100 eyed eggs 72 h before the median hatch date. Fish were acclimated to test water without metals until 72 h after the median hatch date, when exposure to water was started (0 d). The number of fish in each experimental unit was reduced to 50 at swim-up (21 d), and dietary exposure was started. At 42 d, the number of fish was reduced to 25; at 91 d, the test was ended. Temperature was maintained at 10 ± 0.5°C.

Feeding rate was 5% body weight/d between days 21 and 42; after day 42, feeding rate was increased to 6.25% body weight/d to compensate for the lower energy content of the CFI diet. The treatment with the largest biomass was adjusted to 5% or 6.25% body weight/d, and all other treatments

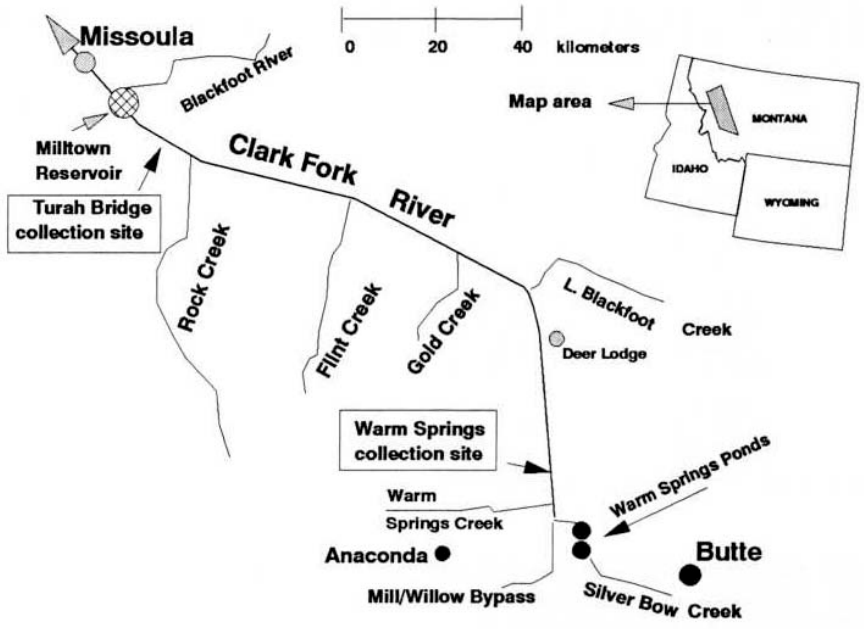

FIGURE 1.—The upper Clark Fork River from Warm Springs Ponds to Milltown Reservoir.

were fed that amount. Therefore, treatments with smaller, slower-growing fish were fed at a rate much higher than 6.25%. Feeding rate was not adjusted for mortality.

The second experiment was conducted with diets that were pasteurized and supplemented with vitamins and minerals (pvm), according to procedures used in standard fish feed formulation (Piper et al. 1982). By testing the pvm diets and using a reference diet collected from the same river, we could eliminate the potential for the introduction of diseases caused by field-collected organisms, for vitamin and mineral deficiencies, and for the influence of water quality variables other than metals on diet quality. We did not attempt to normalize the energy content of the diets because of the possibility of introducing other dietary bias. We used 0× water and three diets: a control diet (BIO pvm), a reference diet (TBI pvm), and the CFI diet remaining from the first experiment (CFI pvm).

In the second experiment, there were 12 experimental units (1 water × 3 diets × 4 replicates). There were 25 rainbow trout fry in each unit and exposure started at exogenous feeding and continued through day 80 posthatch. Other conditions were as described for the first experiment.

Sampling design.—At 21, 42, and 91 d of the first study and 21, 42, and 80 d of the second study, we measured length (mm), weight (mg), and tissue metal content. Fish were starved for 24 h before sampling. Experimental units were checked daily for mortality, and observations on feeding behavior were made following the procedures used in a previous study (Woodward et al. 1989).

At the end of the test, four fish were selected for histopathological examination from each of the water and diet exposures. Whole fish were placed in Bouin's fixative for 24 h, then transferred to 70% ethanol. After dehydration and clearing, these fish were embedded in paraffin and sectioned at 4 μm. Sections were mounted on slides stained with hematoxylin and eosin, and livers were qualitatively analyzed for general pathology. At least five slides of each fish (four sections per slide) were analyzed.

Filtered water samples were taken weekly from the 12 water treatments for metal determinations. The two diets were sampled three times during the study and another three times at the end of the study for metals analysis. Percent moisture and proximate analysis of protein, fat, and ash were determined for each diet at the beginning of the experiment. In the second experiment, only survival, growth, and metal accumulation were measured.

Chemical analysis.—Water was filtered with a Nalgene 300 filter holder with a polycarbonate,

TABLE 1.—Mean (SD) concentrations of metals measured in filtered test water sampled on days 1, 8, 15, 21, 30, 36, 44, 56, 60, 64, 71, 78, and 85. Nominal water concentrations (1×, μg/L) were Cd, 1.1; Cu, 12; Pb, 3.2; and Zn, 50.

Exposure water	Tank			
	1	2	3	4
0×				
Cd	0.06 (0.04)	0.06 (0.04)	0.06 (0.04)	0.06 (0.04)
Cu	0.62 (0.28)	0.71 (0.34)	0.62 (0.28)	0.63 (0.28)
Pb	0.54 (0.22)	0.50 (0.22)	0.50 (0.22)	0.48 (0.21)
Zn	3.39 (2.69)	3.01 (2.82)	2.05 (1.28)	2.37 (2.06)
1×				
Cd	1.04 (0.29)	1.05 (0.20)	1.01 (0.08)	0.99 (0.09)
Cu	10.37 (1.78)	10.61 (2.19)	11.02 (1.19)	10.65 (1.74)
Pb	2.22 (0.24)	2.40 (0.39)	2.41 (0.15)	2.33 (0.22)
Zn	47.87 (4.66)	50.08 (9.60)	48.78 (3.11)	48.21 (5.96)
2×				
Cd	2.02 (0.20)	1.94 (0.23)	2.12 (0.19)	1.86 (0.18)
Cu	21.04 (2.09)	20.11 (2.05)	22.31 (1.75)	20.10 (2.35)
Pb	4.75 (0.28)	4.57 (0.25)	5.08 (0.34)	4.56 (0.23)
Zn	97.12 (3.54)	92.08 (5.61)	102.12 (6.45)	89.78 (7.91)

0.4-μm-pore membrane. Filtered samples (100 mL each) were transferred to precleaned, 125-mL I-Chem polyethylene bottles and preserved by addition of 1 mL Ultrex-II nitric acid. Determinations of Cd, Cu, Pb, and Zn were made with graphite furnace atomic absorption spectrophotometry (GFAAS; Li et al. 1990) or by inductively coupled plasma emission spectroscopy (ICPES).

All fish and diet samples were stored in plastic bags at −25°C until analysis. Digestion for metals determination was accomplished with HNO_3 and $Mg(NO_3)_2$ for As and selenium (Se) (Brumbaugh and Walther 1989) or with HNO_3, H_2O_2, and microwave heating for remaining analytes. Analysis was performed by GFAAS with deuterium arc background correction for aluminum (Al) and Cu or with Zeeman correction for Cd, chromium (Cr), nickel (Ni), and Pb (Slavin et al. 1983). Arsenic, Se, and mercury (Hg) were determined by vapor generation (Welz and Schubert-Jacobs 1991). Zinc was determined by ICPES.

Protein content of fish was determined as 6.25 times Kjeldahl nitrogen. Fat content was determined by Soxhlet extraction, and ash was determined by exposure of samples to 600°C for 2 h in a muffle furnace. Moisture content was determined by weight loss after drying at 105°C for 24 h.

Statistical analyses.—Percent survival, growth, and behavioral data were statistically evaluated with analysis of variance. Percent data were arcsine- and square-root transformed, and behavioral count data were square-root transformed before analysis. The experiment was treated as a completely randomized, two-factor, split-plot design with four replicates. The statistical model included aqueous metal concentration as the main-plot treatment effect and dietary metal concentration and the interaction of aqueous and dietary metals as subplot effects. Means were compared with Fisher's least-significant-difference (LSD) test. Statistical significance in all tests was assigned at $P \leq 0.05$.

Mean tissue metals accumulations were compared for all pairwise combinations of water and diet with two-tailed t-tests and a pooled standard error (M. R. Ellersieck, University of Missouri, personal communication). For comparisons involving means that resulted from data below detection limits (SD = 0), a t-test was constructed as follows: t = (LS mean − detection limit)/(pooled SE of LS mean); LS mean is the mean of values not below detection limit (SD > 0); df = error df of LS mean.

Results and Discussion

Water

The measured concentrations of metals in filtered 1× and 2× exposure water were within 10% of the nominal value except for Pb (Table 1), and were low in part owing to analytical bias; recovery of known concentrations was 90–97%. Lead concentrations were consistently lower than nominal by 25%, which may have been due to precipitation of $PbCl_2$ in the stock solution. Variation within and between water treatments of the same metals concentration was low except for the 0× water,

TABLE 2.—Composition of diets made from benthic invertebrates collected from Clark Fork River and reference locations; SRI = Snake River invertebrates; CFI = Clark Fork invertebrates; BIO = Biodiet number one, a commercial diet; TBI = Turah Bridge invertebrates; and pvm = pasteurized and pelletized, vitamins and minerals added. Values are means with $N = 5$ or 6 for metals except $N = 2$ for Al; $N = 3$ for percent water and $N = 1$ for protein, fat, and ash. Standard deviations are given in parentheses.

Diet	Metal concentration (μg/g dry weight)						Percent composition (dry weight)		
	Al	As	Cd	Cu	Pb	Zn	Protein	Fat	Ash
SRI	333	3.5	0.36	14	<2.0	122	60	15	10
	(23)	(1.0)	(0.23)	(3.1)		(25)			
CFI	1,759	43.1	3.12	381	32.7	528	50	8.7	26
	(385)	(14.0)	(1.31)	(149)	(14.7)	(190)			
BIO pvm	46	2.8	0.50	12	0.36	185	43	14	11
	(3.8)	(0.27)	(0.01)	(1.7)	(0.08)	(3)			
TBI pvm	990	5.0	1.20	109	9.69	655	59	13	14
	(47)	(0.69)	(0.02)	(4)	(0.14)	(19)			
CFI pvm	1,809	42.0	2.39	415	28.4	1,070	50	8.7	26
	(173)	(13.1)	(0.06)	(11)	(0.5)	(37)			

for which large variations can be associated with measurement made near the detection limits.

Diet

The dominant invertebrate genera by weight composing the diets were CFI—*Hydropsyche* and *Tipula*; SRI—*Pteronarcys, Pteronarcella,* and *Arctopsyche*; and TBI—*Pteronarcys, Arctopsyche,* and *Tipula.* Although the diets had differing compositions, their nutritional quality was probably the most important factor controlling ingestion and assimilation by test fish (Piper et al. 1982). Toxicologically, it was important to evaluate the hazard of environmentally contaminated natural foods, which have a greater absorption efficiency than surficially contaminated artificial diets (Harrison and Curtis 1992). It would have been advantageous to have had the same dominant invertebrates in each diet. However, we believe it was more important to use diets containing invertebrates that had accumulated metals in situ, because these would better represent the situation in the Clark Fork River.

Protein content was between 43% and 60% for all diets (Table 2), which equals or exceeds the protein content of trout starter diets used in fish hatcheries (Piper et al. 1982). Recommended fat content is 15% for trout starter diets, and all diets except CFI were at or near that level. The energy content was 14.8 kJ/g for the SRI diet, 25% higher than in the CFI diet (11.1 kJ/g). Energy content was 20% higher in the TBI pvm diet (14.0 kJ/g) than in the CFI pvm diet. In both studies, the reference diet had a higher energy content; however, daily ration was 25% more than recommended (6.25% versus 5% body weight/d) (Piper et al. 1982). The daily ration was usually more than the fish consumed because uneaten food was observed routinely in all treatments. Therefore, the measured daily ration was actually ad libitum feeding.

Diets were initially "scanned" for Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn, and six of these elements (Al, As, Cd, Cu, Pb, and Zn) were selected for subsequent analysis based on their relatively high concentration in the diets (Table 2). The concentrations of metals were higher in the CFI diet than the reference SRI diet by factors ranging from 9-fold for Cd to 27-fold for Cu. In a similar comparison with the pvm diets, CFI pvm was 3–8-fold higher in As, Cu, and Pb than the TBI pvm diet.

Survival and Growth

Survival of rainbow trout was significantly reduced by exposure to the CFI diet (Figure 2). By day 42, survival of fish fed the CFI diets in all water treatments was significantly lower than that of fish fed the reference diet (SRI); by day 91, survival was on average less than 50% (CFI versus SRI). Survival of fish fed the reference diet was not significantly affected by 0×, 1×, or 2× metals in water.

Exposure to metals through the CFI diet significantly reduced growth, regardless of water exposure (Figure 3). By day 42, rainbow trout fed the CFI diet (0× treatment) were 15% smaller by weight than fish fed the SRI diet (0× treatment), and by day 91, they weighed 37% less than the controls. Exposing rainbow trout to metals in water at twice the Environmental Protection Agency water quality criteria for selected metals represen-

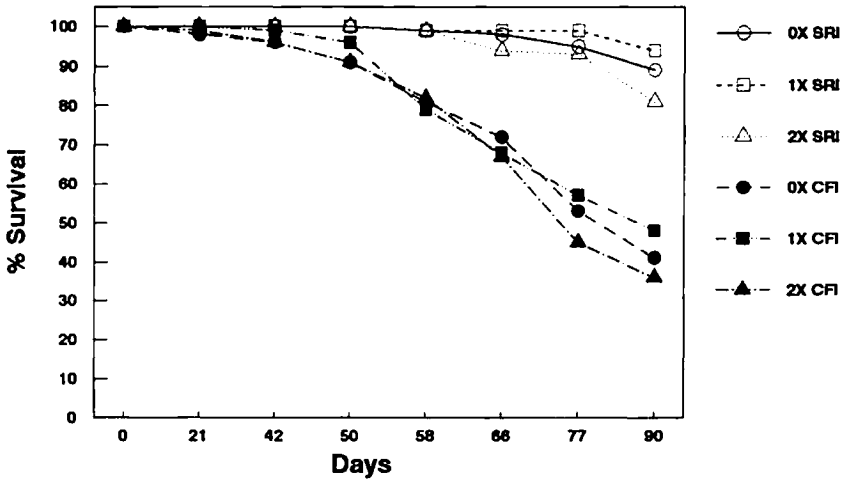

FIGURE 2.—Survival of rainbow trout exposed to 0×, 1×, and 2× metals treatments in water and to metals in SRI and CFI dietary invertebrate sources.

tative of the Clark Fork River (2×) significantly reduced weight at days 21 and 42 compared with fish in the 0× treatment. At 1× metals concentration for all sampling times and at 2× at 91 d, aqueous metal exposure had no significant effect on length or weight.

Feeding rates of fish on SRI and CFI diets did not differ significantly, indicating that fish did not reject the metals-contaminated CFI diet. Therefore, chronic growth and mortality effects observed in this exposure can be ascribed to physiological changes rather than to impaired feeding ability or rejection of contaminated food.

Histopathology

Livers from fish fed the SRI diet were normal and contained mild to moderate glycogen vacuolation (Figure 4), whereas livers of fish fed the CFI diet exhibited degenerative changes and generally lacked glycogen vacuolation. Degeneration consisted of nuclear pleomorphism (variation in size and shape of nuclei), increased cytoplasmic granularity, and degeneration of individual hepatocytes. Metals-induced degeneration of hepatocytes can result in death in severe cases and can divert energy from growth and metabolism during

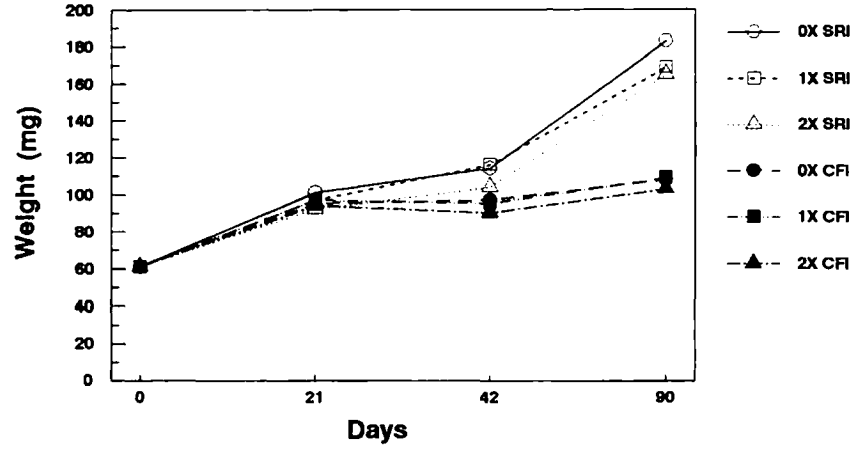

FIGURE 3.—Growth of rainbow trout exposed to 0×, 1×, and 2× metals treatments in water and to metals in SRI and CFI dietary invertebrate sources.

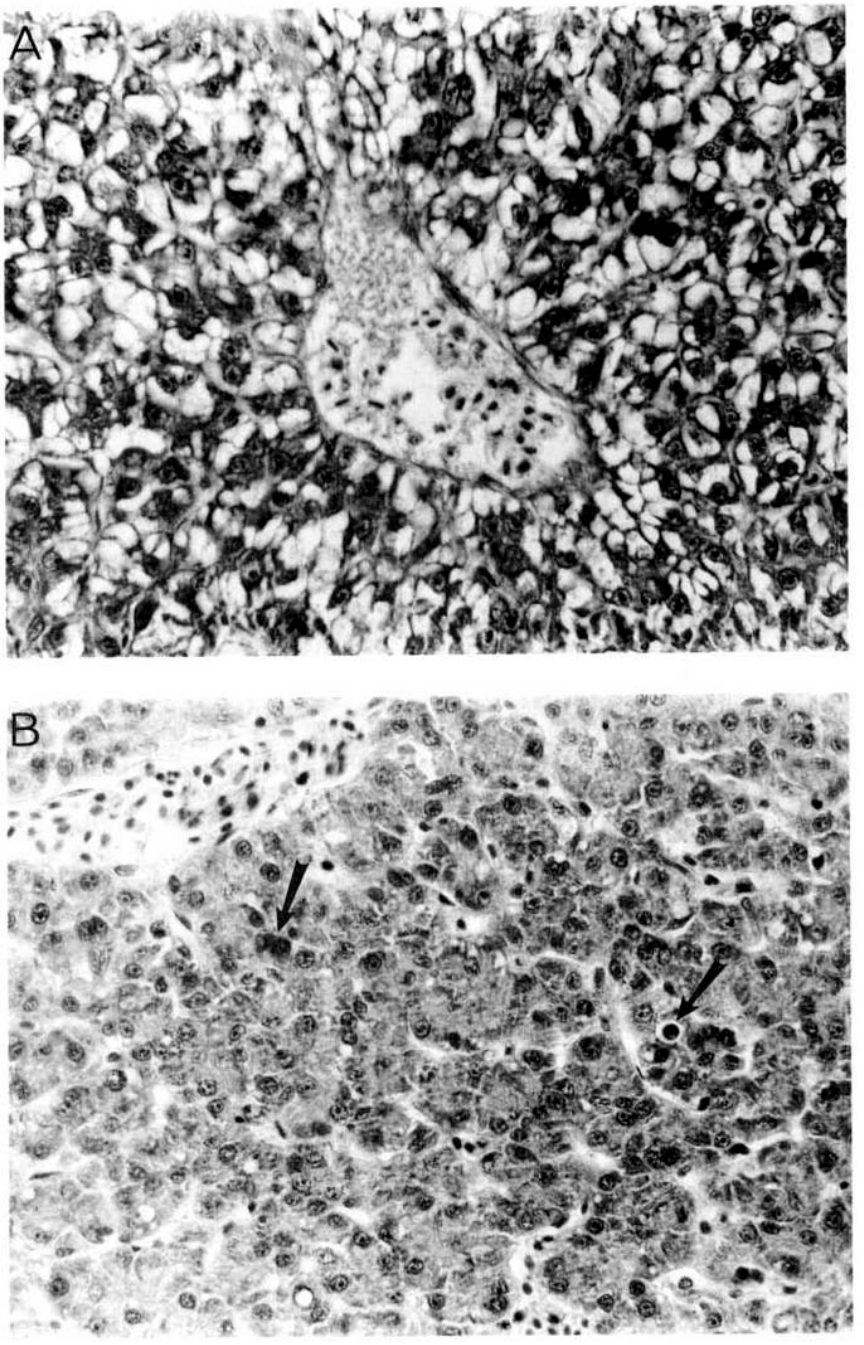

FIGURE 4.—Livers from fish fed invertebrates from the Snake River and Clark Fork River. (A) The SRI-fed fish show moderate glycogen vacuolation. Note large vein in center containing blood cells. (B) Livers of CFR-fed fish display nuclear pleomorphism, little cytoplasmic vacuolation, and scattered degenerate hepatocytes (arrows). (Both figures 450×.)

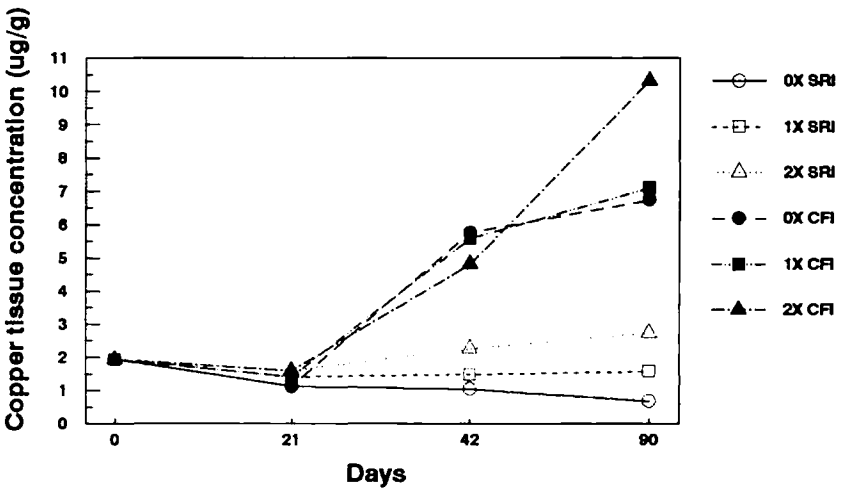

FIGURE 5.—Copper concentrations in rainbow trout exposed to 0×, 1×, and 2× metals treatments in water and to metals in SRI and CFI diets.

sublethal exposure (Hodson 1988). Pathology was not observed in other tissues in this study, but morphological and functional alterations have been induced in trout intestine and gallbladder by dietary metals (Crespo et al. 1986; Cockell and Bettger 1993). These changes might reduce assimilation efficiency (Brafield and Koodie 1991) and growth.

Bioaccumulation of Metals

Concentrations of Cu in tissues of rainbow trout fed the CFI diet were significantly higher than in tissues of fish fed the SRI diet for all water treatments on days 42 and 91 (Figure 5). In 0× water, tissue Cu concentration at 91 d was 6.7 μg/g for fish receiving the CFI diet or about 10-fold higher than in fish fed the SRI diet. Exposure in 1× water did not have a significant effect on tissue Cu accumulation compared with 0× water within a dietary treatment; however, 2× water significantly increased tissue Cu in fish on the SRI diet.

Tissue concentrations of As were significantly greater in rainbow trout receiving CFI diets and 0× and 1× water exposures at day 42, and at all water exposures by day 91 (Figure 6). Dietary exposure was the only route of accumulation for As because it was not an element added in 1× and 2× water exposures. Tissue concentrations of As were near detection limits for fish on the SRI diet, but increased 6-fold for 0×–CFI-exposed fish to 1.4 μg/g and 10-fold to 2.4 μg/g for 2×–CFI-exposed fish. Both As and Cu continued to increase in tissue through 91 d with no indication of a steady state being reached between dietary exposure and tissue accumulation.

Significant increases in tissue Cd were observed at day 91 after exposure to either 1× water alone (1×–SRI, 0.21 μg/g) or CFI diet alone (0×–CFI, 0.16 μg/g) compared with fish in the 0×–SRI treatment (Figure 7). Exposure to both sources (1×–CFI) significantly increased tissue Cd over exposure to either one individually. The bioaccumulation of Cd was greater than for As, Cu, or Pb after 91 d of exposure. Tissue Cd increased 21-fold (1×–SRI versus 0×–SRI) due to water alone, 16-fold due to diet alone (0×–CFI versus 0×–SRI), and 39-fold due to exposure to both water and diet (1×–CFI versus 0×–SRI).

Compared with fish in the 0×–SRI treatment, tissue increase in Pb concentration at day 91 was not significant for diet alone (0×–CFI) or 1× water exposure alone (1×–SRI). However, significant increases were observed when both diet and water exposure occurred together (1×–CFI; Figure 8).

Other investigations of metal contamination like that in the Clark Fork River, where Cu concentrations were low in water but remained elevated in sediments, macrophytes, and benthic invertebrates (Dallinger and Kautzky 1985; Lanno et al. 1987), also indicate diet loading can be an important source of Cu accumulation in fish. The "food chain effect" from metals has been described as a relationship in which biomagnification is not observed and bioconcentration factors are small, but the amount of metal transferred by

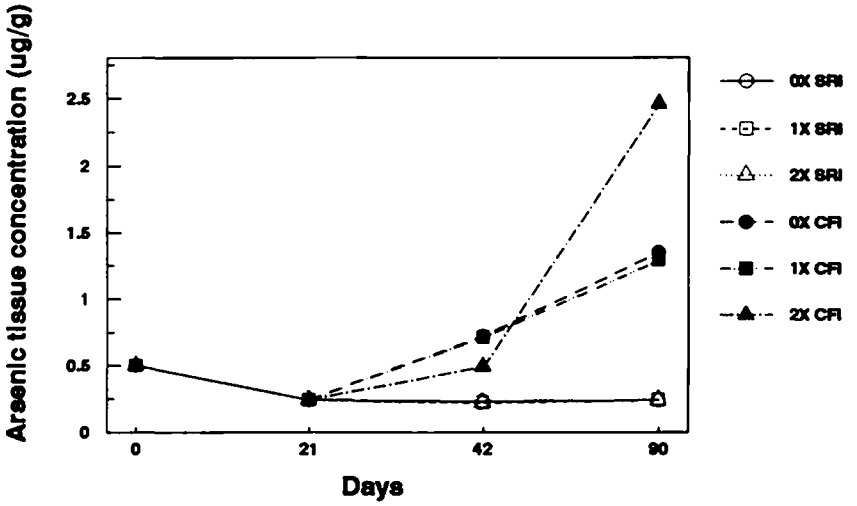

FIGURE 6.—Arsenic concentrations in rainbow trout exposed to 0×, 1×, and 2× metals treatments in water and to metals in SRI and CFI diets.

food can be high enough to attain biologically harmful concentrations in fish (Dallinger et al. 1987). Once in the lumen of fish, heavy metals can be absorbed into gut tissue, where they are distributed to other organs such as liver, kidney, and muscle (Dallinger and Kautzky 1985).

Exposure to Pasteurized Diets with Vitamins and Minerals

Survival was similar among rainbow trout fed the CFI pvm, TBI pvm, and BIO pvm diets. However, growth of rainbow trout on the CFI pvm diet was significantly less than that of fish on the TBI pvm diet (Table 3). Growth of fish to 80 d posthatch on the CFI pvm diet was only 56% by weight of that achieved by fish on the TBI pvm diet and only 48% of growth by fish on the BIO pvm diet.

The TBI pvm diet was collected from a downstream location, where the invertebrates had one-third to one-eighth the As, Cu, and Pb concentrations of the CFI pvm diet (Table 2). These two

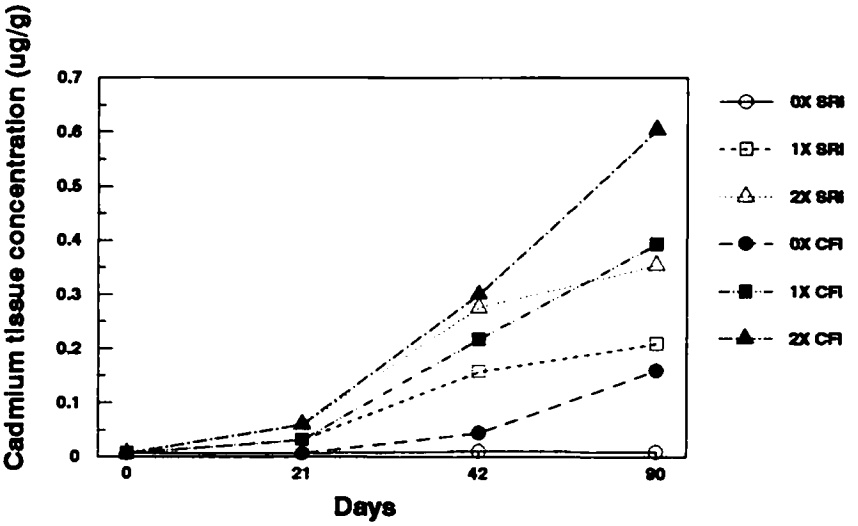

FIGURE 7.—Cadmium concentrations in rainbow trout exposed to 0×, 1×, and 2× metals treatments in water and to metals in SRI and CFI diets.

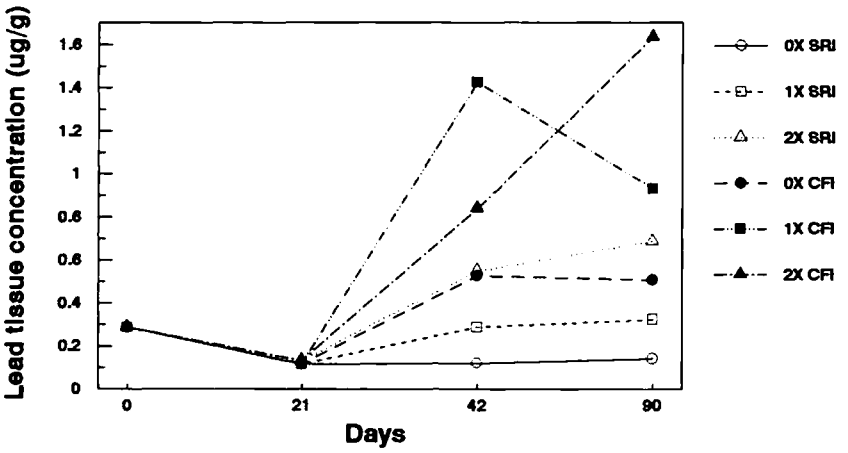

FIGURE 8.—Lead concentrations in rainbow trout exposed to 0×, 1×, and 2× metals treatments in water and to metals in SRI and CFI diets.

diets were standardized to the extent possible and we believe the reduced growth was a result of high concentrations of metals in the CFI pvm diet and not to lower energy content. In similar experiments with brown trout (unpublished data, our laboratory), the energy content in a high-metals test diet collected at Warm Springs was 13.6 kJ/g as compared to 13.2 kJ/g in a low-metals reference diet collected at Turah Bridge. Notwithstanding the higher energy content, growth was significantly lower for brown trout fed the high-metals diet. However, in this experiment, we cannot rule out the possibility that differences in energy content of test diets may have contributed to the growth differences observed for rainbow trout.

Survival was reduced with the CFI diet, but was not affected with the CFI pvm diet. Although disease introduction was possible with the CFI diet, there was no evidence of reduced appetite or abnormal swimming behavior typical of diseased fish. Also, the pathological examination of gills and liver showed no evidence of disease. Although supplementation assured that vitamin and mineral requirements were met and improved overall diet quality with the CFI pvm diet, the raw CFI invertebrates alone should have sustained the test fish (J. Halver, University of Washington, personal communication). The heat of pasteurization may have changed the nature of amino acids and organometal complexes in the CFI pvm diet, making them less toxic (Piper et al. 1982). Vitamin and mineral supplements in the CFI pvm diet might have produced healthier fish that were more capable of withstanding metals exposure than fish on the raw CFI diet. Early life stage trout in the Clark Fork River must sustain themselves on an invertebrate food source that is neither pasteurized nor supplemented with vitamins and minerals. In our attempt to rule out causes of mortality other than metals, we altered and probably improved the natural food source.

Growth of fish on the CFI pvm diet in the second experiment was considerably better than growth of fish fed the CFI diet in the first study.

TABLE 3.—Growth of rainbow trout fry exposed to metals in three pelletized diets. Diet sources were as follows: BIO= Biodiet number one (commercial), TBI = Turah Bridge invertebrates, and CFI = Clark Fork invertebrates (pvm = pelletized and pasteurized, vitamins and minerals added). Values are means (SD), total lengths in mm, weights in mg; $N = 3$ or 4. Same-day values with different letters are significantly different ($P \leq 0.05$).

	Duration of exposure				
	21 d,	42 d		80 d	
Diet	weight	Weight	Length	Weight	Length
BIO pvm	97 (13) z	217 (53) z	30 (2.1) z	854 (237) z	45 (4.0) z
TBI pvm	97 (13) z	216 (31) z	30 (1.0) z	730 (154) y	43 (2.8) y
CFI pvm	97 (13) z	152 (28) y	27 (1.3) y	410 (128) x	37 (6.7) x

TABLE 4.—Metal concentrations in rainbow trout exposed to metals in three pelletized diets. Diet sources were as follows: BIO = Biodiet number one, a commercial trout diet; TBI = Turah Bridge invertebrates; and CFI = Clark Fork invertebrates (pvm = pelletized and pasteurized, vitamins and minerals added). Values are means (SD); $N = 3$ or 4. Within a metal and among diets, values with different letter are significantly different ($P \leq 0.05$).

Diet	Metal concentration in tissue (μg/g, wet weight)				
	As	Cd	Cu	Pb	Zn
BIO pvm	0.23 (0.05) z	0.019 (0.003) z	0.87 (0.13) z	<0.04 z	33 (2.6) z
TBI pvm	0.21 (0.03) z	0.022 (0.002) z	3.2 (0.34) y	0.20 (0.017) z	31 (2.2) z
CFI pvm	1.5 (0.17) y	0.12 (0.16) y	7.8 (0.46) x	0.48 (0.044) z	36 (0.58) z

In the second experiment, the pvm diet was graded to a size suitable for fry as well as fortified with vitamins and minerals; these changes should have improved diet quality and feeding efficiency.

Mean concentrations of As, Cd, and Cu measured in tissue of fish fed the CFI pvm diet were significantly higher than concentrations in fish receiving the TBI pvm diet (Table 4) and similar to the results observed with the CFI diet exposures. The increase was 7-fold for As, 5-fold for Cd, and 2-fold for Cu. Except for Cu, the concentrations of metals in fish fed the BIO pvm (control) and TBI pvm (reference) diets were similar. Concentration of Cu in fish fed the TBI pvm reference diet was 3.2 μg/g, 3.5 times higher than in fish fed the BIO pvm diet.

Metal concentrations in Clark Fork fishes (redside shiner *Richardsonius balteatus*, mountain sucker *Catostomus platyrhynchus*, slimy sculpin *Cottus cognatus*) collected from the Warm Springs area (unpublished data, our laboratory) were 6–13 times higher than the U.S. average for various species shown by Schmitt and Brumbaugh (1990). The mean concentrations (μg/g wet weight, $N = 6$) for Clark Fork fishes were As, 1.5; Cd, 0.11; Cu, 8.6; Pb, 0.71; and Zn, 49. These values are similar to mean measured values in 0×–CFI-exposed rainbow trout (Figures 5–8) at 91 d and in 0×–CFI pvm-exposed fish (Table 4) at 80 d. Therefore, independent of water exposure, accumulation of metals by fish during dietary exposure in the laboratory resulted in metal residues similar to those measured in field-collected fish.

We conclude that Clark Fork invertebrates were the major source of the metals that accumulated in rainbow trout fry used in this study. We also conclude that the accumulated metals in those fry caused decreased growth and survival. To verify the role of dietary metals on fish health, additional studies are needed with other species and different sources of contaminated invertebrates.

Acknowledgments

J. Arms, R. Barrows, J. Hansen, B. Jones, R. Knowlton, E. MacConnell, D. Monda, M. Walther, and R. Wiedmeyer performed the experimental procedures and analyses. M. Ellersieck provided assistance with the experimental design and statistical analyses. Those providing the help necessary to collect food chain organisms from the Clark Fork River included V. Watson and her students from the University of Montana: M. Fox, J. Friedman, N. Gilbertson, D. Jones, and J. Kreilick; D. Palawski, C. DuBois, and J. Malloy from the U.S. Fish and Wildlife Service field office in Helena; and W. Hadley and G. Pearson from the Montana Department of Fish, Wildlife and Parks office in Deer Lodge, Montana. The research was sponsored by the U.S. Environmental Protection Agency under contract 83440-91-035.

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Received October 14, 1992
Accepted July 23, 1993