Arch. Environ. Contam. Toxicol. 26, 169–178 (1994)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Evaluation of the Critical Body Burden Concept Based on Inorganic and Organic Mercury Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)

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Received: 12 May 1993/Revised: 28 July 1993

Abstract. Subadult rainbow trout (*Oncorhynchus mykiss*) were exposed to four waterborne concentrations each of 64–426 μg/L mercuric chloride ($HgCl_2$) and 4–34 μg/L methylmercury chloride (CH_3HgCl) until death to evaluate the critical body burden concept. Mean days to death for fish exposed to the highest and lowest concentrations of $HgCl_2$ were 1 and 58 d, and 2 and >100 d for fish exposed to CH_3HgCl. Time to death was an important factor that influenced Hg tissue concentration, and was most evident among fish that died within a few days of exposure. Critical body burdens for Hg could be difficult to establish at the tissue level because no threshold concentrations were clearly indicated among the liver, kidney, spleen, brain, muscle, and gill that were monitored in this study. A critical burden for Hg was derived on a whole body basis for Hg in its organic form. An evaluation of this and other studies suggests whole body concentrations of 10–20 mg/kg Hg could be lethal to fish. Extrapolation from other studies indicate whole body concentrations of 1–5 mg/kg Hg could have chronic effects on fish and possibly other aquatic organisms. This concept could be used to assess the toxicological significance of chemical concentrations that are monitored in feral aquatic organisms. This tissue-based approach appears to have some advantages over current assessment protocols that focus on waterborne concentrations.

Critical body burden is a concept that examines the relationship between the accumulation of a toxicant and its effects on an organism. It could be derived from radiological studies where the maximum permissible level for a radionuclide is established for a tissue or the whole body (Eisenbud 1973). This concept has a broader application in toxicology where it is based on the premise that a toxicant must reach a threshold concentration before an adverse response is elicited (Foulkes 1990). The response can range from the lowest observed adverse effect to death, and the threshold concentration is the critical or lethal body burden.

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Chemical monitoring programs for the aquatic environment have generally focused on waterborne concentrations for the protection of aquatic organisms and concentrations in edible tissues of some organisms for the protection of human health. Little use is made of most of these chemical measurements that are in compliance. The body burden approach could assess the toxicological significance of these concentrations by comparing the levels monitored in the organisms to those that can cause chronic or lethal effects. This tissue-based approach has some advantages because the relationship between environmental chemical concentration and toxicity can be influenced by physical, chemical, and biological factors. Increasing temperature and decreasing pH, alkalinity, oxygen content, and salinity can enhance the toxicity of some chemicals like Hg to fish (Amend *et al.* 1969; MacLeod and Pessah 1973; Reinert *et al.* 1974; Hamilton and Buhl 1990). The chemical state can also influence toxicity where 48 h LC_{50} of 0.21, 0.01, 0.04, and 0.07 mg/L for rainbow trout (*Oncorhynchus mykiss*) were reported for mercuric chloride ($HgCl_2$), phenyl mercuric acetate, methylmercury chloride (CH_3HgCl), and ethyl mercuric phosphate respectively (Matida *et al.* 1971). These differences in toxicity can be partly attributable to the gill uptake efficiencies of 0.002 for $HgCl_2$ and 0.1–0.2 for CH_3HgCl reported for fish (Hamelink *et al.* 1977; Phillips and Buhler 1978; Rogers and Beamish 1981).

The relationship between chemical body burden and death has been examined for several decades (Mount 1964; Mount and Boyle 1969; Burton *et al.* 1972). More recent studies have examined the body burden concept in greater detail (Friant and Henry 1985; de Bruijn *et al.* 1991). These studies often measure the concentrations on a whole body basis at death and report the lethal body burden. Body burdens for pentachlorophenol of 80–155 mg/kg for goldfish, and 20–200 mg/kg in trout, were reported among fish that died within 2 d (Kobayashi and Kishino 1980; Hattula *et al.* 1981; van den Heuvel *et al.* 1991). The rapid death indicates the fish were exposed to high waterborne concentrations. It has not been well established if body burdens attained under these conditions would be comparable to burdens after a longer exposure period.

Comparisons between short- and long-term lethality studies indicate time to death can influence tissue concentrations although the observations appear to be inconclusive. Studies on

arsenic and several pesticides reported fish that died within a few days had substantially higher body burdens than fish exposed to a lower concentration that died after a longer period (Sorensen 1976; de Bruijn *et al.* 1991). Other studies on nickel and cadmium reported lower concentrations in fish that died within a few days compared to fish exposed to lower concentrations that survived longer (Eaton 1974; Gupta and Rajbanshi 1988; Sreedevi *et al.* 1992). Long-term, non-lethal exposure studies generally indicate the concentrations of persistent chemicals in organisms increase with exposure concentration and time of exposure (Renfro *et al.* 1975; Rhead and Perkins 1974; Oliver and Niimi 1985). This relationship was shown in trout where tissue concentrations of Hg gradually increased over the 39-week study at each of the four waterborne concentrations examined (McKim *et al.* 1976). Hg concentrations were also highest among the few fish exposed to the highest concentration that died after 16–30 weeks. The response shown in the latter study could represent the accumulation pattern one might anticipate in a critical body burden relationship.

This study examines the critical body burden concept by exposing subadult rainbow trout to several waterborne concentrations of $HgCl_2$ and CH_3HgCl until death. Lethality is not the most appropriate criterion to assess this concept in an organism from an ecotoxicological perspective although is a well defined response. Hg was selected because it is a neurotoxin and a xenobiotic chemical whose concentration is not regulated by the organism. CH_3HgCl is toxic at a lower waterborne concentration than $HgCl_2$ to fish, although little is known about their lethal critical body burdens. Exposure concentrations were varied to induce differences in time to death to examine its influence on lethal body burdens. Measurements included Hg concentrations in specific tissues and on a whole body basis to determine if critical tissue burdens can be used. The feasibility of using tissue-based values to assess the toxicological significance of Hg concentrations observed in feral aquatic organisms will also be discussed.

Materials and Methods

Hatchery-reared trout were held for 40–60 d in 440 L circular tanks at 15 ± 2°C and fed *ad libitum* on a commercial dry diet every second day before the study began. Groups of 20 fish, each fish weighing 100–150 g, were transferred into 200 L circular tanks 4–6 d before the exposure started. The experimental fish were also maintained at 15°C and fed *ad libitum* every second day throughout the study. These fish were continuously exposed to waterborne $HgCl_2$ or CH_3HgCl until death. The Hg was added to each tank using a peristaltic pump that added a stock solution at 0.5 ml/min which was diluted by the 3 L/min inflow of water. The stock solution was prepared by dissolving the Hg salt in distilled water acidified with 1 drop of concentrated H_2SO_4 per liter. About 30 ml stock solution was added to the experimental tank at the start to rapidly achieve the desired concentration. Domestic water originating from Lake Ontario was passed through an activated charcoal filter to remove the chlorine for laboratory use. Properties of this water included a pH of 7.6–7.9, hardness of 135 mg/L, dissolved oxygen 8–9 mg/L, and residual chlorine of <10 μg/L. Water samples were taken weekly, or more frequently at the higher Hg concentrations, and preserved with potassium dichromate-sulfuric acid (Environment Canada 1981).

These groups of fish were exposed to 4, 10, 13, and 34 μg/L CH_3HgCl and to 64, 135, 241, and 426 μg/L $HgCl_2$. Dead fish were frozen at −20°C until analyzed. The kidney, liver, spleen, brain, muscle, and gill were dissected from each fish for Hg analyses. Muscle was taken from the hypaxial area below the dorsal fin.

A group of 20 fish was also exposed to 9 μg/L CH_3HgCl until death following the same procedure. These fish were analyzed for whole body Hg concentration by homogenizing each fish to a uniform consistency using an Oster blender. Another group of 20 fish that were held under the same conditions, but not exposed to Hg, served as controls. Six fish were sampled at the beginning of the study, and seven fish each after 30 and 60 d. Samples of the six tissues monitored in experimental fish were also taken from the control fish for Hg analysis. Nine rainbow trout that weighed 1.7–4.6 kg each were also collected from the Ganaraska River, a tributary of Lake Ontario. These fish were also analyzed for Hg to compare tissue distribution patterns between feral and experimentally exposed fish.

Tissue samples were prepared for Hg analyses using a wet digestion method, and analyzed by cold vapor spectrophotometry using a Pharmacia Mercury Monitor (Knechtel and Fraser 1979). Detection limit in water was 0.1 μg/L, and recovery was 101% for spiked water samples. Detection limit of a standard dogfish tissue was 0.01 mg/kg, and recovery was 95% based on analyses of the National Research Council of Canada Certified Reference Material DORM-1. All Hg concentrations in fish are expressed on a wet weight basis, and the mean ± standard deviation is reported in most cases. Analysis of variance (ANOVA) was used to compare Hg concentrations in tissues among treatments and linear regression to examine trends. Mercury uptake rates by fish were calculated from tissue or whole body Hg concentrations at death based on the time to death and exposure concentration.

Results

No gross external anomalies were observed among all fish exposed to Hg. Fish exposed to the highest concentrations of both Hg forms exhibited erratic swimming behavior characterized by short bursts several hours before death. Fish exposed to the lower concentrations gradually reduced food intake and became lethargic several days before death. No abnormal feeding or swimming behavior, or external anomalies, were observed among the control fish that were held up to 60 d. There were no significant differences in Hg tissue concentrations among these fish at the three sample intervals. Hg concentrations in control fish were 0.042 ± 0.003, 0.037 ± 0.009, 0.034 ± 0.003, 0.013 ± 0.002, 0.033 ± 0.002, and 0.022 ± 0.003 mg/kg in kidney, liver, spleen, brain, muscle, and gill, respectively.

Mean days to death decreased as concentrations increased among fish exposed to both forms of Hg (Table 1). No mean value was estimated for the 64 μg/L $HgCl_2$ treatment because of delayed mortality. Six fish were sampled after 60 d, and seven fish after 90 d, because no mortalities occurred during this period. The remaining seven fish died after 94–130 d exposure. The time interval between the first and last fish to die at each treatment increased from less than 3 to over 60 d with decreasing concentrations. This time interval resulted in a relationship between cumulative mortality and days to death that changed from a linear to nonlinear response (Figure 1).

Mercury concentrations that were measured in tissues and whole fish are reported in Tables 2 and 3. ANOVA indicated significant differences ($P \leq 0.05$) in Hg tissue concentrations among the treatments. Mean tissue concentrations markedly increased with decreasing exposure concentrations among most tissues except the gill of fish exposed to CH_3HgCl that remained at about 50–65 mg/kg. There were differences in Hg concentrations between $HgCl_2$ and CH_3HgCl exposed fish where higher levels were generally found in fish exposed to

Table 1. Waterborne Hg concentrations and days to death of rainbow trout exposed to $HgCl_2$ and CH_3HgCl

Hg conc (μg/L)			Days to death			
Mean	SD	n	Mean	SD	Range	n
$HgCl_2$						
64	11	23	—	—	94–130	7[a]
135	32	13	55.4	16.9	14–75	20
241	23	11	15.0	12.4	4–47	20
426	—	2	2.5	1.2	1–4	20
CH_3HgCl						
4	0.7	20	58.2	21.4	30–98	20
9	1.2	5	24.2	5.6	12–33	20
10	1.0	5	21.7	6.0	10–32	20
13	0.8	6	7.6	5.1	3–23	20
34	—	2	1	—	1	20

[a]Does not include the 13 fish that were sampled after 60 and 90 d exposure

$HgCl_2$. Mercury tissue distribution patterns were also different between $HgCl_2$ and CH_3HgCl exposed fish. Comparisons among the three lowest exposure levels generally indicated the lowest concentrations in muscle of fish exposed to both forms of Hg, but $HgCl_2$-exposed fish had the highest concentrations in kidney compared to spleen in CH_3HgCl-exposed fish. The Hg concentrations in adult rainbow trout collected from Lake Ontario were 0.28 ± 0.14, 0.21 ± 0.10, 0.26 ± 0.06, 0.07 ± 0.02, 0.22 ± 0.05, and 0.11 ± 0.04 mg/kg in kidney, liver, spleen, brain, muscle, and gill, respectively. The concentrations were also considerably lower than those observed in this study, although their tissue distribution patterns are similar.

There were generally little or no differences in Hg concentrations among fish exposed to 64 μg/L $HgCl_2$ that were sampled after 60 and 90 d compared to those that died after 94–130 d exposure (Table 2). There were also similarities in Hg tissue concentrations among these fish and those exposed to 64 μg/L and 135 μg/L that died after 14–75 d exposure. Fish exposed to 9 μg/L CH_3HgCl that died after 12–33 d had a mean concentration of 11 mg/kg Hg on a whole body basis (Table 3).

Differences in Hg tissue concentrations would indicate Hg uptake rates among tissues will vary. Uptake rates would be comparable on a more equitable basis when expressed as mg Hg/kg/d/μg Hg/L to account for differences in exposure concentrations and days to death among treatments. Comparisons among uptake rates generally indicated the rates for kidney, liver, and spleen were higher than brain and muscle for both forms of Hg, and the rates for CH_3HgCl were about 10 fold greater than $HgCl_2$ (Tables 2 and 3). Uptake rates for gill were the greatest at the highest exposure concentrations for both forms of Hg; this response was different from the other tissues, where uptake rates were similar or slightly increased with decreasing exposure concentrations.

Regression analyses were used to examine the relationship between time to death and lethal body burden for each tissue. Mercury concentrations significantly increased ($P \leq 0.05$) with time to death among all tissues of fish exposed to $HgCl_2$, and tissues of fish exposed to CH_3HgCl except the gill (Figures 2, 3). The influence of time to death was examined further by using the results of fish that died after 20 or more days exposure. Regression analyses on this data set indicated significant increases ($P \leq 0.05$) in Hg concentrations in most tissues of fish exposed to $HgCl_2$ except liver and gill, but increases only in liver and muscle in fish exposed to CH_3HgCl (Figures 2, 3). The regression coefficients (b) among the tissues of fish that died after 20 d exposure were also substantially lower than those for all fish for each of the tissues for both forms of Hg.

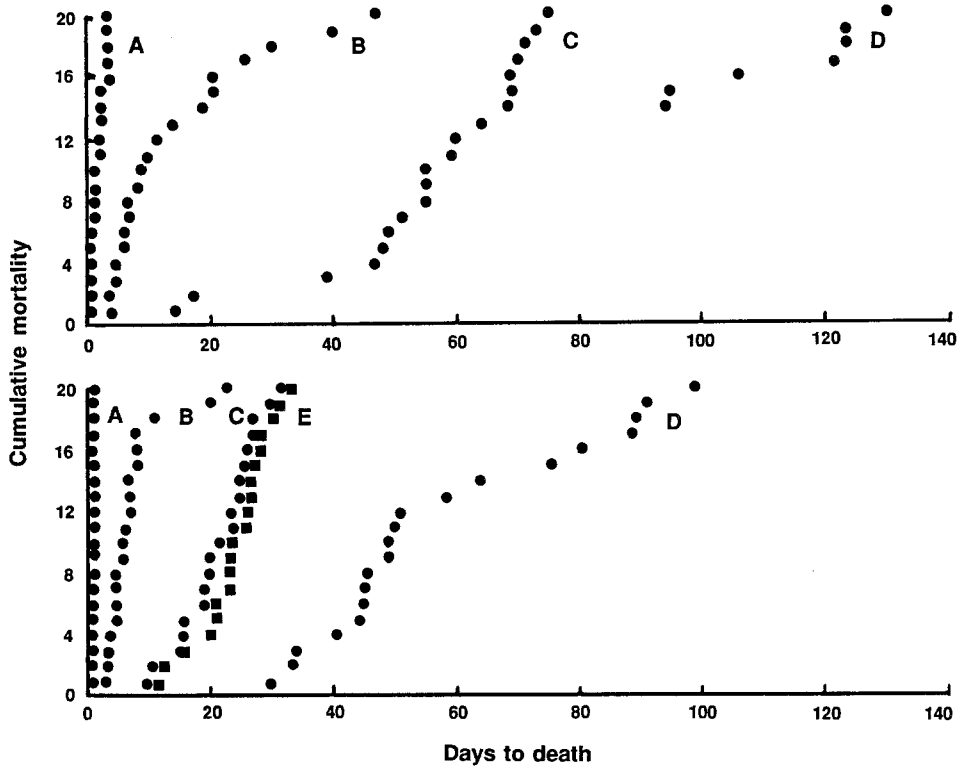

Fig. 1. Relationship between days to death and cumulative mortality of 20 rainbow trout exposed to 426 (A), 241 (B), 135 (C), and 64 (D) μg/L $HgCl_2$ that were used for Hg tissue analyses (upper panel). Only the last seven fish that died from exposure to 64 μg/L $HgCl_2$ are shown. The same relationship is shown for trout exposed to 34 (A), 13 (B), 10 (C), and 4 (D) μg/L CH_3HgCl (lower panel). Fish exposed to 9 μg/L CH_3HgCl (E) were analyzed on a whole body basis

Table 2. Mercury (Hg) tissue concentration and uptake rate in rainbow trout exposed to 64–426 μg/L $HgCl_2$. Concentration is reported as the mean ± SD and range and expressed in mg/kg for the 20 fish in each treatment. The mean ± SD of the rate of uptake is expressed as mg Hg/kg/d/μg Hg/L. Values for fish exposed to 64 μg/L represents live fish sampled after 60 d (n = 6) and 90 d (n = 7), and fish that died after 94–130 d exposure (n = 7)

	Exposure concentration (μg/L)					
	64			135	241	426
Tissue	60 d	90 d	94–130 d			
Kidney						
Conc	262 ± 69[a]	395 ± 110[b]	448 ± 154[b]	269 ± 110[a]	117 ± 84[c]	29 ± 30[d]
	157–361	206–508	268–662	78–496	10–289	2–105
Uptake	0.07 ± 0.02[a]	0.07 ± 0.02[a]	0.06 ± 0.01[a]	0.04 ± 0.01[b]	0.04 ± 0.02[b]	0.02 ± 0.02[b]
Liver						
Conc	202 ± 47[a]	233 ± 106[a]	213 ± 43[a]	271 ± 115[a]	93 ± 82[b]	18 ± 15[c]
	136–270	125–371	136–264	42–483	6–275	2–62
Uptake	0.05 ± 0.01[a]	0.04 ± 0.02[a]	0.03 ± 0.01[ab]	0.04 ± 0.01[b]	0.02 ± 0.01[b]	0.02 ± 0.01[b]
Spleen						
Conc	102 ± 19[a]	117 ± 8[a]	120 ± 35[a]	138 ± 64[a]	60 ± 43[b]	15 ± 11[c]
	82–131	104–127	72–163	20–257	6–154	1–42
Uptake	0.03 ± 0.01[a]	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.01 ± 0.01[b]
Brain						
Conc	51 ± 17[a]	72 ± 16[a]	70 ± 27[a]	67 ± 28[a]	30 ± 24[ab]	3.9 ± 3.2[c]
	29–81	47–100	41–113	9–124	2–87	0.6–11
Uptake	0.013 ± 0.004[a]	0.012 ± 0.003[ab]	0.009 ± 0.003[b]	0.009 ± 0.002[b]	0.009 ± 0.004[b]	0.004 ± 0.002[c]
Muscle						
Conc	6.2 ± 1.5[a]	2.9 ± 1.1[b]	7.1 ± 2.5[a]	8.2 ± 4.8[a]	3.1 ± 2.3[b]	0.7 ± 0.6[c]
	4.4–8.4	1.9–5.0	3.8–11	1.2–21	0.5–8.8	0.1–2.5
Uptake	0.002 ± 0.001[a]	0.001 ± 0.001[b]	0.001 ± 0.001[b]	0.001 ± 0.001[b]	0.001 ± 0.001[b]	0.001 ± 0.001[b]
Gill						
Conc	115 ± 18[a]	76 ± 15[b]	127 ± 63[a]	159 ± 59[a]	116 ± 52[a]	62 ± 28[b]
	82–135	56–95	66–257	87–284	48–226	23–112
Uptake	0.03 ± 0.01[a]	0.01 ± 0.01[b]	0.02 ± 0.01[b]	0.02 ± 0.01[ab]	0.04 ± 0.02[ac]	0.07 ± 0.01[d]

Values with the same superscripts are not significantly different ($P > 0.05$) among the treatments

Discussion

The results indicate the Hg tissue concentrations observed in trout would not fully support the body burden concept. Time to death was an important factor that influenced the lethal burden because fish that died within a few days had lower Hg concentrations than those that survived longer. This response was shown by the differences in regression relationships between all fish that died from Hg exposure compared to those that died ≥20 d whose regression coefficients (b) were lower or the relationship was not significant ($P > 0.05$). The premise that the critical concentration should not be influenced by the time to death was more evident among fish exposed to CH_3HgCl than $HgCl_2$, where Hg concentrations in kidney, spleen, brain, and gill did not increase significantly among those that died ≥20 d. The increase of Hg in liver among these fish could be due to the sequestering action of Hg by metallothionein (Hamilton and Mehrle 1986; Hogstrand and Haux 1991). The increase of Hg in muscle as time to death increased suggest it is less sensitive to Hg toxicity than the other tissues examined.

Mercury concentrations at death among trout exposed to $HgCl_2$ were at least several-fold higher in most tissues than fish exposed to CH_3HgCl. This difference in tissue concentrations appear smaller than the waterborne concentrations required to induce a similar response between the two forms of Hg. Trout in this study exposed to 135 μg/L $HgCl_2$ that died after an average 55 d exposure had 1.6–3.6-fold more Hg in the kidney, liver, spleen, brain, and gill than fish exposed to 4 μg/L CH_3HgCl that died after 58 d. These differences in tissue concentrations are less than the differences in 48 h LC_{50} of 0.21 mg/L for $HgCl_2$ in trout which was fivefold higher than the value reported for CH_3HgCl (Matida *et al.* 1971). $HgCl_2$ at 0.72 μg/L required to significantly reduce *Daphnia* reproduction was 18-fold higher than the concentration required by CH_3HgCl (Biesinger *et al.* 1982). Hg distribution patterns were also different between $HgCl_2$ and CH_3HgCl exposed fish. Trout exposed to waterborne $HgCl_2$ in this and other studies reported the highest concentrations in kidney, lower levels in liver, spleen, and brain, and lowest concentration in muscle (Boudou and Ribeyre 1983). Fish exposed to dietary $HgCl_2$ had higher Hg concentrations in kidney and spleen than the other tissues (Boudou and Ribeyre 1983).

Mercury concentrations among fish exposed to 64 μg/L $HgCl_2$ that were sampled after 60 and 90 d were similar those that died after 94–130 d exposure. Other studies have reported similar observations between fish that survived and died after being exposed to Hg. Brook trout (*Salvelinus fontinalis*) that died 14–28 weeks after exposure to 3 μg/L CH_3HgCl had 15–20 mg/kg Hg while surviving fish had 12 mg/kg Hg after 39 weeks (McKim *et al.* 1976). Trout exposed to the same concentration, that died after 26–96 weeks, had 10 mg/kg Hg while those that survived after 108 weeks exposure had 8 mg/kg Hg in

Table 3. Mercury (Hg) tissue concentration and uptake rate in rainbow trout exposed to 4–34 μg/L CH_3HgCl until death. Concentration is reported as the mean ± SD and range and expressed as mg/kg for the 20 fish in each treatment. The rate of Hg uptake is expressed as mg Hg/kg/d/μg Hg/L and the mean ± SD is reported for each treatment

	Exposure concentration (μg/L)				
Tissue	4	10	13	34	9
Kidney					
Conc	74 ± 30[a]	64 ± 20[a]	39 ± 21[b]	6.2 ± 2.7[c]	
	16–116	40–116	19–91	2.3–10	
Uptake	0.34 ± 0.15[a]	0.34 ± 0.06[a]	0.42 ± 0.12[b]	0.18 ± 0.08[c]	
Liver					
Conc	76 ± 19[a]	47 ± 10[b]	42 ± 27[b]	7.2 ± 2.8[c]	
	32–114	27–65	16–129	3.0–12	
Uptake	0.35 ± 0.11[a]	0.23 ± 0.03[b]	0.44 ± 0.09[c]	0.21 ± 0.08[b]	
Spleen					
Conc	89 ± 38[a]	72 ± 22[b]	51 ± 38[c]	6.4 ± 3.2[d]	
	32–118	37–112	19–194	2.7–14	
Uptake	0.41 ± 0.17[a]	0.34 ± 0.07[a]	0.52 ± 0.16[b]	0.19 ± 0.09[c]	
Brain					
Conc	19 ± 8[a]	13 ± 3[b]	7.7 ± 5.6[c]	1.1 ± 0.3[d]	
	7–32	7–19	2.3–22	0.6–1.5	
Uptake	0.09 ± 0.04[a]	0.09 ± 0.07[a]	0.08 ± 0.03[a]	0.03 ± 0.01[b]	
Muscle					
Conc	31 ± 12[a]	18 ± 5[b]	6.2 ± 7.7[c]	0.7 ± 0.3[d]	
	9–52	9–27	1.2–26	2.7–14	
Uptake	0.14 ± 0.06[a]	0.08 ± 0.02[b]	0.05 ± 0.02[c]	0.02 ± 0.01[d]	
Gill					
Conc	66 ± 15[a]	51 ± 12[b]	64 ± 15[ac]	56 ± 12[bcd]	
	42–93	34–85	36–98	29–73	
Uptake	0.32 ± 0.12[a]	0.25 ± 0.2[b]	0.79 ± 0.31[c]	1.64 ± 0.36[d]	
Whole fish					
Conc					11.2 ± 6.1
					4.0–27.3
Uptake					0.09 ± 0.02

Values with the same superscripts are not significantly different ($P > 0.05$) among the treatments

another aspect of that study. Similar chemical concentrations between dead and live fish were also reported by other studies on copper, cadmium, and pentachlorophenol (Brungs *et al.* 1973; Eaton 1974; van den Heuvel *et al.* 1991).

Laboratory studies often expose fish to chemicals through waterborne exposure, although other means such as dietary or injection methods have been used. An important ecotoxicological consideration in applying the body burden approach is the chemical distribution pattern between laboratory exposed and feral fish. Some evidence suggests fish exposed to waterborne and dietary CH_3HgCl have similar tissue distribution patterns after long exposure. Trout used in this and other studies exposed to waterborne CH_3HgCl had higher Hg concentrations in kidney, liver, spleen, and gills than brain and muscle (McKim *et al.* 1976; Boudou and Ribeyre 1983). Trout fed dietary CH_3HgCl for 4–40 weeks had Hg concentrations in the liver and kidney that were several-fold higher than brain and muscle (Matida *et al.* 1971; Boudou and Ribeyre 1983). A similar tissue distribution pattern was also observed in the Lake Ontario rainbow trout analyzed in this study.

Relatively few studies have exposed fish to dietary Hg and reported death or other adverse effects. Trout fed a diet with 25–100 mg/kg CH_3HgCl that died or exhibited neurological effects after 34–72 d had 11–21 mg/kg Hg on a whole body basis (Matida *et al.* 1971). Walleye (*Stizostedion vitreum vitreum*) fed food with 8 mg/kg Hg that died near the end of a 300 d study had 20–40 mg/kg Hg in muscle, 20–30 mg/kg in brain, and 30–60 mg/kg Hg in the liver (Scherer *et al.* 1975). These results are consistent with the observations on Hg concentrations at death among fish exposed to waterborne Hg, and similarities in tissue distribution patterns among fish exposed to long-term dietary and waterborne Hg.

There were differences in the rate of chemical uptake in trout where CH_3HgCl accumulated faster than $HgCl_2$ at comparable waterborne concentrations. Hg uptake rates calculated for brook trout exposed for 39 weeks to 0.03–0.93 μg/L CH_3HgCl were 2–3 times lower than the rates for the same six tissues reported in this study (McKim *et al.* 1976). One may expect uptake rates could be lower in this study because of the greater water hardness, although both studies reported lethal body burdens of 10–11 mg/kg Hg.

The toxicity of Hg to trout was greater when exposed as CH_3HgCl than $HgCl_2$. The analytical method used in this study measured total mercury, and it was assumed that the Hg measured was in the same state as that exposed. Studies have indicated fish have little or no capability to methylate inorganic Hg (Pentreath 1976; Huckabee *et al.* 1978). Mercury in its organic form has a greater toxicological significance in the

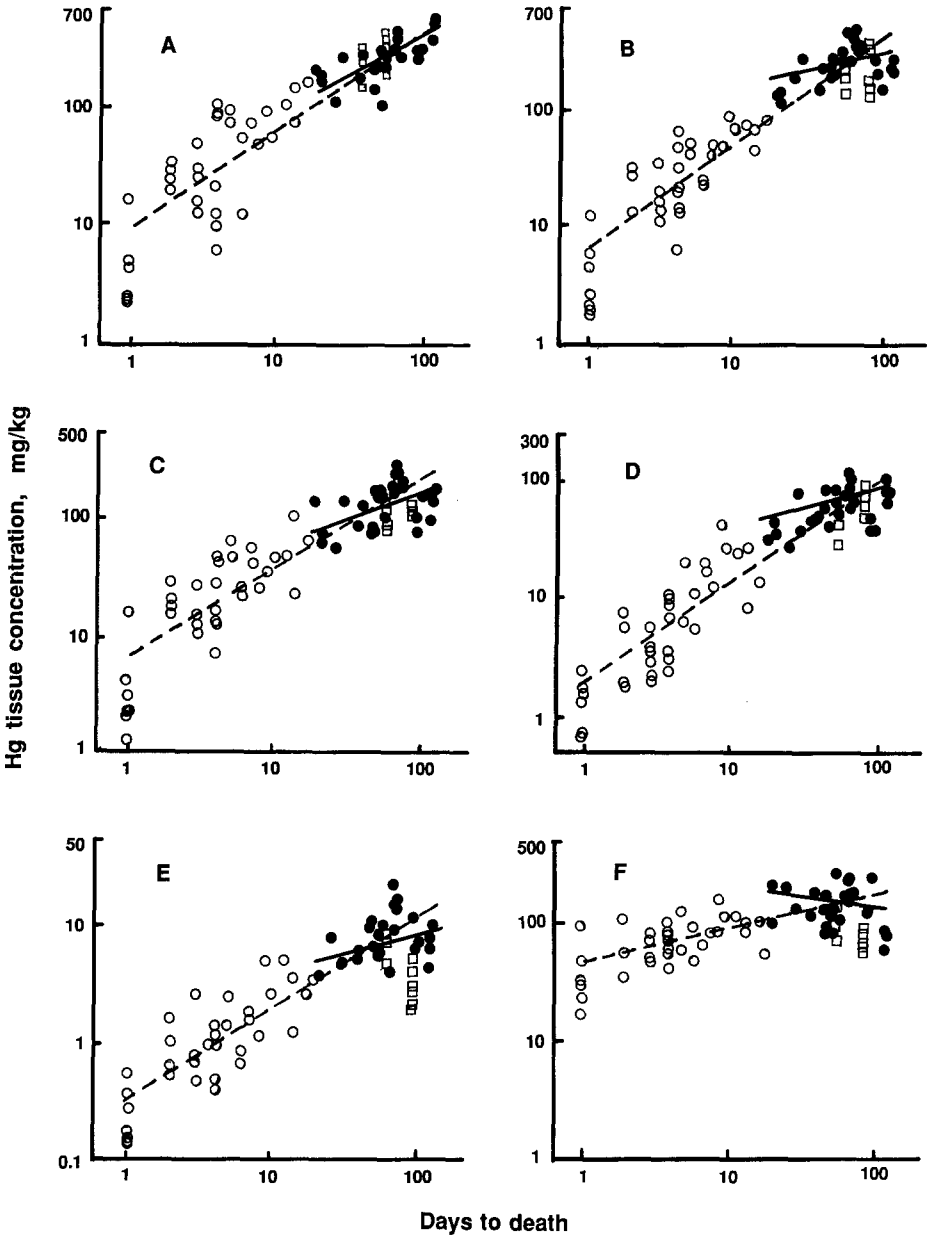

Fig. 2. Relationship between days to death and tissue concentration in mg/kg among rainbow trout exposed to 64–426 μg/L $HgCl_2$ for kidney (A), liver (B), spleen (C), brain (D), muscle (E), and gill (F). Open circles represent fish that died after 1–19 d exposure, and closed circles fish that died after ≥20 d exposure. Open squares are the 13 fish exposed to 64 μg/L that were sampled after 60 and 90 d. Regression analyses for the 67 fish that died after 1–130 d exposure for the six tissues examined, indicated by the broken line, are: log A = 0.943 + 0.865(±0.096)log X, r^2 = 0.83*; log B = 0.808 + 0.887(+0.084)log X, r^2 = 0.87*; log C = 0.769 + 0.756(±0.094)log X, r^2 = 0.80*; log D = 0.234 + 0.893(±0.084)log X, r^2 = 0.87*; log E = −0.477 + 0.747(±0.082)log X, r^2 = 0.83*; log F = 1.669 + 0.279(±0.066)log X, r^2 = 0.53*; where X is days to death and A–F the Hg concentration in mg/kg for the respective tissues. Regression analyses for the 31 fish that died after ≤20 d exposure, indicated by the solid line, are: log A = 1.271 + 0.663(±0.238)log X, r^2 = 0.53*; log B = 1.922 + 0.260(±0.260)log X, r^2 = 0.13; log C = 1.342 + 0.418(±0.293)log X, r^2 = 0.23*; log D = 1.243 + 0.321(±0.249)log X, r^2 = 0.19*; log E = 0.258 + 334(±0.326)log X, r^2 = 0.13*; log F = 2.441 − 0.153(±0.288)log X, r^2 = 0.04. Regression equations whose regression coefficient (±95% confidence limits) are significantly different from zero ($P \leq 0.05$) are identified with an asterisk

aquatic environment. Trophodynamic studies on Hg in aquatic ecosystems generally indicate biomagnification becomes a more important pathway than bioconcentration as trophic levels increases (Potter *et al.* 1975; Francesconi and Lenanton 1992). The percent organic Hg of the total Hg concentration is higher in aquatic vertebrates than invertebrates (Kumagai and Saeki 1978). Organic Hg can represent over 80% of total Hg in fish at higher trophic levels (Westöö 1973; Hattula *et al.* 1978). These observations indicate it would be more appropriate to develop a critical body burden for Hg in aquatic organisms based on its organic rather than the inorganic form.

It cannot be determined if any of the tissues monitored in this study contributed to, or was the cause of death, although it is presumed that exposure to Hg was the cause of death. Toxicity

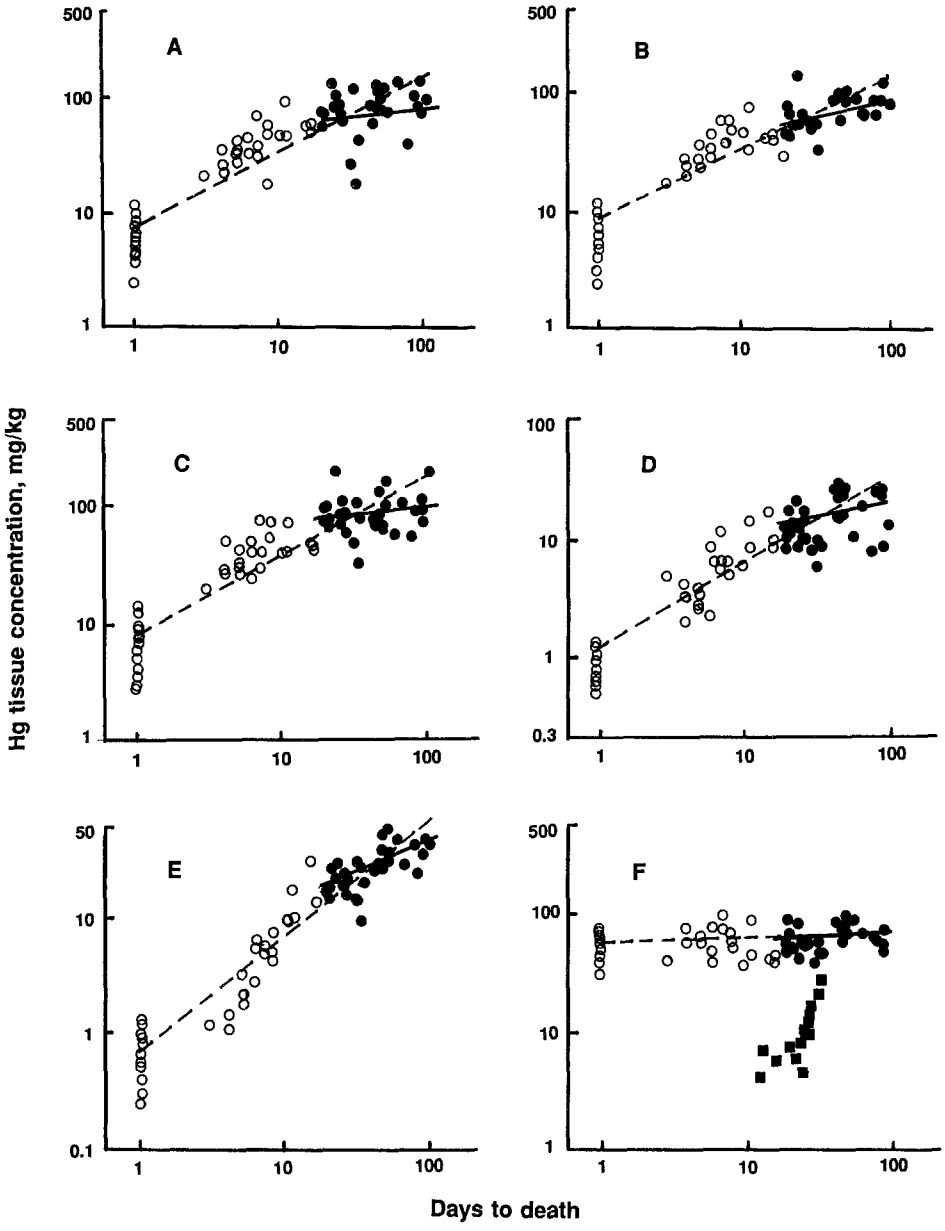

Fig. 3. Relationship between days to death and Hg tissue concentration in mg/kg among rainbow trout exposed to 4–34 μg/L CH_3HgCl for kidney (A), liver (B), spleen (C), brain (D), muscle (E), and gill (F). Open circles represent fish that died after 1–19 d exposure, and closed circles are fish that died after ≥20 d exposure. Regression analyses for the 80 fish that died after 1–98 d exposure for the six tissues examined, indicated by the broken line, are: log A = 0.851 + 0.643(±0.071)log X, r^2 = 0.81*; log B = 0.900 + 0.596(±0.006)log X, r^2 = 0.85*; log C = 0.874 + 0.682(±0.007)log X, r^2 = 0.87*; log D = 0.100 + 0.715(±0.062)log X, r^2 = 0.93*; log E = −0.184 + 0.991 (±0.062)log X, r^2 = 0.93*; log F = 1.729 − 0.029(±0.037)log X, r^2 = 0.03, where X is days to death and A–F the Hg concentration in mg/kg for the respective tissues. Regression analyses for the 35 fish that died after ≥20 d exposure, indicated by the solid line, are: log A = 1.581 + 0.160(±0.313)log X, r^2 = 0.03; log B = 1.285 + 0.334(±0.190)log X, r^2 = 0.28*; log C = 1.716 + 0.128(±0.258)log X, r^2 = 0.03; log D = 0.828 + 0.241(±0.276)log X, r^2 = 0.09; log E = 0.598 + 0.499(±0.220)log X, r^2 = 0.39*; log F = 1.638 + 0.092(±0.165)log X, r^2 = 0.03. Regression equations whose regression coefficient (±95% confidence limits) are significantly different from zero ($P \leq 0.05$) are identified with an asterisk. Values for fish exposed to 9 μg/L CH_3HgCl and analyzed on a whole body basis are shown in panel F as closed squares

studies on fish have reported Hg can impair the respiratory and osmoregulatory functions of the gill (Burton *et al.* 1972; Evans 1987). Mercury can also reduce monoamine and cholinesterase activities in neural tissues (Kirubagaran and Joy 1990; Shaw and Panigrahi 1990). Liver and kidney membrane transport is impaired by Hg through reduced acid and alkaline phosphatase activity (Hinton and Koening 1975; Lakshmi *et al.* 1991). Enzymes in liver and muscle associated with protein synthesis are also adversely affected in fish exposed to Hg (Nicholls *et al.* 1989). A number of other enzyme systems in fish including transferases, dehydrogenases, and other biochemical processes are also affected by Hg (Gill *et al.* 1990). These observations indicate application of the body burden concept for Hg to fish should be based on a whole body rather than a tissue basis

because of the difficulty in determining the specific cause of death. It could be difficult to establish which tissue, organ, or biochemical system was the cause of death, and whether the same system would again be the cause if the fish was exposed to a different Hg concentration. Hence, a lethal body rather than lethal tissue burden for Hg may be more suitable for aquatic organisms because of the large degree of uncertainty in identifying the critical tissue.

Lethal and Chronic Body Burden of Hg

The results of laboratory and field observations on Hg toxicity indicate a lethal body burden of 10–20 mg/kg Hg could be proposed for fish. Long-term laboratory studies have reported lethal concentrations on a whole fish basis in this range (Matida *et al.* 1971; McKim *et al.* 1976, present study). Naturally contaminated walleye fed Hg contaminated food in the laboratory that became adversely affected had 20–40 mg/kg Hg in muscle (Scherer *et al.* 1975). This estimate is also consistent with field observations where fish from Minamata Bay, Japan that were lethargic had 20 mg/kg Hg in muscle (Matida and Kumada 1969). A related study reported trout that receive an intraperitoneal injection equivalent to 15 mg/kg CH_3HgCl could die after 15 d (Hawryshyn and Mackay 1979). Other studies have reported no adverse effects among fish to CH_3HgCl with similar Hg concentrations. Trout that accumulated 10 mg/kg Hg were not adversely affected during a 84 d study (Lock 1975). No mortalities were reported for trout with 30 mg/kg Hg in muscle after 105 d exposure (Wobeser 1975).

An estimate of the chronic body burden for Hg could provide a useful index for assessing Hg concentrations monitored in organisms from different water bodies. Chemical concentrations in most aquatic ecosystems would likely represent a greater concern at the chronic rather than lethal levels to many organisms. Such an estimate should be determined at a general organism level at this time because of the limited information available to develop concentrations for specific taxonomic groups. A number of studies have reported the maximum allowable toxicant concentration (MATC) and no observable effect concentration (NOEL) for Hg among different organisms. A MATC for CH_3HgCl on brook trout was estimated to be 3 mg/kg Hg in muscle based on a 108 weeks life-cycle study (McKim *et al.* 1975). Fathead minnows (*Pimephales promelas*) exposed to CH_3HgCl for 48 weeks showed no observable effects on survival, behavior, growth, and appearance, even though whole fish concentrations ranged from 1.4 to 10.9 mg/kg (Olson *et al.* 1975). Another study reported a significant reduction in young *Daphnia magna* exposed to CH_3HgCl that contained 16 mg/kg Hg, but no impairment among those that contained 0.9 mg/kg Hg (Biesinger *et al.* 1982). The same study also exposed *Daphnia* to $HgCl_2$ and reported no impairment among animals with 8.6 mg/kg Hg. A MATC for reproductive impairment of 1.4 mg/kg Hg was reported for fathead minnows exposed to $HgCl_2$ for 41 weeks (Snarski and Olson 1982). Based on these observations, a chronic body burden of 1–5 mg/kg could be proposed as a threshold concentration for Hg in aquatic organisms where adverse effects may occur.

Use of Hg Body Burden for Assessment Purposes

This tissue-based approach could evaluate the toxicological significance of chemical concentrations that are observed in feral animals, an aspect not done in most hazard assessment protocols. Recent surveys of Hg indicate concentrations in fish can range from the low μg/kg to mg/kg range. Hg concentrations in muscle of cod (*Gadus morhua*) from the northwest Atlantic Ocean ranged from 0.01–0.2 mg/kg (Hellou *et al.* 1992). Three species of fish from the northeast Irish Sea had Hg concentrations of 0.1–2.3 mg/kg in muscle (Leah *et al.* 1991). Four invertebrate and fish species from the Tyrrhenian Sea had Hg concentrations in muscle of 0.1–3.2 mg/kg (Barghigiani and de Ranieri 1992). Based on the lethal and chronic body burdens for Hg estimated in this study, it could indicate some species from the Irish and Tyrrhenian Seas have attained Hg concentrations that could be of toxicological significance, while others would be below levels of concern.

A more specific application of this concept could be difficult because of a number of biological variables. Chemical measurements are often reported on muscle samples. The results of this study indicate the differences between muscle and whole fish Hg concentrations are small. Concentrations of some chemicals such as Hg can increase with the size of the organism which would require the critical body burdens be estimated at certain life stages (Westöö 1973; Barghigiani and Ranieri 1992). Lethal and chronic body burdens derived for chemicals should be based on the younger life stages of a species, or in mature fish if reproductive impairment is a concern. The early life stages are the most sensitive to toxicants in the life history of a species (Akiyama 1970). The large number of taxonomic groups found in an aquatic community would also limit the use of the concept to a general level of application; body burdens for some of the more relevant taxonomic groups could be derived as more information becomes available.

Comparisons between tissue residue levels and critical body burdens in feral organisms would be particularly useful in multichemical monitoring programs because they might identify the relative significance of each chemical from a toxicological perspective. This level of assessment is not being done at the present time. Nevertheless, further studies among a large variety of species would be required to demonstrate the contributions of a tissue-based approach to the assessment process. A compilation of the waterborne toxicity of $HgCl_2$ among nine taxonomic groups from bacteria to amphibians, representing 20 species, reported no effect concentration (NOEC) or nonlethal concentration (NOLC) that ranged from 0.006 to 0.46 mg/L (Sloof and Hermens 1983). The 48-h LC_{50} values for six groups from crustaceans to amphibians ranged from 0.005 to 1.05 mg/L. No Hg measurements were reported among the organisms to determine if the range of critical or lethal body burdens was less than the waterborne concentrations among the taxonomic groups.

References

Akiyama A (1970) Acute toxicity of two organic mercury compounds to the teleost, *Oryzias latipes*, in different stages of development. Bull Jpn Soc Sci Fish 36:563–570

Amend DF, Yasutake WT, Morgan R (1969) Some factors influencing susceptibility of rainbow trout to the acute toxicity of ethyl mercury phosphate formulation (Timsan). Trans Am Fish Soc 98:419–425

Barghigiani C, de Ranieri S (1992) Mercury content in different size classes of important edible species of the northern Tyrrhenian Sea. Mar Pollut Bull 24:114–116

Biesinger KE, Anderson LE, Eaton JG (1982) Chronic effects of inorganic and organic mercury on *Daphnia magna:* Toxicity, accumulation, and loss. Arch Environ Contam Toxicol 11:769–774

Boudou A, Ribeyre F (1983) Contamination of aquatic biocenoses by mercury compounds: an experimental ecotoxicological approach. In: Nriagu JO (ed) Aquatic toxicology. John Wiley & Sons, NY, pp 73–116

de Bruijn J, Yedema E, Seinen W, Hermens J (1991) Lethal body burdens of four organophosphorous pesticides in the guppy (*Poecilia reticulata*). Aquat Toxicol 20:111–122

Brungs WA, Leonard EN, McKim J (1973) Acute and long-term accumulation of copper by the brown bullhead, *Ictalurus nebulosus*. J Fish Res Board Can 30:583–586

Burton DT, Jones AH, Cairns J Jr (1972) Acute zinc toxicity to rainbow trout (*Salmo gairdneri*): confirmation of the hypothesis that death is related to tissue hypoxia. J Fish Res Board Can 29:1463–1466

Eaton JG (1974) Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Trans Amer Fish Soc 103:729–735

Eisenbud M (1973) Environmental radioactivity. Academic Press, NY

Environment Canada (1981) Analytical methods manual update, 1981. Water Quality Branch, Ottawa, Canada

Evans DH (1987) The fish gill: Site of action and model for toxic effects of environmental pollutants. Environ Health Perspect 71:47–58

Foulkes EC (1990) The concept of critical levels of toxic heavy metals in target tissues. Crit Rev Toxicol 20:327–340

Francesconi KA, Lenanton RC (1992) Mercury contamination in a semi-enclosed marine embayment: organic and inorganic mercury content of biota, and factors influencing mercury levels in fish. Mar Environ Res 33:189–212

Friant SL, Henry L (1985) Relationship between toxicity of certain organic compounds and their concentrations in tissues of aquatic organisms: a perspective. Chemosphere 14:1897–1907

Gill TS, Tewari H, Pande J (1990) Use of the fish enzyme system in monitoring water quality: Effects of mercury on tissue enzymes. Comp Biochem Physiol 97C:287–292

Gupta AK, Rajbanshi (1988) Acute toxicity of cadmium to *Channa punctatus* (Bloch). Acta Hydrochim Hydrobiol 16:525–535

Hamelink JL, Waybrant RC, Yant PR (1977) Mechanisms of bioaccumulation of mercury and chlorinated hydrocarbon pesticides by fish in lentic ecosystems. In: Suffet IH (ed) Fate of pollutants in the air and water environments, Vol 8, Part 2. John Wiley & Sons, NY, pp 261–281

Hamilton SJ, Buhl KJ (1990) Safety assessment of selected inorganic elements to fry of chinook salmon (*Oncorhynchus tshawytscha*). Ecotoxicol Environ Safety 20:307–324

Hamilton SJ, Merhle PM (1986) Metallothionein in fish: review of its importance in assessing stress from metal contaminants. Trans Am Fish Soc 115:596–609

Hattula ML, Sarkka J, Janatuinen J, Paasivirta J, Roos A (1978) Total mercury and methyl mercury contents in fish from Lake Paijanne. Environ Pollut 17:19–29

Hattula ML, Wasenius VM, Reunanen H, Arstila AU (1981) Acute toxicity of some chlorinated phenols, catechols and cresols to trout. Bull Environ Contam Toxicol 26:295–298

Hawryshyn CW, Mackay WC (1979) Toxicity and tissue uptake of methylmercury administered intraperitoneally to rainbow trout (*Salmo gairdneri* Richardson). Bull Environ Contam Toxicol 23:79–86

Hellou J, Warren WG, Payne JF, Belkhode S, Lobel P (1992) Heavy metals and other elements in three tissues of cod, *Gadus morhua* from the northwest Atlantic. Mar Pollut Bull 24:452–458

Hinton D, Koening JC (1975) Acid phosphatase activity in the subcellular fractions of fish liver exposed to methylmercury chloride. Comp Biochem Physiol 50:621–625

Hogstrand C, Haux C (1991) Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. Comp Biochem Physiol 100C:137–141

Huckabee JW, Janzen SA, Blaylock BG, Talmi Y, Beauchamp JJ (1978) Methylated mercury in brook trout (*Salvelinus fontinalis*): Absence of an *in vivo* methylating process. Trans Am Fish Soc 107:848–852

Kirubagaran R, Joy KP (1990) Changes in brain monoamine levels and monoamine oxidase activity in the catfish, *Clarias batrachus*, during chronic treatments with mercurials. Bull Environ Contam Toxicol 45:88–93

Knechtel JR, Fraser JL (1979) Wet digestion method for the determination of mercury in biological and environmental samples. Anal Chem 51:315–317

Kobayashi K, Kishino T (1980) Effect of pH on the toxicity and accumulation of pentachlorophenol in goldfish. Bull Jpn Soc Sci Fish 46:167–170

Kumagai H, Saeki K (1978) Contents of total mercury, alkyl mercury and methyl mercury in some coastal fish and shells. Bull Jpn Soc Sci Fish 44:807–811

Lakshmi R, Kundu R, Thomas E, Mansuri AP (1991) Mercuric chloride induced inhibition of acid and alkaline phosphatase activity in the kidney of mudskipper, *Boleophthalmus dentatus*. Acta Hydrochim Hydrobiol 19:341–344

Leah RT, Evans SJ, Johnson MS, Collings S (1991) Spatial patterns in accumulation of mercury by fish from the NE Irish Sea. Mar Pollut Bull 22:172–175

Lock RAC (1975) Uptake of methylmercury by aquatic organisms from water and food. In: Koeman JH, Strik JJTWA (eds) Sublethal effects of toxic chemicals on aquatic organisms. Elsevier Sci Publ Co, Amsterdam, pp 61–79

MacLeod JC, Pessah E (1973) Temperature effects on mercury accumulation, toxicity, and metabolic rate in rainbow trout (*Salmo gairdneri*). J Fish Res Board Can 30:485–492

Matida Y, Kumada H (1969) Distribution of mercury in water, bottom mud and aquatic organisms of Minamata Bay, the River Agano and other water bodies in Japan. Bull Freshw Fish Res Lab 19:73–90

Matida Y, Kumada H, Kimura S, Saiga Y, Nose T, Yokota M, Kawatsu H (1971) Toxicity of mercury compounds to aquatic organisms and accumulation of the compounds by the organisms. Bull Freshw Fish Res Lab 21:197–227

McKim JM, Olson GF, Holcombe GW, Hunt EP (1976) Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): Toxicity, accumulation, distribution, and elimination. J Fish Res Board Can 33:2726–2739

Mount DI (1964) An autopsy technique for zinc-caused fish mortality. Trans Am Fish Soc 93:174–182

Mount DI, Boyle HW (1969) Parathion—use of blood concentration to diagnose mortality of fish. Environ Sci Technol 3:1183–1185

Nicholls DM, Teichert-Kuliszewska K, Girgis GR (1989) Effect of chronic mercuric chloride exposure on liver and muscle enzymes in fish. Comp Biochem Physiol 94C:265–270

Oliver BG, Niimi AJ (1985) Bioconcentration factors of some halogenated organics for rainbow trout: Limitations in their use for prediction of environmental residues. Environ Sci Technol 19:842–849

Olson GF, Mount DI, Snarski VM, Thorslund TW (1975) Mercury residues in fathead minnows, *Pimephales promelas* Rafinesque, chronically exposed to methylmercury in water. Bull Environ Contam Toxicol 14:129–134

Pentreath RJ (1976) The accumulation of inorganic mercury from sea water by the plaice, *Pleuronectes platessa* L. J Exp Mar Biol Ecol 24:103–119

Phillips GR, Buhler DR (1978) The relative contributions of methylmercury from food or water to rainbow trout (*Salmo gairdneri*) in a controlled laboratory environment. Trans Am Fish Soc 107:853–861

Potter L, Kidd D, Standiford D (1975) Mercury level in Lake Powell, bioamplification of mercury in man-made desert reservoir. Environ Sci Technol 9:41–46

Reinert RE, Stone LJ, Willford WA (1974) Effect of temperature on accumulation of methylmercuric chloride and *p,p'* DDT by rainbow trout. J Fish Res Board Can 31:1649–1652

Renfro WC, Fowler SW, Heyraud M, La Rosa J (1975) Relative importance of food and water in long-term zinc^{-65} accumulation by marine biota. J Fish Res Board Can 32:1339–1345

Rhead MM, Perkins JM (1984) An evaluation of the relative importance of food and water as sources of *p,p'*-DDT to the goldfish, *Carassius auratus* (L). Water Res 18:719–725

Rodgers DW, Beamish FWH (1981) Uptake of waterborne methylmercury by rainbow trout (*Salmo gairdneri*) in relation to oxygen consumption and methylmercury concentration. Can J Fish Aquat Sci 38:1309–1315

Scherer E, Armstrong FAJ, Nowak SH (1975) Effects of mercury-contaminated diet upon walleyes, *Stizostedion vitreum vitreum* (Mitchell). Fish Mar Ser Tech Rpt, No 597, Ottawa, Canada, 21 pp

Shaw BP, Panigrahi AK (1990) Brain AChE activity studies in some fish species collected from a mercury contaminated estuary. Water Air Soil Pollut 53:327–334

Slooff W, Canton JH, Hermens JLM (1983) Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I. (Sub)acute toxicity tests. Aquat Toxicol 4:113–128

Snarski VM, Olson GJ (1982) Chronic toxicity and bioaccumulation of mercuric chloride in the fathead minnow (*Pimephales promelas*). Aquat Toxicol 2:143–156

Sorensen EMB (1976) Toxicity and accumulation of arsenic in green sunfish, *Lepomis cyanellus*, exposed to arsenate in water. Bull Environ Contam Toxicol 15:756–761

Sreedevi P, Suresh A, Sivaramakrishna B, Prabhavathi B, Radhakrishnaiah K (1992) Bioaccumulation of nickel in the organs of the freshwater fish, *Cyprinus carpio*, and the freshwater mussel, *Lamellidens marginalis*, under lethal and sublethal nickel stress. Chemosphere 24:29–36

van den Heuvel MR, McCarty LS, Lanno RP, Hickie BE, Dixon DG (1991) Effect of total body lipid on the toxicity and toxicokinetics of pentachlorophenol in rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 20:235–252

Westöö G (1973) Methylmercury as percentage of total mercury in flesh and viscera of salmon and sea trout of various ages. Science 181:567–568

Wobeser G (1975) Prolonged oral administration of methyl mercury chloride to rainbow trout (*Salmo gairdneri*) fingerlings. J Fish Res Board Can 32:2015–2023