

DYNAMICS OF DIETARY METHYLMERCURY IN RAINBOW TROUT, *SALMO GAIRDNERI*

D.W. RODGERS¹ and F.W.H. BEAMISH

Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

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Rainbow trout, *Salmo gairdneri*, were fed diets containing 0, 25, 45, 75 or 95 mg Hg·kg⁻¹ methylmercury for 84 days at meal sizes of 1 and 2%·day⁻¹ and to satiation. Uptake and depuration of methylmercury was followed in fish fed fixed rations by feeding some of these fish diets containing ²⁰³Hg-labeled methylmercury.

Appetite, apparent digestibility of ration and growth decreased in fish fed diets containing added methylmercury. Whole body mercury concentrations of trout fed diets containing added methylmercury increased throughout the first 56 days of feeding, then generally stabilized from 56–85 days. The total amount of mercury in the fish, however, increased throughout the 84 days.

Trout generally assimilated 70–80% of the labelled methylmercury they were fed. Among fish fed the 75 mg Hg·kg⁻¹ diet for 9 wk, assimilation decreased to < 50%, indicating either a saturation of uptake or an induction of a specific block to methylmercury assimilation.

Labeled methylmercury was depurated faster in fish fed the higher meal size. Methylmercury depuration in rainbow trout would thus seem related to metabolic rate. Trout fed diets containing added methylmercury depurated labeled methylmercury more rapidly than fish fed diets without added methylmercury, suggesting that methylmercury depuration may be induced by continued exposure to the compound.

Key words: assimilation, bioenergetics; bioaccumulation; depuration; growth

INTRODUCTION

The accumulation of methylmercury by fish results from efficient uptake from food and water (Olson et al., 1973; DeFreitas et al., 1975; Phillips and Buhler, 1978) and the slow depuration of assimilated methylmercury (Jarvenpää et al., 1970; Ruohutula and Miettinen, 1975; Sharpe et al., 1977).

The uptake and depuration of ingested methylmercury appears to follow a two-stage process (DeFreitas et al., 1975). A portion of the ingested methylmercury remains associated with dietary components which are not assimilated and is

¹ Present address: Biological Research Section, Chemical Research Division, Ontario Hydro, 800 Kipling Ave., Toronto, Ontario, Canada M8Z 5S4.

eliminated rapidly with the feces. The remaining fraction of ingested methylmercury is absorbed across the gut and incorporated into tissues. This assimilated fraction of ingested methylmercury is depurated much more slowly (DeFreitas et al., 1975; Sharpe et al., 1977). There is considerable variation in the fraction of dietary methylmercury reported as assimilated from diet; with values ranging from approximately 20% (Phillips and Gregory 1979) to 70–90% (DeFreitas et al., 1975; Sharpe et al., 1977; Phillips and Buhler, 1978). The factors contributing to these differences in assimilation of dietary methylmercury, particularly the effects of meal size, methylmercury concentration and duration of exposure are not known.

Methylmercury in fish is depurated by the kidney, liver and possibly gills (Burrows and Krenkel, 1973; Renfro et al., 1974; Olson et al., 1978). Although the biochemical sequences leading to methylmercury depuration are not known in detail, demethylation of methylmercury to inorganic mercury is seen as an important step (Burrows and Krenkel, 1973; Olson et al., 1978). Reports that the rate of methylmercury depuration is directly proportional to the quantity of methylmercury ingested (DeFreitas et al., 1975; Ruohtula and Miettinen, 1975) suggest an induction of depuration of methylmercury. Many of the reported rates of methylmercury depuration, however, are based on a single exposure of short duration to relatively low concentrations of methylmercury (Jarvanpaa et al., 1970; Giblin and Massaro, 1973; Sharpe et al., 1977). The above evidence for an induction of methylmercury depuration suggests that the reported rates may underestimate the abilities of fish to eliminate methylmercury.

Growth may be considered as an integration of the stresses affecting the metabolism of the fish (Beamish et al., 1975) and provides a sensitive index of environmental effects. Reports of apparent reductions in growth of fish fed diets containing methylmercury (Matida et al., 1971; Wobeser, 1975) indicate the adverse effects of this compound. Unfortunately, meal size was neither measured nor controlled in the above studies and differences in growth due to changes in appetite cannot be distinguished from changes in growth per se.

Our research investigated the effects of methylmercury in the diet and duration of exposure on appetite, growth and methylmercury assimilation and depuration in rainbow trout, *Salmo gairdneri*.

MATERIALS AND METHODS

Fingerling rainbow trout purchased from a local hatchery (Goosen's Trout Farm, Otterville, Ontario) were held in circular tanks (50 l) supplied with aerated ground water at 10.5 ± 1.5 °C. Fish were kept to the outside perimeter of the tanks by a central, circular plastic partition and forced to swim against a current of 5–10 $\text{cm} \cdot \text{s}^{-1}$. As the tanks had central drains, the partitions also separated the fish from their feces, which were removed daily. Throughout all experiments, dissolved oxygen content of the water in the tanks was > 80% of air saturation, total hardness

380–390 mg·l⁻¹ and pH 7.9–8.1 (APHA et al., 1971). Photoperiod was maintained at 16 h of light with a gradual change of intensity for 0.5 h at each end of the light regime.

Experimental diets were prepared from a commercial trout food (GRT-78G, Martin's Feed Mills, Elora, Ontario) ground to a homogenous powder, then mixed, 2:1, with an aqueous solution containing the required quantities of methylmercuric chloride (ICN Pharmaceuticals, Plainview, N.Y.). The wet diet was mixed and extruded in a food mixer, lyophilized and kept frozen in plastic bags until used.

Total mercury content of fish and diets was determined by cold vapor atomic spectrometry (Hatch and Ott, 1968). Mean recovery of spiked samples was $97 \pm 1\%$. The coefficient of variation of the mean of 5 replicate samples was $\approx 10\%$ for samples with < 0.5 mg Hg·kg⁻¹ and $< 5\%$ for samples with > 2 mg Hg·kg⁻¹. Limits of detection were 0.1 mg Hg·kg⁻¹ wet weight. Mercury in rainbow trout fed diets containing methylmercury remains almost entirely in the form of methylmercury (Matida et al., 1971). Hence it was assumed that all mercury detected in the fish was methylmercury.

Appetite of fish fed diets containing different concentrations of methylmercury was determined over a period of 12 wk. Fish (initial weight 5.5 ± 0.5 g) were maintained at an initial density of 65 fish per tank and fed once daily. Appetite was determined as the quantity of food the fish voluntarily consumed in a 5-min daily feeding interval. Appetite was expressed as a percentage of total food consumed to the wet weight of all fish in the tank for the previous weighing interval. Fish were weighed weekly. Nominal mercury concentrations were 0 (no methylmercury added), 25, 45 and 95 mg Hg·kg⁻¹, while measured mercury levels were < 0.1 , 23.9 ± 0.4 , 46.9 ± 0.9 and 94.8 ± 1.5 mg Hg·kg⁻¹ respectively (mean \pm SE of 5 samples). These treatments are subsequently referred to as 0/ad lib., 25/ad lib., 45/ad lib., and 95/ad lib. respectively. Fish in each of four tanks were fed the 0 mg Hg·kg⁻¹ diet for 3 wk. The tanks were then randomly assigned to the experimental diets and the fish fed that diet for the next 12 wk. The day before commencing experimental feeding and at 14, 28, 56 and 84 days after initiation of experimental feeding, a sample of 10 fish was removed at random from each tank. Fish were frozen, then lyophilized, homogenized and analyzed for mercury content.

The effects of dietary methylmercury on growth rate and methylmercury dynamics of fish fed at fixed meal sizes were also determined over a period of 12 wk. Individual fish were anesthetized with tricane methane-sulphonate (MS222) at 50–74 mg·l⁻¹, weighed and tagged (Floy fingerling tags – Floy Tag Manufacturing, Seattle, Wa.). Fish (5.7 ± 0.6 g) were maintained in tanks at an initial density of 65 fish per tank and fed the equivalent of 1% wet weight per day with control diet for 2 wk. Nominal mercury concentrations of experimental diets were 0, 25 and 75 mg Hg·kg⁻¹ with measured levels of < 0.1 , 23.2 ± 1.0 and 76.5 ± 1.3 mg Hg·kg⁻¹ respectively. Meal sizes were 2%·day⁻¹ (2% wet weight daily) and 1%·day⁻¹ (2% wet weight every second day). These treatments are

subsequently referred to as 0/2%, 25/2%, 75/2%, 0/1%, 25/1% and 75/1% for fish fed diets containing nominal methylmercury concentration at specified meal size. The tanks were then randomly assigned to combinations of dietary methylmercury concentration and meal size, with two replicates of each of the six treatment combinations. Fish were then fed the prescribed experimental diet and meal size for 12 wk. Fish were anesthetized with MS222 and weighed at 3, 14, 28, 42, 56, 70 and 84 days after initiation of experimental regime, with rations adjusted after each weighing. Fish fed the 75 mg Hg · kg⁻¹ diet at 2% · day⁻¹ did not always consume the full ration from days 71–85 after initiation of experimental diets; otherwise fish consumed all ration as it was offered. The day before commencing experimental feeding and at 14, 28, 56 and 84 days after initiation of experimental regimes, a random sample of 5 fish was removed from each tank. Fish were frozen, lyophilized and analyzed for mercury content.

At the termination of the experiment (85 days), an additional 5 fish were selected randomly for blood and tissue samples. Blood was collected in non-heparinized capillary hematocrit tubes from the severed caudal peduncle of fish anesthetized with MS222. Blood was left to clot (20 min at 4°C), then centrifuged at 500 g for 5 min and hematocrit measured. Serum was separated from cells by breaking tubes above the cell layer. The serum was then refrigerated and analyzed within 2 days. Serum osmolality was determined with a vapor pressure osmometer (model 1500, Westcor Inc.) and serum sodium and potassium measured by flame photometry (FLM-2, Radiometer Inc.).

Tissues (gill, posterior kidney, liver and gut) from fish sampled for blood, were preserved in Karnovsky's fixative for 5 days; then transferred to 70% ethanol, until they were embedded in either wax or Epon 812. Epon-embedded tissues were sectioned at 1–2 μm and stained with azure blue. Wax-embedded tissues were sectioned at 5 μm and stained with haematoxylin and eosin. Sections were examined using a light microscope.

Methylmercury assimilation and depuration were determined for groups of fish removed from their treatment tanks to separate containers. At 1, 28 and 56 days after the initiation of experimental diets, a random sample of 10 fish per tank was transferred to separate 15-l containers supplied with aerated well water (30 l · h⁻¹). The 10 fish in each container were fed at the same meal size and methylmercury concentration as the tank from which they were removed. Fish were fed two meals containing ²⁰³Hg-labeled methylmercury. Fish fed at 2% · day⁻¹ were given labeled diets on days 5 and 6 after transfer to the containers, while those fed at 1% · day⁻¹ were given labeled diets on days 5 and 7. The fish were then fed their non-labeled diets, for the subsequent 20 days.

The ²⁰³Hg-labeled methylmercury diets were of similar mercury concentration to their non-labeled counterparts. Methylmercury chloride labeled with ²⁰³Hg was purchased from New England Nuclear (Montreal, P.Q.) at an activity of 20–85 MB · mg⁻¹ Hg and diets made up to an initial specific activity of ≈ 10 kBq · g⁻¹.

New diets were prepared at intervals of 45 days, approximately equivalent to the half-life of ^{203}Hg . Fish transferred to the containers at 1 and 28 days after the initiation of experimental diets were fed from the first set of labeled diets, while those transferred to the containers 56 days after the initiation of experimental diets were fed from the second set of labeled diets. Nominal and measured mercury concentrations in the first and second sets of labeled diets, respectively, were:

0 mg Hg · kg⁻¹—0.48 ± 0.07 and 0.13 ± 0.01 mg Hg · kg⁻¹
 25 mg Hg · kg⁻¹—21.5 ± 0.5 and 23.3 ± 0.7 mg Hg · kg⁻¹
 75 mg Hg · kg⁻¹—73.8 ± 0.8 and 75.2 ± 2.9 mg Hg · kg⁻¹

The concentrations of ^{203}Hg -labeled impurities in the diets remained at < 4% as measured by extraction with benzene (Westoo 1973).

The quantities of ^{203}Hg -labeled methylmercury in food and fish were determined by gamma spectrometry. Food samples were counted in an automated gamma counter with a 7.5 cm diameter, 7.5 cm high well shaped NaI(Tl) crystal (nuclear Chicago model 4233). Fish were counted in a whole body counter with two rectangular 5 × 5 × 15 cm (NaI(Tl)) crystals as the detection unit (Rodgers and Beamish, 1981). Each fish was counted for three consecutive 1-min intervals. The coefficient of variation of the mean was < 3% for fish counting at 2500 cpm or greater. Counting efficiency was independent of weight of fish for trout from < 5 to > 50 g. Fish transferred to the containers 1 day after the initiation of experimental diets were counted 1, 3, 6, 11, 16 and 20 days after the last labeled meal; while fish transferred to the containers at 28 and 56 days after initiation of experimental diets were counted 1, 3, 10, 15 and 20 days after the last labeled meal. Internal standards containing known quantities of nuclide were prepared from each isotope shipment. They were used to correct for radioactive decay and interrelate counts on the deep well and whole body counters.

Labeled methylmercury did not appear to be transferred among fish in the containers. In some of the groups fed at 1% · day⁻¹, one or two of the fish counted at background rates at all times. This suggests that the fish did not consume appreciable amounts of labeled diet. The fact that these fish did not subsequently accumulate radioactivity indicates that recontamination with labeled mercury either through coprophagy or uptake across body surfaces was not significant.

The quantity of labeled methylmercury in the fish decreased exponentially with time after the last isotope meal. Linear regression analysis of log_e of the quantity of labeled methylmercury in all fish in the container surviving the 20-day counting period and time after the last meal of labeled methylmercury yielded R² > 0.8 for each group of ^{203}Hg exposed fish. Measurements of labeled methylmercury 1 d after the last labeled meal were not included in the above regressions as this measurement includes activity associated with the digestive tract but not assimilated (DeFreitas et al., 1975).

The rate of depuration of labeled methylmercury was estimated from the slope of the above regression of log_e of the quantity of labeled methylmercury in the fish

and time. Weight-corrected rates of depuration of labeled methylmercury were obtained by dividing the above depuration rate by the mean weight of the trout in the container, raised to the exponent -0.58 (DeFreitas et al., 1975; Sharpe et al., 1977). Assimilation of labeled methylmercury was determined from the ratio of the intercept of the above regression to the quantity of labeled methylmercury the fish were fed (all labeled diet was consumed). If fish died during the counting period and were not included in the regression equation, the intercept was adjusted by:

$$\text{adjusted intercept} = \text{intercept} + (\text{intercept} \times B)$$

where:

$$B = \frac{\text{methylmercury on day 1 of fish not in regression equation}}{\text{methylmercury on day 1 of fish included in regression equation}}$$

This adjustment was warranted as the ratio of labeled methylmercury of an individual fish to the quantity of labeled methylmercury in all fish remained stable throughout the 20-day counting period.

Mortality was observed in 5 of the 36 treatment combinations but did not appear to be related to treatment. At least 8 of the 10 fish in each container survived the 20-day counting period.

Apparent digestibility of dry material of 0 and 75 mg Hg · kg⁻¹ diets was determined over a 10-wk period. Trout (13.8 ± 0.5 g wet weight) were kept individually in 2-l plastic containers supplied with well water (4 l · h⁻¹). Diets (0 and 75 mg Hg kg⁻¹) were prepared as above but with 2% chromic oxide (J.T. Baker Chemical Co., Phillipsburg, N.J.) added. Measured mercury concentrations were < 0.1 and 73.0 ± 1.2 mg Hg · kg⁻¹. Fish were fed control diet daily at 1.5% wet weight for 2 wk, then randomly assigned to control and methylmercury diets, with 10 fish per treatment. Trout were fed at 1.5% wet weight · day⁻¹ for 7 wk, then at 1% wet weight · day⁻¹ for the next 3 wk. Feces were collected for 2 consecutive days at 1, 2, 4, 6, 8 and 10 wk after the initiation of experimental diets. Feces were aspirated with a glass syringe from the bottom of the container, filtered on No. 1 filter paper, dried at 50°C for 1 day and desiccated. Chromium concentration in food and feces was determined by spectrophotometry following nitric and perchloric-sulphuric acid digestion of samples (Czarnocki et al., 1961). Estimates of apparent digestibility using dry weights of food and feces were 3–5% lower than those obtained using the ratio of chromium in food to feces, which is attributed to incomplete collection of feces. The present analysis employs values obtained using the chromium indicator method.

Experiments were analyzed using the appropriate analysis of variance model. When repeated measurements were made on the same experimental unit (tank), the experiment was analyzed as a split plot in time using conservative degrees of freedom (Steele and Torrie, 1960). Cell means were compared using the least

significant difference with t and t' values calculated as appropriate (Steele and Torrie 1960), if the F -ratio was significant ($P < 0.05$). In all statistical tests of significance, differences between cells means were considered significant if $P \leq 0.05$.

In growth analysis of fish at fixed rations, 10 of the fish remaining in each experimental tank at the termination of the experiment (84 days), were randomly selected. Over the 84-day feeding period, the growth of individual fish was apparently exponential. The change in wet weight with time of each fish was analyzed by fitting a linear regression of \log_e of the wet weight of the fish at time (t) and time after the initiation of experimental feeding. Growth rate of individual fish, considered as the slope of this regression, was then employed in a two-factorial nested analysis of variance.

RESULTS

Both behavior and appearance changed in some of the trout fed large meals of diets containing high concentrations of methylmercury. Among fish fed the 75/2% or 95/ad lib. diets for > 8 wk, ≈ 20 –30% of the trout were much darker than other fish in the tank, and fish at other treatment levels. The darker fish seemed lethargic and at times would drift with the current rather than hold their position as did other fish.

Appetites of fish fed to satiation with diets containing added methylmercury were initially slightly greater than those of fish fed the 0/ad lib. diet. However, appetites of fish fed diets containing methylmercury subsequently declined while the appetite of fish fed the 0/ad lib. diet remained stable (Fig. 1). The depression in appetite of fish fed methylmercury diets increased with duration of experimental feeding while the rate of decline in appetite was proportional to dietary methylmercury concentration. The decrease in food consumption by fish fed methylmercury diets was paralleled by an apparent decrease in growth. Thus the mean weight that the trout attained after 84 days of feeding was significantly influenced by concentrations of methylmercury in the diet. The final mean weight of trout fed the 0/ad lib. diet (54.8 ± 2.3 g wet weight, mean \pm SE, $n = 15$) was significantly greater than that of fish fed diets with added methylmercury. Among fish fed diets containing added methylmercury, the mean weights of fish fed the 25/ad lib. and 45/ad lib. diets (45.1 ± 3.1 and 39.3 ± 2.1 g wet weight respectively) were not significantly different. Both groups were significantly heavier than trout fed the 95/ad lib. diet (21.9 ± 1.6 g wet weight).

Among trout fed the higher fixed ration ($2\% \cdot \text{day}^{-1}$), both final weight and growth rate of fish fed diets containing added methylmercury (25/2% and 75/2%) were significantly reduced relative to fish fed the 0/2% diet. In contrast, at the lower meal size ($1\% \cdot \text{day}^{-1}$), final weights and growth rates of fish fed the 0/1%, 25/1% and 75/1% diets were not significantly different. The effects of dietary methylmercury on growth were not directly proportional to quantity ingested as the

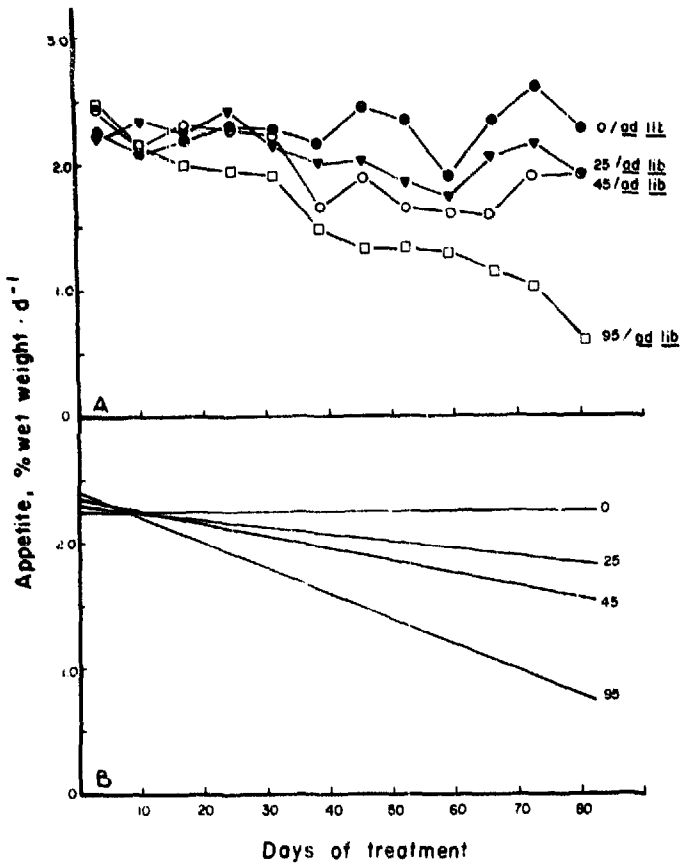


Fig. 1. Decrease in appetite of rainbow trout fed diets containing indicated concentrations of methylmercury. A. Weekly mean appetite. B. Regression lines fit to changes in daily appetite with time. The slope of regression equation for fish fed 0 mg Hg · kg⁻¹ diet was not significantly different from zero ($P < 0.05$) and mean value was used.

effects of the 25 and 75 mg Hg · kg⁻¹ diets at a given meal size were not significantly different.

The mean weight attained by the trout after 84 days of consuming fixed rations was significantly influenced by both meal size and concentration of methylmercury in the diet (Fig. 2). Although initial weights did not vary significantly, fish fed at 2% · day⁻¹ were approximately twice as large as those fed 1% · day⁻¹ at the termination of the experiment. Among fish fed the higher ration, the mean weight of trout fed the 2% diet (28.2 ± 1.9 g wet weight, mean ± SE, $n = 20$) was significantly greater than that of fish fed the 25/2% and 75/2% diets (19.7 ± 2.2 and 17.9 ± 1.2 g wet weight respectively). Mean weights of fish fed the 25/2% and 75/2% diets were not significantly different. At the lower meal size, fish fed the

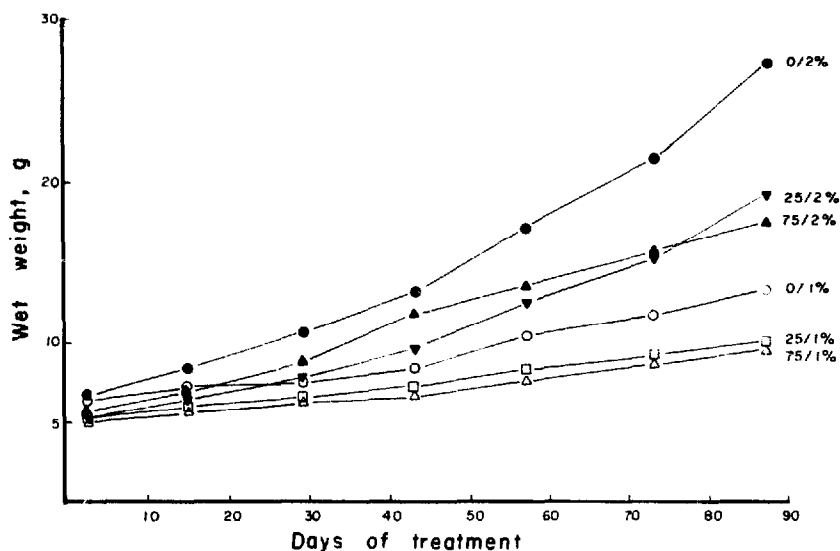


Fig. 2. Mean weight of rainbow trout fed fixed rations of diets containing methylmercury at indicated concentrations and meal sizes.

0/1%, 25/1% and 75/1% diets did not differ significantly in final weight (12.4 ± 1.2 , 10.2 ± 0.9 and 9.7 ± 1.0 g wet weight respectively), although the pattern of final weights was similar to that at the higher ration.

Mean growth rates of fish fed fixed rations varied with meal size and dietary methylmercury concentration in a fashion similar to final weights. Mean growth rates of trout fed at $2\% \cdot \text{day}^{-1}$ were approximately twice those of fish fed at $1\% \cdot \text{day}^{-1}$. At the higher meal size, the mean growth rate of trout fed the 2% diet ($16.7 \pm 0.4 \times 10^{-3} \cdot \text{day}^{-1}$) was significantly greater than the mean growth rates of fish fed the 25/2% and 75/2% diets (15.8 ± 0.8 and $14.1 \pm 0.3 \times 10^{-3} \cdot \text{day}^{-1}$). Growth rates of fish fed the 25/2% and 75/2% diets were not significantly different. Among fish fed at the lower ration, mean growth rates of trout fed 0/1%, 25/1% and 75/1% diets (7.2 ± 0.7 , 7.4 ± 0.5 and $7.1 \pm 0.6 \times 10^{-3} \cdot \text{day}^{-1}$ respectively) were not significantly different.

Apparent digestibility of ration was significantly higher in trout fed the 0 mg $\text{Hg} \cdot \text{kg}^{-1}$ diet than in fish fed the diet containing methylmercury at 75 mg $\text{Hg} \cdot \text{kg}^{-1}$ (Fig. 3). The reduction in apparent digestibility was observed throughout the 10-wk feeding period, with the mean apparent digestibility of fish fed the 0 mg $\text{Hg} \cdot \text{kg}^{-1}$ diet ($67.9 \pm 0.2\%$) significantly higher than that of fish fed the 75 mg $\text{Hg} \cdot \text{kg}^{-1}$ diet ($66.1 \pm 0.2\%$). The reduction in apparent digestibility of fish fed the 75 mg $\text{Hg} \cdot \text{kg}^{-1}$ diet appeared to increase slightly with exposure duration, however, this trend was not statistically significant.

Measure blood parameters were similar among fish fed the 0, 25 and 75 $\text{Hg} \cdot \text{kg}^{-1}$

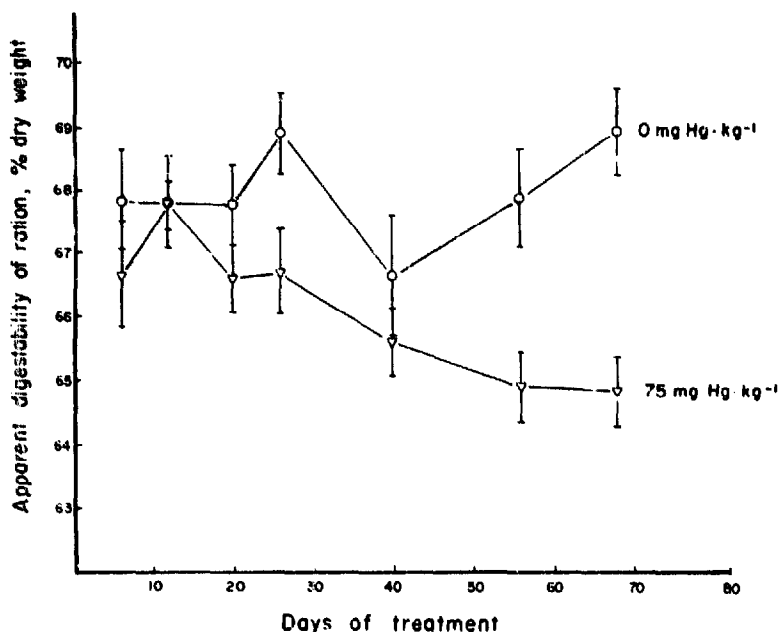


Fig. 3. Apparent digestibility of ration for rainbow trout fed diets containing different concentrations of methylmercury (mean \pm SE).

diets for 85 days, with significant variation observed only in hematocrit. Trout fed the 75/1% diet had a lower hematocrit (34.2 ± 1.9 , $n = 10$) than fish fed 0 and 25 mg Hg \cdot kg⁻¹ diets at both meal sizes (39.6 ± 0.6 , $n = 30$). In contrast, fish fed the 75/2% diet had an increased hematocrit (46.1 ± 2.7 , $n = 10$). Serum osmolality and concentrations of sodium and potassium ions were not significantly different among treatments with mean values of 275 ± 2 mOsm \cdot kg⁻¹, 151 ± 2 and 3.2 ± 0.4 mEq \cdot l⁻¹ respectively.

Tissues of trout fed diets containing added methylmercury for 85 days were similar in appearance and morphology to those of fish fed the 0 mg Hg \cdot kg⁻¹ diet and did not exhibit apparent pathological changes. The mean diameter of cell nuclei from the proximal convoluted tubule of the posterior kidney was not significantly different between fish fed the 0/2% and 75/2% diets (7.55 ± 0.14 and 7.48 ± 0.17 μ m respectively, mean \pm SE, $n = 50$). Other tissues examined (liver, intestine and gill) were also of similar appearance and morphology among fish fed 0, 25 and 75 mg Hg \cdot kg⁻¹ diets.

In general, the concentrations of mercury in trout fed diets containing added methylmercury increased throughout the first 56 days of feeding, then stabilized between 56 and 85 days (Fig. 4). Fish fed the 75 mg Hg \cdot kg⁻¹ diet at fixed rations were an exception to this pattern, with mercury concentrations continuing to

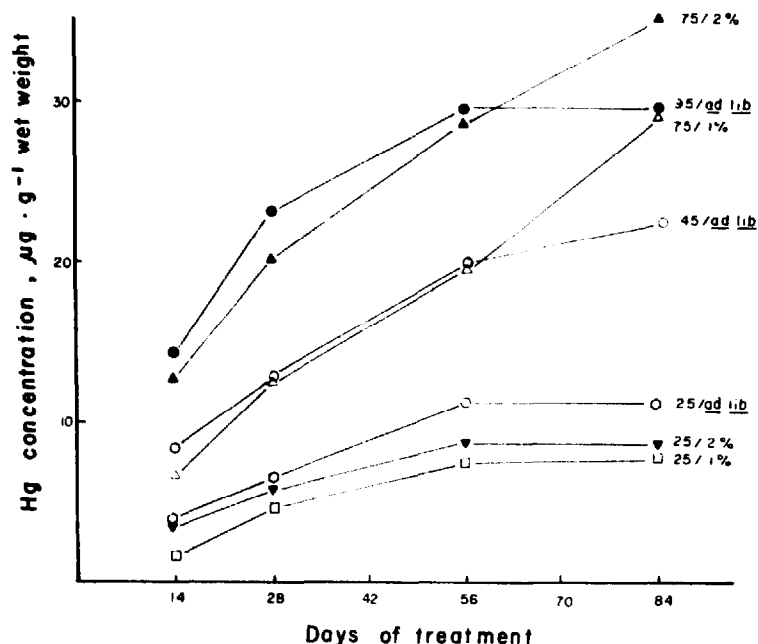


Fig. 4. Mercury concentration in whole body tissues of rainbow trout fed diets containing methylmercury at indicated concentrations and meal sizes.

increase throughout the 85-day feeding period. Mercury concentrations of trout before experimental feeding and of fish fed the $0 \text{ mg Hg} \cdot \text{kg}^{-1}$ diet were $< 0.1 \text{ mg Hg} \cdot \text{kg}^{-1}$ wet weight. Among fish fed the $25 \text{ mg Hg} \cdot \text{kg}^{-1}$ diet, mercury concentrations stabilized in the range of $10 \text{ mg Hg} \cdot \text{kg}^{-1}$ wet weight at all meal sizes. In trout fed the 45/ad lib. and 95/ad lib. diets, mercury concentrations levelled off in the range of 20 and $30 \text{ mg Hg} \cdot \text{kg}^{-1}$ wet weight respectively. Mercury concentrations of fish fed the 75/2% diet were 5–6 $\text{mg Hg} \cdot \text{kg}^{-1}$ wet weight higher than those of fish fed the 75/1% diet from 14 to 84 days.

The total amount of mercury in fish fed diets containing added methylmercury increased throughout the feeding period (Fig. 5). As this quantity is the product of mercury concentration and wet weight, changes in amount of mercury in fish with time reflect changes in both mercury concentration and growth. The increase in quantity of mercury with time in fish fed fixed rations was apparently linear. The amount of mercury in trout fed $2\% \cdot \text{day}^{-1}$ was slightly greater than twice that of fish fed $1\% \cdot \text{day}^{-1}$ at an equivalent mercury concentration and time (Fig. 5). Among fish fed to satiation, the pattern was more complex. Trout fed the 45/ad lib. diet were larger than those fed the 95/ad lib. diet from 42–84 days of experimental feeding. Even though the concentration of mercury in fish fed the 95/ad lib. diet was $\approx 10 \text{ mg Hg} \cdot \text{kg}^{-1}$ wet weight greater than that of fish fed the 45/ad lib. diet,

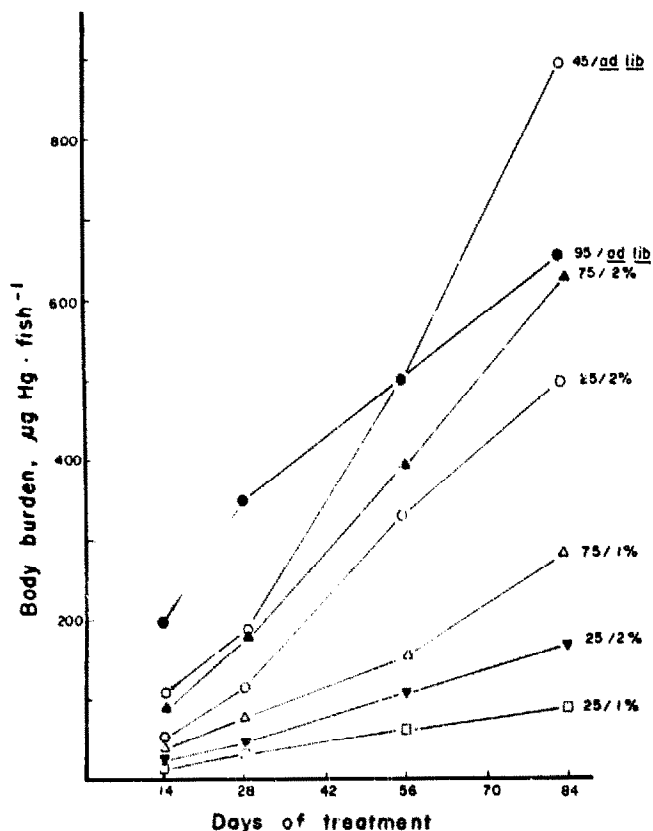


Fig. 5. Total amount of mercury (body burden) in rainbow trout fed diets containing methylmercury at indicated concentrations and meal sizes.

the latter fish had approximately the same quantity of mercury after 56 days of exposure and $\approx 35\%$ more mercury after 84 days.

Generally, trout assimilated 70–80% of the labeled methylmercury which they were fed (Table I). Assimilation of labeled methylmercury by fish fed the 75 mg $\text{Hg} \cdot \text{kg}^{-1}$ diet was significantly outside this range in some time periods. In the third interval (57–84 days after initiation of experimental feeding) fish fed the 75 mg $\text{Hg} \cdot \text{kg}^{-1}$ diet at either meal size assimilated $< 50\%$ of the labeled methylmercury which they were fed. The assimilation of $\approx 85\%$ of labeled methylmercury in fish fed the 75/1% diet in the first test interval (1–28 days) was slightly but significantly above the 70–80% range.

The rate of depuration of ^{203}Hg -labeled methylmercury varied significantly among treatments (Table I). In general, methylmercury was depurated more rapidly in fish fed the larger meal size. In addition, trout fed diets containing added

TABLE 1
Retention and depuration rate of ^{203}Hg -labeled methylmercury in rainbow trout fed diets containing cold methylmercury for 84 days (mean of 2 replicate tanks).

| Dietary Hg conc. ($\mu\text{g Hg} \cdot \text{g}^{-1}$) | Meal size (% $\cdot \text{day}^{-1}$) | Exposure duration (days) | | |
|---|--|--|---|---|
| | | 1-28 | 29-56 | 57-84 |
| 0 | 1 | 72.9% ^a | 72.8% | 79.2% |
| | | $6.47 \times 10^{-3} \cdot \text{day}^{-1b}$ | $5.16 \times 10^{-3} \cdot \text{day}^{-1}$ | $6.47 \times 10^{-3} \cdot \text{day}^{-1}$ |
| | | $1.78 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1c}$ | $1.70 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $2.42 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ |
| 25 | 1 | 76.7% | 77.2% | 75.9% |
| | | $8.32 \times 10^{-3} \cdot \text{day}^{-1}$ | $6.00 \times 10^{-3} \cdot \text{day}^{-1}$ | $6.54 \times 10^{-3} \cdot \text{day}^{-1}$ |
| | | $2.23 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $1.87 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $2.19 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ |
| 75 | 1 | 84.8% | 75.2% | 32.7% |
| | | $10.1 \times 10^{-3} \cdot \text{day}^{-1}$ | $6.68 \times 10^{-3} \cdot \text{day}^{-1}$ | $7.33 \times 10^{-3} \cdot \text{day}^{-1}$ |
| | | $2.82 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $2.18 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $2.39 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ |
| 0 | 2 | 72.1% | 71.0% | 71.5% |
| | | $9.39 \times 10^{-3} \cdot \text{day}^{-1}$ | $6.17 \times 10^{-3} \cdot \text{day}^{-1}$ | $6.45 \times 10^{-3} \cdot \text{day}^{-1}$ |
| | | $2.67 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $2.18 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $3.27 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ |
| 25 | 2 | 75.0% | 72.1% | 72.4% |
| | | $12.2 \times 10^{-3} \cdot \text{day}^{-1}$ | $9.06 \times 10^{-3} \cdot \text{day}^{-1}$ | $7.32 \times 10^{-3} \cdot \text{day}^{-1}$ |
| | | $3.06 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $3.28 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $3.54 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ |
| 75 | 2 | 69.8% | 71.2% | 47.4% |
| | | $12.7 \times 10^{-3} \cdot \text{day}^{-1}$ | $8.77 \times 10^{-3} \cdot \text{day}^{-1}$ | $7.18 \times 10^{-3} \cdot \text{day}^{-1}$ |
| | | $3.47 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $2.99 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ | $3.53 \times 10^{-2} \text{g}^{0.58} \cdot \text{day}^{-1}$ |

^a Retention (% of ingested).

^b Depuration rate (day^{-1}).

^c Weight-corrected depuration rate ($\text{g}^{0.58} \cdot \text{day}^{-1}$).

methylmercury depurated labeled methylmercury faster than fish fed the 0 mg Hg · kg⁻¹ diet. These trends, however, became less pronounced with the duration of experimental feeding. The average rate of depuration of labeled methylmercury of fish fed at 2% · day⁻¹ was significantly higher than that of fish fed at 1% · day⁻¹ in the first two intervals (1–28 days and 29–56 days) but in the third interval (57–84 days) rates of depuration of labeled methylmercury were not significantly different between meal sizes. In the first interval (1–28 days, the rate of depuration of labeled methylmercury of fish fed the 75 mg Hg · kg⁻¹ diet was significantly higher than that of fish fed the 25 mg Hg · kg⁻¹ diet, which in turn was significantly higher than the rate of labeled methylmercury depuration in fish fed the 0 mg Hg · kg⁻¹ diet. Rates of depuration of labeled methylmercury of fish fed the 75 and 25 mg Hg · kg⁻¹ diets in the second interval (29–56 days) were not significantly different but were significantly higher than the rate of depuration of labeled methylmercury in fish fed the 0 mg Hg · kg⁻¹ diet. In the third interval (57–84 days) the rates of depuration of labeled methylmercury of fish fed 0, 25 and 75 mg Hg · kg⁻¹ diets were not significantly different.

The patterns obtained using weight-corrected depuration rates (observed rate divided by weight to the exponent -0.58) were generally similar to the patterns obtained with observed rates of elimination of labeled methylmercury (Table I). Weight-corrected elimination rates of fish fed at 2% · day⁻¹ were significantly greater than those of fish fed at 1% · day⁻¹; while those of fish fed the 25 and 75 mg Hg · kg⁻¹ diets were significantly higher than those of fish fed the 0 mg Hg · kg⁻¹ diet. No consistent pattern was apparent in changes in weight-corrected elimination rate with duration of feeding.

DISCUSSION

Changes in appearance, behavior, appetite, growth and digestibility of ration were manifest in trout fed diets containing added methylmercury. In these same fish, serum ionic and osmotic concentrations and cellular appearance and morphology were similar to fish fed diets without added methylmercury. The latter criteria would thus seem to be relatively insensitive as indices of the effects of methylmercury on rainbow trout.

The darkened color and listlessness observed in some of the fish fed diets containing 75 or 95 mg Hg · kg⁻¹ methylmercury were similar to signs previously reported in rainbow trout after prolonged exposure to high concentrations of methylmercury in food or water (Matida et al., 1971). The lack of effects of dietary methylmercury on serum ionic or osmotic concentrations is in accord with previous reports that prolonged exposure to methylmercury in food or water did not affect the concentrations of plasma electrolytes and serum proteins (O'Connor and Fromm, 1975; Wobeser, 1975). The absence of apparent pathological changes in fish fed dietary methylmercury is consistent with the findings of Wobeser (1975)

who detected only a slight gill hyperplasia in rainbow trout fed diets containing added methylmercury for 15 wk.

The increased hematocrit observed in fish fed the 72/2% diet for 12 wk is in agreement with previous reports of increased hematocrit in rainbow trout following prolonged exposure to elevated concentrations of methylmercury in food or water (O'Connor and Fromm, 1975; Wobeser, 1975). The decreased hematocrit of trout fed the 75/1% diet is contrary to the above results. Mercurials have been reported to cause hemolysis (Webb, 1966) which may account for the observed decrease in hematocrit. The increased hematocrit observed in this and other studies suggests that methylmercury may also affect hematopoiesis. As serum ionic and osmotic concentrations did not differ significantly among fish fed the 0, 25 and 75 mg Hg · kg⁻¹ diets, changes in serum water content do not seem to be a factor in the observed changes in hematocrit.

Appetite appears to be one of the most sensitive indicators of the effects of dietary methylmercury. Decreases in appetite, apparently proportional to concentration of methylmercury in the diet were observed at all methylmercury concentrations tested. The progressive nature of the observed decrease in appetite, with the reduction becoming more pronounced with increased duration during feeding, suggests a cumulative effect of methylmercury on appetite. This pattern is in marked contrast to that observed in salmonids exposed to sublethal concentration of copper and zinc, where appetite was initially depressed and then returned to control values (Lett et al., 1976; Farmer et al., 1979). Food intake in teleosts is influenced by a variety of neurological, endocrinological, nutritional and social factors (Peter, 1979) which may account for its sensitivity to stress.

Differences in growth among fish fed to satiation are indicative of the effects of methylmercury when food is not limiting. In the 12 wk of feeding, there was a three-fold difference in growth between fish fed the 0/ad lib. and 95/ad lib diets. Similar reductions in growth of trout fed diets containing added methylmercury have been previously reported, although food consumption was not measured (Matida et al., 1971; Wobeser, 1975). As changes in growth are concomitant with changes in food consumption, differences in growth in these experiments do not assess the effects of methylmercury on growth per se.

The decreased growth of trout fed diets containing added methylmercury at the higher fixed ration demonstrates that methylmercury affects growth as well as appetite. While we have insufficient information to construct detailed energy budgets, the 2% decrease in apparent digestibility observed in fish fed the 75 mg Hg · kg⁻¹ diet appears adequate to account for ≈ 50% of the observed reduction in growth. The decrease in apparent digestibility observed in fish fed the 75 mg Hg · kg⁻¹ diet is consistent with observations that inorganic mercury inhibited intestinal transport of nutrients in teleosts *in vitro* (Miller, 1981). The remaining differences in growth are assumed to reflect changes in other components of the energy budget. Standard metabolic rate was not measured but is expected to be

higher in methylmercury treated fish due to methylmercury induced impairment of mitochondrial functions (Miller and Miller, 1979). Metabolism associated with swimming should be similarly elevated above that of control fish. Thus increases in metabolic rate may explain the remaining differences in growth rates.

The lack of significant differences in growth among trout fed the lower ration likely results from the low growth rates and higher relative variability of growth rate of these fish obscuring the effects of methylmercury. Growth rates of trout fed the lower ration were approximately one-half those of fish fed the higher ration, while the coefficients of variation of mean growth rate of trout at the lower meal size were approximately twice that of fish at the higher meal size. Differences in growth rates between rations would further compound differences in amount of food consumed. After 43 days of experimental feeding, the mean weights of fish fed at 1 and 2% $\cdot \text{day}^{-1}$ were \approx 6.5 and 12 g respectively. Thus the ration of fish fed at 1% $\cdot \text{day}^{-1}$ (65 mg $\cdot \text{day}^{-1}$) at this time would be less than one-third that of fish fed at 2% $\cdot \text{day}^{-1}$ (240 mg $\cdot \text{day}^{-1}$). The effects of methylmercury on energetic costs such as apparent digestibility would be similarly compounded at the higher meal size.

The whole body methylmercury concentrations of 30–35 mg Hg $\cdot \text{kg}^{-1}$ wet weight attained by fish fed the 75 and 95 mg Hg $\cdot \text{kg}^{-1}$ diets for 8–12 wk are comparable to highest concentrations reported in either experimentally or naturally contaminated fish (Matida et al., 1971; Wobeser, 1975; Armstrong, 1979). In our experiments and those of Matida et al. (1971), mercury concentrations in fish fed the 25 mg Hg $\cdot \text{kg}^{-1}$ diets tended to stabilize at \approx 10 mg Hg $\cdot \text{kg}^{-1}$ wet weight after 8–12 wk exposure; while fish fed rations containing > 45 mg Hg $\cdot \text{kg}^{-1}$ attained residues in the range of 20–35 mg Hg $\cdot \text{kg}^{-1}$ wet weight. In contrast, Wobeser (1975) reported that rainbow trout fed diets containing 4–24 mg Hg $\cdot \text{kg}^{-1}$ attained mercury concentrations greater than their respective diets in 9–12 wk. Fish fed the 24 mg Hg $\cdot \text{kg}^{-1}$ diet attained mercury concentrations of \approx 30 mg Hg $\cdot \text{kg}^{-1}$ wet weight after 16 wk feeding. These discrepancies are at least in part due to differences in diet composition and ration size. Wobeser's fish were fed a wet diet at an estimated ration size of 3–4% wet weight $\cdot \text{day}^{-1}$ whereas our fish were fed a dried diet at meal sizes ranging from 1–2.4% wet weight $\cdot \text{day}^{-1}$. Wobeser's fish would thus receive from 2–4 times the amount of methylmercury per day at equivalent dietary mercury concentrations. The attainment of mercury concentrations would thus seem to be substantially modified by experiment specific factors such as initial fish size, feeding rate, growth rate, diet composition and mercury concentration.

Fish fed the 0 and 25 mg Hg $\cdot \text{kg}^{-1}$ diets consistently assimilated \approx 75% of the labeled methylmercury ingested at both meal sizes. These values are in agreement with the range of 70–90% previously reported (DeFreitas et al., 1975; Sharpe et al., 1977). The constant assimilation of labeled methylmercury, combined with the relative ease with which it may be measured in intact fish suggests that measurements of assimilated methylmercury could be used to provide an estimate

of food consumption of individual fish. This technique may be particularly useful in estimating the ingestion of other non-labeled compounds (Roberts et al., 1977) or in estimating food consumption among individual fish.

The marked decrease to < 50% assimilation of labeled methylmercury observed in fish fed the 75 mg Hg·kg⁻¹ diet for 8 wk, indicates significant changes in the fish's processing of methylmercury. As a coincident decrease in apparent digestibility was not observed and the gut did not exhibit apparent pathological changes, either a saturation of uptake or an induction of a specific block to methylmercury assimilation is indicated. Although this decrease in methylmercury assimilation was observed only in fish fed the 75 mg Hg·kg⁻¹ diet for 8 wk, the possibility of a similar decrease with continued exposure to lower concentrations of methylmercury remains. The low (15–20%) assimilation of methylmercury obtained in a study using chronically exposed predator and prey fish (Phillips and Gregory, 1979) may reflect this induction, although differences in diet composition may also be involved. If a similar reduction in methylmercury assimilation occurs with prolonged exposure at lower dietary concentrations, models of accumulation of dietary methylmercury should employ an assimilation efficiency less than the 0.8 employed by Norstrom et al. (1976).

Trout fed the larger meal size should have a higher metabolic rate (Beamish, 1974) in addition to their faster rate of methylmercury depuration. The suggestion that methylmercury depuration is in part determined by metabolic rate is consistent with reports that methylmercury depuration rates in a number of teleosts decreased with increased body weight (DeFreitas et al., 1975; Sharpe et al., 1977). The observation that rainbow trout at 16–19°C cleared methylmercury more rapidly than fish at 0.5–4°C (Ruohtula and Miettinen, 1975) also supports a metabolic dependence of methylmercury depuration. In contrast, the report that in goldfish, *Carassius auratus*, the rate of methylmercury depuration did not vary significantly among fish at 5, 10 and 20°C (Sharpe et al., 1977) may indicate that methylmercury depuration in goldfish is not strictly dependent on metabolic rate. This discrepancy may indicate that the relationship between methylmercury depuration and metabolic rates is more species-specific than previously supposed (Norstrom et al., 1976). The pronounced effects of meal size on methylmercury depuration suggest that studies of methylmercury dynamics in non-feeding fish (Olson et al., 1978) may underestimate the abilities of the fish to eliminate the compound.

The decrease with time of differences between meal sizes of observed rates of methylmercury depuration likely results from differences in growth rate. In the third interval (57–84 days) trout fed the lower ration were ≈ 1/3–1/2 as heavy as fish fed the higher ration. The decrease in methylmercury depuration rate of fish at the higher ration due to their larger body size appears to have compensated for the increase in depuration rate due to meal size. The differences between meal sizes were maintained throughout the study, however, when weight-corrected depuration rates were compared.

The higher rates of methylmercury depuration observed in trout fed diets containing added methylmercury suggest that the quantity of methylmercury consumed by the fish may affect the rate at which it is eliminated. Previous reports that the rate of methylmercury depuration in fish was proportional to the quantity ingested (DeFreitas et al., 1975; Ruohtula and Miettinen, 1975) are also consistent with an induction of methylmercury depuration. Methylmercury within the tissues will bind to both protein and lipid, but is specifically bound by low molecular weight proteins (Clison et al., 1978; Thomas and Smith, 1979). Similar low molecular weight proteins are induced in fish previously exposed to inorganic metals such as copper and mercury and appeared to increase the tolerance of the fish to the metal (Bouquegneau, 1979; Dixon and Sprague, 1981). Methylmercury in fish is eliminated by the liver, kidney and possibly gills (Burrows and Krenkel, 1973; Olson et al., 1978). Demethylation of methylmercury to inorganic mercury is an important and possibly rate limiting step in methylmercury depuration (Burrows and Krenkel, 1973; Rentro et al., 1974). The enzymes central to xenobiotic depuration are inducible, with induction effecting both qualitative and quantitative changes in xenobiotic accumulation (Lech and Bend, 1980). An induction of the processes of methylmercury depuration would thus be consistent with the observed increased rates of depuration in fish consuming diets with added methylmercury. The decrease in differences in rate of methylmercury depuration among fish fed the 0, 25 and 75 mg Hg · kg⁻¹ diets with time may represent a saturation or injury to a component or components of the processes of methylmercury elimination.

In our studies, growth, assimilation and rate of depuration of dietary methylmercury each varied by factors of two to four. The pattern of mercury accumulation was essentially linear however; with the rate of increase in quantity of mercury of fish fed at 2% · day⁻¹ approximately twice that of trout at 1% · day⁻¹. The increased rate of methylmercury depuration of trout fed at the higher meal size would seem to have offset the compounding of differences in absolute meal size with time, resulting in the appearance that methylmercury accumulation was directly proportional to meal size.

In sensitivity analysis of their model of methylmercury accumulation, Norstrom et al. (1976) indicated that a doubling of either depuration rate or growth rate produces a change of approximately 50% in methylmercury concentration attained at a given weight. The observed changes in methylmercury dynamics, coupled with the effects of growth could thus result in changes of 100–200% in methylmercury concentration attained at a given weight. For compounds metabolized and eliminated at a faster rate than methylmercury, depuration will exert a far greater effect on accumulation (Southworth et al., 1980) and an induction of depuration would affect accumulation of these compounds to an even greater degree.

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