Neves

RICHARD J. NEVES

Smith et al 1975

Uptake of Mercury by Freshwater Clams (Family Unionidae)

ANNETTE L. SMITH AND ROGER H. GREEN

Department of Zoology, University of Manitoba, Winnipeg, Man. R3T 2N2

AND ANDREW LUTZ

Department of the Environment, Fisheries and Marine Service, Freshwater Institute, Winnipeg, Man. R3T 2N6

SMITH, A. L., R. H. GREEN, AND A. LUTZ. 1975. Uptake of mercury by freshwater clams (family Unionidae). J. Fish. Res. Board Can. 32: 1297–1303.

Mercury concentrations were measured in water, sediments, and three species of clams from lakes with and without reported mercury contamination. Elevated mercury levels in clams were associated with elevated mercury levels in water and sediments.

Uncontaminated clams were exposed to three mercury compounds at 1.0, 10, 50, and 100 μg Hg/liter for up to 3 wk in the laboratory. The clams concentrated the metal in the order methylmercuric chloride > phenylmercuric acetate > mercuric chloride. The rate of uptake of mercury increased with increasing mercury concentration in the water. In most cases, temperature had no effect on the rate of mercury uptake or elimination.

The distribution among organs depended on the compound to which the clams had been exposed. Only methylmercuric chloride was concentrated extensively in foot muscle. Transfer of methylmercuric chloride among organs apparently continued after exposure to the compound had ended.

SMITH, A. L., R. H. GREEN, AND A. LUTZ. 1975. Uptake of mercury by freshwater clams (family Unionidae). J. Fish. Res. Board Can. 32: 1297–1303.

Les auteurs ont mesuré les concentrations de mercure dans l'eau, les sédiments et trois espèces de coques provenant de lacs où on avait signalé ou non une contamination au mercure. Les hauts niveaux de mercure dans les coques sont liés aux niveaux élevés de mercure dans l'eau et les sédiments.

Des coques non contaminées furent exposées à trois composés de mercure à des concentrations de 1.0, 10, 50 et 100 μg Hg/litre durant des périodes allant jusqu'à 3 semaines au laboratoire. Les coques assimilent le mercure dans l'ordre chlorure méthylmercurique > acétate phénylmercurique > chlorure mercurique. Le taux d'assimilation du mercure augmente en fonction de la concentration du mercure dans l'eau. Dans la plupart des cas, la température n'affecte pas le taux d'assimilation ou d'élimination du mercure.

La répartition dans les organes dépend du composé auquel la coque a été exposée. Seul le chlorure méthylmercurique se concentre à un haut degré dans le muscle du pied. Le transfert du chlorure méthylmercurique d'un organe à l'autre semble se poursuivre après la fin de l'exposition au composé.

Received January 31, 1974
Accepted March 11, 1975

Reçu le 31 janvier 1974
Accepté le 11 mars 1975

BIVALVES are noted for their ability to concentrate trace metals (Pringle et al. 1968). Fimreite et al. (1971) have found mercury levels of 0.93–3.59 μg/g in both freshwater and marine clams collected near pulp mills and chlor-alkali factories.

Few studies have dealt with conditions influencing rates of uptake and release of mercury by molluscs. Hannerz (1968) reported concentration factors for mercury of up to 3570 times in gastropods exposed to inorganic and organic mercury compounds. Irukayama et al. (1962) found greater accumulation of alkyl mercury compounds than of inorganic or other organic mercury compounds by *Venus japonica*. Although some authors (Irukayama et al. 1962; Yoshida et al. 1967) have recorded varying mercury levels in different organs of bivalves depending upon the mercury compound to which the animals were exposed, no papers have dealt with differences in patterns of uptake and release by specific organs.

We have attempted to determine the effects of temperature, type of mercury compound, and mercury concentration on the rates of uptake and release of this metal by freshwater clams, and have considered variations in mercury levels and uptake–release patterns in several organs. Although clams can obtain mercury both from water and from food, only uptake from water is considered here.

Printed in Canada (J3301)
Imprimé au Canada (J3301)

Materials and Methods

Sampling Procedures and Mercury Analyses

During the summers of 1970–72, *Anodonta grandis* were collected from several locations in Manitoba and northwestern Ontario, both with and without reported mercury contamination. In late summer 1971 and in 1972, sampling was concentrated in Minnedosa Lake, Man. (50°16′N, 99°48′W), and Clay Lake, Ont. (50°03′N, 93°30′W). Mercury levels in water, sediments, and the unionid clams *Lampsilis radiata* and *Lasmigona complanata*, in addition to *A. grandis* were determined.

In order to avoid loss of mercury, water samples were acidified in the field using 5 ml of concentrated H_2SO_4 for each 500 ml of water. Clams and sediments were frozen upon return to the laboratory.

Mercury analyses were performed on foot muscle, other individual organs, or whole clam. For whole clam determinations, soft tissues of individual clams were homogenized in a Waring blender, and samples of the homogenate analyzed. Frozen clams were dissected for analyses of separate organs. Mercury in clam tissues was determined by the wet digestion and flameless atomic absorption method of Armstrong and Uthe (1971).

Mercury was extracted from water samples by the dithizone method of Chau and Saitoh (1970). Sediments were prepared by Dow (1970) method. Aliquots of mercury extracts from water and sediments were used for mercury determination by flameless atomic absorption spectrophotometry (Armstrong and Uthe 1971).

For mercury uptake experiments, *A. grandis* from Mad Dog Lake, Ont., and Minnedosa Lake, Man., were acclimated to laboratory conditions for at least 2 wk and then placed in 30-liter aquaria containing dissolved mercury compounds. The water in each aquarium was changed daily and fresh mercury compound added, in order to assure that the mercury remained at the desired concentration. During mercury release experiments, the clams were held in constantly flowing clean water. Since the mercury concentration in the water might be altered by the formation and subsequent precipitation of mercury–food particle complexes, the clams were not fed during any of the experiments.

Laboratory Studies of Mercury Uptake and Release

The rates of uptake and release of mercury were first studied by exposing the clams to mercuric chloride ($HgCl_2$), phenylmercuric acetate (PMA), and methylmercuric chloride (MMC), at 10, 50, and 100 μg Hg/liter at 10 and 20 C for 2 wk. The clams were then allowed to release mercury at these temperatures for an additional 2 wk. One clam per treatment was sampled at days 1, 4, 7, and 14 of the uptake and release periods. Background mercury determinations were performed on 18 clams prior to any exposure. Mercury analyses for this experiment were performed on foot muscle.

When subsequent experiments suggested that uptake and release of MMC by muscle tissue differs from uptake and release by other organs, a modified version of the first experiment was performed using homogenized whole clam for mercury analysis. Clams were exposed to MMC at 1.0 and 10.0 μg Hg/liter for 3 wk at 12 or 20 C and allowed to release mercury for 4 wk. Four clams (one per analytical sample) were used to determine background mercury levels, and individuals were sampled at uptake days 1, 4, 7, 14, and 21 and release days 2, 7, 14, 21, and 28.

From these two experiments we attempted to build a mathematical model to relate mercury concentrations in clams to the mercury concentration in the water from which the animals were collected.

The distribution of mercury among organs was studied using contaminated clams from Clay Lake. Initial mercury levels were measured in the mantle, gill, liver, and adductor muscle of five clams and the foot of seven clams. After the animals had been allowed to release mercury at 20 C for 8 wk, mercury concentrations in the same tissues of four additional clams were measured.

An experiment was designed to compare uptake and release of the three mercury compounds in three organs. After background mercury levels in the gills, foot, and liver of six replicate individuals were determined, clams were exposed to $HgCl_2$, PMA, and MMC at 50 μg Hg/liter for 4 days at 20 C, with two individuals per treatment sampled at days 1 and 4. The remaining clams were allowed to excrete mercury for 1 wk, with samples taken at 2, 4, and 7 days.

Mercury uptake data were analyzed by factorial analysis of variance and split-plot analysis of variance. Since release of Hg was assumed to follow an exponential curve, release data were treated by covariance analysis as linear regression of log (Hg) against time.

Results

Mercury concentrations were about 10 times higher in *A. grandis* from Clay Lake than from other waters (Table 1). Clams from the Winnipeg River system (Dorothy and Nutimik lakes) did not contain higher mercury levels than animals from the remaining locations, although mercury contamination has been reported in the Winnipeg River (Bligh 1971). Water apparently must be severely polluted before mercury concentrations in clams become elevated.

Clams, water, and wet sediments from Minnedosa Lake showed concentrations of mercury only 5–12% of those measured in Clay Lake (Table 2). There were highly significant ($P < 0.005$) differences in mercury levels in whole clams between Minnedosa and Clay lakes. Differences among clam species were also highly significant, *A. grandis* concentrating less mercury than either *Lampsilis radiata* or *Lasmigona complanata*.

TABLE 1. Concentration of mercury in *Anodonta grandis* from various locations.

Location	Latitude	Longitude	Body part	Hg (μg/g)	No. specimens	SE
Red Rock L.	50°00′N	95°32′W	whole clam	<0.01	1	—
White L.	50°02′N	95°32′W	" "	<0.01	1	—
Betula L.	50°05′N	95°34′W	" "	<0.01	1	—
Mad Dog L.	49°36′N	93°48′W	foot	0.06	18	0.01
Minnedosa L.	50°16′N	99°48′W	foot	0.05	6	0.01
Minnedosa L.	"	"	gill	0.07	6	0.02
"	"	"	liver	0.06	6	<0.01
"	"	"	whole clam	0.01	6	<0.01
Nutimik L.	50°10′N	95°42′W	whole clam	0.01	1	—
Dorothy L.	50°11′N	95°45′W	" "	0.04	1	—
Dorothy L. narrows	50°11′N	95°45′W	liver	0.06	1	—
"	"	"	adductor	0.07	1	—
"	"	"	foot and visceral mass	0.07	1	—
Clay L.	50°03′N	93°30′W	mantle	0.47	5	0.03
"	"	"	gills	0.51	5	0.02
"	"	"	adductor	0.83	5	0.06
"	"	"	liver	0.78	5	0.07
"	"	"	foot	0.62	7	0.05
"	"	"	visceral mass	0.65	5	0.23
"	"	"	decalcified shell	0.08	4	0.03
"	"	"	whole clam	0.18	6	0.02

TABLE 2. Mercury concentrations of water, sediment, and clams from two lakes. Values are given as micrograms Hg per gram wet weight, except where noted. Values in parentheses are Hg levels expressed as a percentage of the mercury concentration of the same substance from Clay Lake.

Substance analyzed	Year	Lakes	
		Minnedosa	Clay
A. grandis (foot)	1971	0.05 (8.1%)	0.62
A. grandis (foot)	1972	—	0.40
A. grandis (homogenate)	1972	0.01 (5.6%)	0.18
L. radiata (foot)	1971	—	0.74
L. radiata (homogenate)	1972	0.03 (12.0%)	0.25
L. complanata (homogenate)	1972	0.03 (9.1%)	0.33
Water[a]	1972	0.01 (5.0%)	0.20
Sediment, wet	1972	0.01 (10.0%)	0.10
Sediment, dry	1972	0.04 (33.3%)	0.12

[a]Water (Hg) is given as μg Hg/liter.

UPTAKE AND RELEASE OF MERCURY

The mercury compound to which the clams were exposed was the most significant source of variation in uptake of mercury by clam foot muscle ($P < 0.01$). However, the concentration of mercury in the water was also significant ($P < 0.05$). Differences in temperature at which the experiment was run did not contribute significantly to differences in mercury uptake. There were no significant differences in slopes of the release curves among the treatments, and the pooled regression coefficient estimate of −0.037 per day was significantly different from zero. The test for differences in intercepts of the release curves, which is actually a test of mercury uptake, indicated ($P < 0.01$) that PMA is concentrated to a greater extent at 10 C than at 20 C. All other intercept tests were in agreement with the analyses of the uptake data.

Our model of uptake and release of mercury by clam foot muscle was similar to Ulfvarson's

(1962) model for mercury uptake and release in rats, except that his dosage constant was replaced by the uptake rate, which is a function of the mercury concentration in the water. The model with slight modifications fit the data, but the equation we obtained greatly underestimated the mercury levels in lakes to which the model was applied.

Both the length of exposure and the concentration significantly ($P < 0.01$) influenced uptake of MMC by whole clam, but temperature influence was not significant. Release rates were not significantly different from zero, nor did the slopes of the release curves differ with either temperature or concentration of mercury to which the clams had previously been exposed.

According to the Ulfvarson model, if the release rate of Hg were zero, then uptake would be described by a simple linear regression. This equation implies infinite capacity for uptake of mercury, which in practice would be modified if the number of binding sites for mercury in clam tissue approached saturation before the body burden of mercury proved fatal. For our data there was no significant deviation from a straight line regression. Thus, saturation of binding sites for mercury apparently was not approached during this experiment.

Since the release rate of mercury was negligible in this experiment, it was impossible to develop an equilibrium model of MMC uptake and release by whole clam.

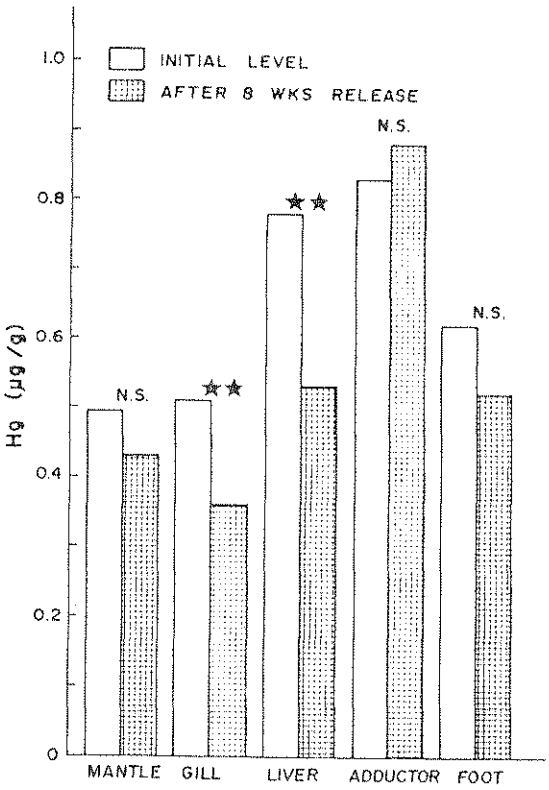

FIG. 1. Change in mean mercury concentration in several tissues of *Anodonta grandis* from Clay Lake after an 8-wk period in uncontaminated water.

DISTRIBUTION OF MERCURY AMONG ORGANS

Mercury was distributed unequally among the organs of *A. grandis* from Clay Lake. Significantly more mercury ($P < 0.01$) was concentrated per unit weight of adductor muscle, liver, and foot than of gill or mantle, while adductor also contained a significantly higher mercury concentration than did the visceral mass (Table 3).

TABLE 3. Distribution of mercury in the organs of *A. grandis* collected from Clay Lake. Means and standard errors of mercury concentrations are given as micrograms Hg per gram wet weight. The number of clams in each sample is given in parentheses.

	Year clams collected	
Organ	1971	1972
Mantle	0.47±0.01 (5)	—
Gill	0.51±0.01 (5)	—
Liver	0.78±0.02 (5)	0.47±0.07 (6)
Adductor	0.83±0.03 (5)	0.63±0.03 (6)
Foot	0.62±0.05 (7)	0.40±0.02 (14)
Visceral mass	0.65±0.08 (5)	—

In contaminated clams allowed to release mercury in the laboratory, elimination of the metal over the 8-wk period varied among tissues (Fig. 1). Although the mercury concentration of gill and liver decreased significantly ($P < 0.01$), change in mercury concentration of mantle, adductor muscle, and foot was not statistically significant.

The patterns of uptake and release of mercury by various tissues in the laboratory suggested a complex pattern of mercury transfer (Fig. 2). Among-organ differences in mercury uptake were highly significant ($P < 0.01$), with liver and gill concentrating more mercury per unit weight than foot muscle. During the 4-day exposure, the form in which the mercury was administered ($HgCl_2$, PMA, or MMC) did not significantly influence uptake.

The decrease in mercury concentration after transfer to clean water was highly dependent ($P < 0.01$) upon the compound to which the animals had been exposed, with the order of retention of

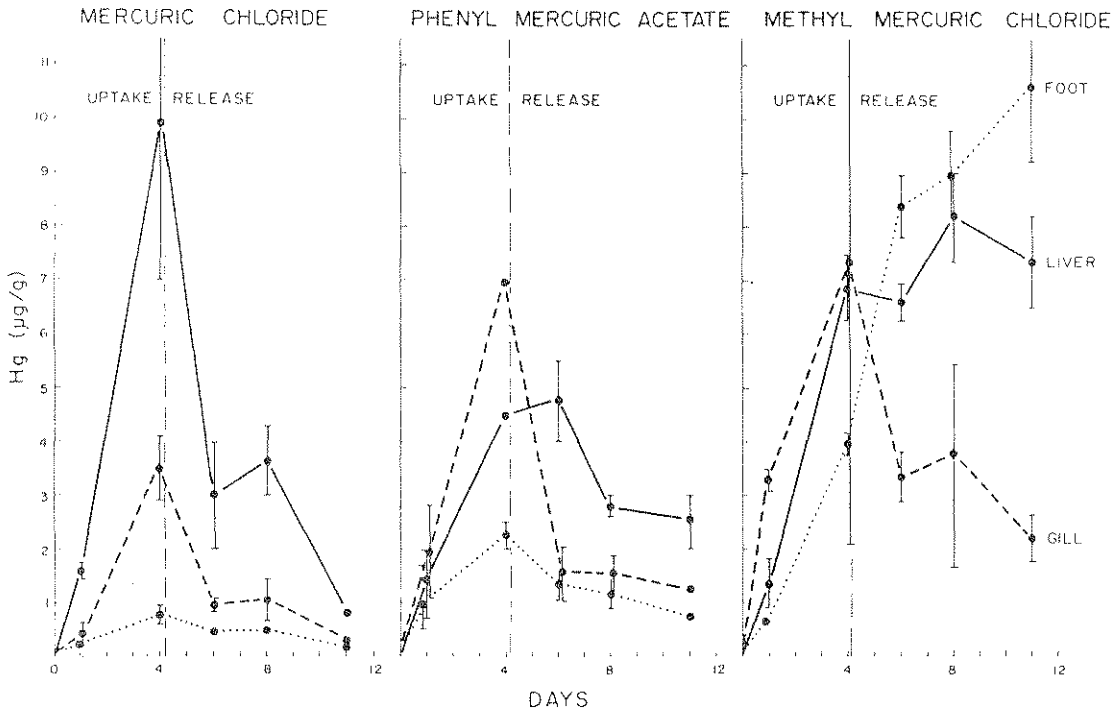

FIG. 2. Uptake and release of three mercury compounds by *A. grandis* gill, foot, and liver at 20 C. Mercury concentration in the water during the uptake portion of the experiment was 50 µg/liter. Bars represent standard errors.

compounds being MMC>PMA>$HgCl_2$. Individual organs also varied in their release of mercury. In general, gill lost the most mercury per unit weight and liver the least. A strong interaction ($P < 0.01$) between mercury compound and organ was present. Figure 2 suggests that this interaction is due to the rapid loss of $HgCl_2$ from the liver while MMC is retained by that organ, and to the increase in the concentration of MMC in foot muscle after termination of exposure.

The mercury concentration in clams at the end of the uptake experiment was significantly higher than that in clams after transfer to clean water for 2 days; but there was no significant change in mercury levels between day 2 and the end of the release experiment. Apparently there is a change in the slope of the release curve, with rapid loss of mercury during the first 2 days after exposure ends, followed by a period of much slower release or no release of the remaining mercury.

Discussion

Uptake and Release of Mercury

The concentration of mercury in clam tissue depends on the compound to which the animals have been exposed but two experiments suggest conflicting reasons for compound-dependent differences. The study involving only foot muscle suggests that each compound is taken up at a different rate, but that there is no significant difference in the rates at which different compounds are released. However, the experiment including several organs implies that there is no difference in the instantaneous rate of uptake but that long-term differences in the tissue concentrations of mercury are dependent upon the degree of retention of each compound.

The second of these conclusions seems the more reasonable since several researchers have found that in mammals methyl mercury is excreted more slowly than PMA (Gage 1964) or inorganic mercury (Berlin and Ullberg 1963; Takahashi and Hirayama 1971). The compound-dependent differences in mercury retention in different organs apparently are due to a rapid (48 h or less) loss of unbound mercury, followed by slower release of the remaining metal. Methyl mercury binds more tightly to tissues than inorganic forms (Brown and Kulkarni 1967), which accounts for the greater retention of that compound.

Compound-dependent differences in the release of unbound mercury from foot muscle may not have been detected because release was not measured until the clams had been in clean water for 24 h. Further experiments of several months' duration might demonstrate differences in the long-term release rates of the bound mercury compounds.

Pringle et al. (1968) have reported that temperature is closely related to uptake rate and ultimate concentration of a given metal. However, the results of our experiments do not support this observation. No significant differences in uptake rates or concentrations attained were observed at different temperatures, except that PMA was concentrated in foot muscle to a greater extent at 10 C than at 20 C.

Lack of temperature dependence of uptake rates suggests that uptake is by diffusion followed by the formation of stable complexes within the animals, a process considered likely by Craig (1967). The other possible mechanism of concentration, active uptake, is linked with the metabolic activities of the cells and has a higher temperature coefficient than simple diffusion (Pringle et al. 1968). Accumulation of mercury apparently depends on the strength and degree of binding, which is consistent with the formation of stable complexes.

The higher uptake of PMA by foot muscle at 10 C was unexpected and is difficult to explain. It may represent organ-specific temperature dependence. Vernberg and O'Hara (1972) have shown that the gills of *Uca pugilator* concentrate mercury to a greater extent at low temperature than at high temperature because the animal is better able to transfer mercury from the gills at higher temperature. Although no temperature-dependent differences in the release of bound PMA were observed, the higher concentration of mercury in foot muscle at 10 C may represent diminished ability to transport the unbound mercury compound from that organ.

Variations in the release rates at different temperatures were expected and have been reported in fish (Jarvenpaa et al. 1970). We could not demonstrate these differences, but if the half-life of bound mercury in clams is on the order of several hundred days, as the MMC data suggest, temperature-dependent differences would be difficult to detect in experiments of 14–28 days' duration.

Distribution of Mercury Among Organs

High initial accumulation of mercury in the gills of clams exposed to mercury in the laboratory suggests that some uptake occurs across these organs. Diffusion of mercury from the water across the gills has been reported in several aquatic organisms (Corner 1959; Jernelov and Lann 1971; Jones et al. 1972). Korringa (1952) found that positive polyvalent ions adhere to the mucous feeding sheets of oysters. Since the mucous sheets of *A. grandis* pass across the gills, some of the mercury concentration measured in the gills might be attributed to contaminated mucus.

Methylated mercury is metabolized by the liver of rats (Takahashi and Hirayama 1971). The sustained high level of MMC in the liver of *A. grandis* suggests similar importance of clam liver in the metabolism of MMC.

Only small amounts of $HgCl_2$ and PMA were accumulated in foot muscle during the 1-wk exposure, and these apparently were excreted in clean water. However, the mercury concentration in the foot muscles of animals exposed to MMC continued to increase after exposure had ended. A similar situation is suggested by Jernelov and Lann (1971), who report that methyl mercury, but not inorganic mercury, is slowly eliminated from liver and subsequently accumulates in muscle. They explain that any change in the relation between accumulation and excretion is observed in the liver before it is seen in the muscle due to the higher metabolic rate of liver. Apparently there is a complicated system of transfer of methylated mercury in and out of the organism. However, in our study it is also possible that, because the clams were not fed, loss of weight contributed to the observed increase in mercury concentration.

Acknowledgments

We would like to express our appreciation to F. A. J. Armstrong for advice, and for critically reviewing this manuscript in an earlier draft. This research was supported by a grant from the Department of the Environment.

Armstrong, F. A. J., and J. F. Uthe. 1971. Semi-automated determination of mercury in animal tissue. At. Absorpt. Newsl. 10: 101–103.

Berlin, M., and S. Ullberg. 1963. Accumulation and retention of mercury in the mouse. III. An autoradiographic comparison of methylmercuric dicyandiamide with inorganic mercury. Arch. Environ. Health 6: 610–616.

Bligh, E. G. 1971. Mercury in Canadian fish. Pacific Fisheries Technologists Conference, Victoria, B.C. March 22–24, 1971.

Brown, J. R., and M. V. Kulkarni. 1967. A review of the toxicity and metabolism of mercury and its compounds. Med. Serv. J. Can. 23: 786–808.

Chau, Y. K., and H. Saitoh. 1970. Determination of

submicrogram quantities of mercury in lake waters. Environ. Sci. Technol. 4: 839–841.

CORNER, E. D. S. 1959. The poisoning of *Maia squinado* (Herbst) by certain compounds of mercury. Biochem. Pharmacol. 2: 121–132.

CRAIG, S. 1967. Toxic ions in bivalves. J. Am. Osteopath. Assoc. 66: 1000–1002.

DOW CHEMICAL COMPANY. 1970. Dow–Sarnia Analytical Laboratory Report Method, CAS-AM-70. 10.

FIMREITE, N., W. N. HOLSWORTH, J. A. KEITH, P. A. PEARCE, AND I. M. GRUCHY. 1971. Mercury in fish and fish-eating birds near sites of industrial contamination in Canada. Can. Field-Nat. 85: 211–220.

GAGE, J. C. 1964. Distribution and excretion of methyl and phenyl mercury salts. Brit. J. Ind. Med. 21: 197–202.

HANNERZ, L. 1968. Experimental investigations on the accumulation of mercury in water organisms. Rep. Inst. Freshwater Res. Drottningholm 48: 120–176.

IRUKAYAMA, K., M. FUJIKI, F. KAI, AND T. KONDO. 1962. Studies on the origin and causative agent of Minamata disease. II. Comparison of the mercury compound in the shellfish from Minamata Bay with mercury compounds experimentally accumulated in control shellfish. Kumamoto Med. J. 15: 1–12.

JARVENPAA, T., M. TILLANDER, AND J. K. MIETTINEN. 1970. Methylmercury: half-time of elimination in flounder, pike, and eel. Suom. Kemistil. B. 43: 439–442.

JERNELOV, A., AND H. LANN. 1971. Mercury accumulation in food chains. Oikos 22: 403–406.

JONES, A. M., Y. JONES, AND W. D. P. STEWART. 1972. Mercury in marine organisms of the Tay region. Nature 238: 164–165.

KORRINGA, P. 1952. Recent advances in oyster biology. Q. Rev. Biol. 27: 266–308.

PRINGLE, B. H., D. E. HISSONG, E. L. KATZ, AND S. T. MULAWKA. 1968. Trace metal accumulation by estuarine mollusks. Am. Soc. Civil Eng. Proc. J. Sanitary Eng. Div. 94(SA3), Paper 5970: 455–475.

TAKAHASHI, H., AND K. HIRAYAMA. 1971. Accelerated elimination of methyl mercury from animals. Nature 232: 201–202.

ULFVARSON, U. 1962. Distribution and excretion of some mercury compounds after long term exposure. Int. Arch. Gewerbepath. Gewerbehyg. 19: 412–422.

VERNBERG, W. B., AND J. O'HARA. 1972. Temperature-salinity stress and mercury uptake in the fiddler crab, *Uca pugilator*. J. Fish. Res. Board Canada 29: 1491–1494.

YOSHIDA, T., T. KAWABATA, AND Y. MATSUE. 1967. Transference mechanism of mercury in marine environments. Tokyo Univ. Fish. 53: 73–84.