THE UNIVERSITY OF MANITOBA

UPTAKE OF MERCURY BY FRESHWATER CLAMS (FAMILY UNIONIDAE)

by

ANNETTE L. SMITH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

May 1973

ABSTRACT

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 various concentrations and temperatures 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 half-time of elimination for all three mercury compounds was 18.9 days, although the half-time of elimination for methylmercuric chloride may be much longer.

Two models related the mercury concentration in clam tissues to mercury levels in water. Model 1 was based on the uptake and release of all three mercury compounds by foot muscle, and Model 2 was based on the uptake and release of methylmercuric chloride by whole clam. Although Model 2 provided the better estimate of environmental mercury levels, both models underestimated those levels.

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.

Exposure to methylmercuric chloride at 0.01 mg Hg/l. depressed respiration rates in clams by 0.108 mg O_2 consumed/gram dry weight/hour.

ACKNOWLEDGEMENTS

I am grateful to my supervisor Dr. R. H. Green for his invaluable suggestions throughout this study, and to Dr. J. G. Eales and Mr. F. A. J. Armstrong for reading the thesis and offering constructive criticism.

The study could not have been completed without the help of A. Lutz and C. Tam, who performed mercury analyses.

Field assistance by P. Smith, B. DeMarch, G. Vascotto, E. Gyselman, B. Seghers and D. Hunka was most appreciated.

This research was financed by a grant to Dr. R. H. Green from the Fisheries Research Board of Canada.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

INTRODUCTION

Contamination of the natural environment by mercury has been recognized as a real and potential health hazard since the 1953-1957 poisoning, in Minamata, Japan, of over one hundred people who had ingested fish and shellfish containing methyl mercury (Kurland et al., 1960). Widespread contamination has more recently been reported in Scandinavia. Borg et al. (1966) observed elevated mercury levels and nerve damage suggestive of mercury poisoning in birds, foxes, polecats and martins found dead in Sweden. Feathers of Swedish birds collected since 1940, when the use of alkyl mercury seed dressings was initiated, revealed an increase in mercury content of ten to twenty times over mercury concentrations in birds collected during the previous hundred years (Berg et al., 1966; Johnels and Westermark, 1969). Swedish freshwater fish have been found to contain 0.4 - 5.0 mg Hg/kg wet weight (all Hg values will be given as wet weight unless otherwise noted) and measurements as high as 10.0 mg/kg have been recorded (Johnels et al., 1967; Westoo, 1969). In Norway, salmonids collected below a pulp mill which used phenylmercuric acetate contained up to 7.38 mg/kg mercury (Underdal and Hastein, 1971). These samples show considerable enrichment in

mercury over background levels of 0.028 to 0.18 mg/kg recorded in European freshwater fish (Stock and Cucuel, 1934).

The possibility of a mercury problem in North America was suggested by Fimreite (1970), who pointed out uses of mercury which could result in pollution. Fimreite et al. (1970) observed higher mercury levels in seed-eating and predatory birds from Alberta where mercury seed dressings are frequently used, than in similar birds from Saskatchewan where such seed dressings are less common. Wobeser et al. (1970) provided the first report of elevated mercury levels in Canadian fish, noting that fish from the Saskatchewan River contained more than 1 mg/kg mercury, which corresponded to the levels reported in Swedish fish from areas of industrial contamination. Subsequently, mercury levels above the 0.5 mg/kg legal limit for human consumption established by the Canadian government, and ranging as high as 12.13 mg/kg have been reported in freshwater fish from the Winnipeg River and English-Wabigoon River systems of Manitoba and Northwestern Ontario (Bligh, 1971), the Great Lakes, Ottawa River, and St. Clair River systems (Seagram, 1970; Uthe and Bligh, 1971; Fimreite et al., 1971), and in some marine invertebrates, fish and sharks from the British Columbia and New Brunswick coasts (Bligh, 1971; Fimreite et al., 1971; Forrester et al., 1972). Elevated mercury levels have also been measured in Lake Erie sediments,

plankton and algae (Pillay et al., 1972). Evans and his colleagues (1972) have suggested that pollution of freshwater by mercury has been a recent occurrence. They observed that seventy per cent of preserved fish collected in the St. Clair and Detroit River systems and the Great Lakes between 1920 and 1965 contained less than 0.20 mg Hg/kg (approximately the range reported by Stock and Cucuel, 1934), while the increase in mercury content of these organisms between 1964 and 1970 appears to be related to mercury losses from chlor-alkali factories.

In order to evaluate the extent of mercury contamination in freshwater it seems useful to choose one or more indicator organisms in which mercury levels can be used as an index of environmental pollution. Johnels et al. (1967) have used pike (Esox lucius) as an indicator species and related mercury concentration in its axial muscle to known mercury-containing effluents and areas of industrialization. They selected pike because of "(1) stationary habits, which provide definite geographic information; (2) life span of several years which will serve to integrate temporary variations in the occurrence of accumulative substances in the environment; and (3) wide distribution which permits comparative studies over extensive geographical areas."

The unionid clams Anodonta grandis and Lampsilis radiata meet the criteria of suitable indicator organisms stated by Johnels and his colleagues. Several species of

pelecypods have already been suggested as indicators of hydrocarbon pollutants (Butler, 1969; Lee et al., 1972). In addition, bivalves have long been noted for their ability to concentrate trace elements. Pringle et al. (1968) asserted that "shellfish are unique in their ability to selectively concentrate materials within their environment." In a study of twelve trace elements Brooks and Rumsby (1965) found metal ions enriched in bivalves as compared with the marine environment. In several instances bivalves have been shown to concentrate mercury. Irukayama et al. (1961, 1962) demonstrated that the shellfish of Minamata Bay contained an organic mercury compound. Dry weight mercury concentrations from five to 102 mg/kg (Kurland et al., 1960; Matida and Kumada, 1969) and wet weight concentrations from 11 to 17 mg/kg (Yoshida et al., 1967; Seagram, 1970) have been measured in the soft tissues of bivalves from Minamata Bay. Average mercury levels greater than 0.8 mg/kg in Spisula sp. and greater than 2.0 mg/kg in Venus mercenaria from Woods Hole have been recorded (Craig, 1967), while lower mercury levels (0.0031 - 0.19 mg/kg) have been found in oysters from a region of Chesapeake Bay considered subject to contamination (Birkner et al., 1972). Fimreite et al. (1971) found that Mya arenaria taken below a pulpmill using mercury slimicides and a chlor-alkali plant contained 0.93 and 3.59 mg Hg/kg respectively, while Margaritifera margaritifera collected upstream from the pulpmill and Mytilus edulis

obtained 11 km away from the chlor-alkali plant contained only 0.08 and 0.11 mg/kg respectively. High mercury levels have also been measured in Mytilus edulis from the Tay River estuary (Jones et al., 1972).

While mercury levels have been measured in bivalves from several areas, few studies have dealt with conditions influencing rates of uptake and release of mercury by molluscs. Hannerz (1968) has reported concentration factors for mercury in the gastropods Lymnaea stagnalis, Physa fontinalis, and Planorbis sp. held in ponds containing several organic and inorganic mercury compounds. Irukayama et al. (1962) found greater accumulation of alkyl mercury compounds than of inorganic or other organic mercury compounds by the clam Venus japonica. Jones et al. (1972) reported rapid accumulation and loss of mercury by the gills of Mytilus edulis over a nine-day period, and Yoshida et al. (1967) found different rates of release of mercury by clams depending upon the mercury compound to which they had been exposed and whether uptake occurred from water or from food. Although several 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 mercury uptake and release by specific organs.

Therefore, in the following series of experiments, I

attempt to determine the effects of temperature, mercury compound and mercury concentration on the rates of uptake and release of this metal by freshwater clams and to use this information to develop a mathematical model which can be used to predict the concentration of mercury in water from which contaminated clams are collected. I also consider variations in mercury levels and uptake-release patterns of several organs. Although clams can obtain mercury both from water and from their food, only uptake from the water is considered here.

Since populations of clams may be found in highly contaminated lakes, it also seems worthwhile to determine whether exposure to sublethal quantities of mercury can affect the physiology of these animals.

MATERIALS AND METHODS

1. Field Mercury Levels in Clams, Water and Sediments

During the summer of 1971, Anodonta grandis were collected from Mad Dog Lake (49°36'N, 93°48'W) and from Minnedosa Lake on the Minnedosa River (50°16'N, 99°48'W). No mercury contamination in either of these lakes has been recorded. Both A. grandis and Lampsilis radiata were collected from Clay Lake on the English-Wabigoon River system (50°03'N, 93°30'W), where excessive mercury concentrations in fish have been reported (Bligh, 1971). A. grandis and L. radiata as well as Lasmigona complanata were again collected from Minnedosa and Clay Lakes in 1972. Water and sediment samples from both lakes were taken during the 1972 collections.

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 homogenized whole clam. Homogenates were prepared by liquefying thawed clams (excluding shells) in a Waring commercial blendor.

The wet weights (excluding shells) and lengths of

twelve of the A. grandis collected from Clay Lake in 1972 were recorded for determination of the relationship between clam size and mercury content.

2. Analytical Techniques

Clam tissues were prepared for mercury analysis by wet digestion in hot nitric and sulfuric acids and oxidation with $KMnO_4$ (Armstrong and Uthe, 1971). Mercury was extracted from water samples with dithizone according to the method of Chau and Saitoh (1970). Sediments were prepared by boiling a 5-g sample for one minute in 10 ml of aquaregia (3:1 hydrochloric:nitric acid). The cooled extract was transferred to a volumetric flask and made up to 50 ml with distilled water. Aliquots of tissue, water and sediment mercury extracts were transferred to autoanalyzer cups for mercury determination by flameless atomic absorption spectrophotometry (Armstrong and Uthe, 1971). All mercury analyses were performed at the Freshwater Institute of the Fisheries Research Board, Winnipeg. The limits of sensitivity for these tests are 0.01 mg/kg for tissue and 0.02 µg/l for water mercury levels.

3. Factors Affecting Uptake and Release of Mercury by Clams

Factors influencing uptake and release of mercury were studied in a factorial experiment in the laboratory. Eighteen 30-liter aquaria contained mercuric chloride,

phenylmercuric acetate (PMA) and methylmercuric chloride (MMC) at 0.01, 0.05 and 0.10 mg Hg/l. The organic mercurials were dissolved in 40% acetone prior to addition to the aquaria. (Thus, 0.07 to 0.12 ml of acetone were added to each aquarium depending on the concentration of mercury and the compound involved.) Nine aquaria (representing each compound at each concentration) were held at 10 C and nine at 20 C.

Uncontaminated A. grandis from Mad Dog Lake were acclimated at these temperatures for three weeks prior to experimentation. Eighteen clams were sacrificed at the beginning of the experiment for background mercury level determinations, and thirteen clams were added to each aquarium to be exposed to the mercurials for two weeks, with individuals sacrificed for mercury determinations at one, four, seven and fourteen days. Since initial determinations had revealed that the mercury concentration of the water in aquaria containing clams dropped by an order of magnitude in two days (Table 1), the water in each aquarium was changed daily, and mercury was added again to insure that mercury concentrations remained at the desired levels throughout the experiment. On the fourteenth day the surviving clams were transferred to continuously flowing laboratory water at 10 C or 20 C. Complete turnover of water in the aquaria occurred in twenty minutes. The clams were held for an additional two weeks with individuals

TABLE 1. Daily changes in mercury concentration in water in a 30-liter aquarium containing 15 Lampsilis radiata at 20 C.

Day	Hg (mg/l)
0	0.50
1	0.31
2	0.03
3	0.01
4	0.01

sacrificed by freezing at one, four, seven and fourteen days. Mercury determinations for this experiment were performed on foot muscle to allow comparisons with fish in which muscle tissue is generally used for mercury analyses.

Since the mercury concentration in the water might be altered by the formation of mercury-food particle complexes, the clams were not fed during this or any of the subsequent experiments.

When subsequent experiments suggested that uptake and release of MMC by foot and adductor muscle differs from uptake and release by other organs, a modified version of the first experiment was performed using homogenized whole clams for mercury analyses. Following three weeks acclimation at 12 C or 20 C, uncontaminated clams from Minnedosa Lake were exposed to MMC at 0.001 or 0.01 mg Hg/l for up to three weeks at these temperatures. Individuals exposed to each temperature-mercury concentration combination were sacrificed at one, four, seven, fourteen and twenty-one days. Four clams were sacrificed prior to any mercury exposure. After three weeks, surviving clams were held for four weeks in continuously flowing water at 12 C or 20 C, and individuals were sacrificed by freezing at two days and one, two, three and four weeks.

4. Uptake and Release of Mercury by Various Organs

Contaminated A. grandis from Clay Lake were returned

to the laboratory for a study of the pattern of mercury release. Initial mercury levels of five clams were measured in mantle, gill, liver and adductor muscle. After the animals had been held in continuously flowing laboratory water at 20 C for eight weeks, mercury concentrations in the same tissues of four additional clams were measured. Initial mercury concentrations in the foot of seven clams were determined. Mercury in foot muscle from single clams was measured at two-week intervals throughout the eight-week release period.

When the preliminary observation of mercury level change in foot muscle revealed that more than simple log-linear release was involved, the experiment was repeated using a second group of contaminated A. grandis from Clay Lake. In order that changes in mercury levels of other organs could more clearly be related to changes in the mercury concentration of foot muscle, initial mercury determinations were performed on adductor, foot, liver and homogenate of the remaining soft tissues of two individuals. The animals were held at 20 C in continuously flowing water for up to six weeks. Two individuals were sacrificed by freezing at day four and at weekly intervals thereafter. Foot and liver were dissected from frozen clams, and a homogenate of the remaining soft tissue was prepared. In addition, adductor muscles from the one-,three-and six-week samples were removed for separate analysis. All tissues

were weighed so that the total wet weight mercury concentration of each clam could be calculated.

An experiment was designed to compare uptake and release of different mercury compounds in various organs. Aquaria containing $HgCl_2$, PMA and MMC at 0.05 mg Hg/l were prepared as previously described and held at 20 C. Clams had been acclimated at this temperature for two weeks prior to the experiment. Six clams were frozen for background mercury level determinations at Day 0. The animals were then allowed to take up mercury for four days, with two individuals from each aquarium sacrificed by freezing at days one and four. Subsequently, the remaining clams were alloweed to excrete mercury in continuously flowing water for one week, with two replicate clams sacrificed at two, four and seven days. Gills, foot and liver were dissected from the frozen clams for mercury determinations.

5. Sublethal Effects of Mercury

Possible sublethal effects of mercury on A. grandis were studied using heart and respiration rates as indicators of the general physiological condition of the animals.

Holes were filed in the shells of uncontaminated clams from Minnedosa Lake directly over the pericardium so that heart beats could be observed. Initially, the holes were covered with transparent polyethlene strips, but when shell regeneration began to obscure the hearts, these strips

were removed. The decision to remove the coverings was based on Wilbur's (1964) observation that most molluscs are unable to repair holes in their shells unless such holes are first covered. After the clams had been allowed to recover for two weeks, shells were marked with nail polish to identify individuals, and heart rates were determined. Individual clams were placed in 24-fl-oz refrigerator jars of clean water to facilitate observation, and the time required for ten heart beats was measured and used to calculate the number of beats per minute. Three observations per clam were made at ten minute intervals to provide an average heart rate. Individuals with extremely slow or irregular heart rates were eliminated, and the 24 clams remaining were evenly divided among a control tank and experimental aquaria containing MMC at 0.001 and 0.01 mg Hg/l. Since the MMC stock solution had been made with 40% acetone, a volume of acetone equal to that added to the experimental aquaria (0.06 ml) was added to the control tank. Clams were held for one week. The water was changed daily with fresh mercury and acetone added. After seven days, surviving clams were transferred to clean water, and heart rates were measured immediately.

Another thirty clams of approximately equal size were used for determination of respiration rates before and after exposure to mercury. These animals were treated in the manner previously described, except that no holes were

filed in the shells. Respiration rates were calculated by placing the clams in 24-fl-oz refrigerator jars filled with clean water of known oxygen concentration and sealing the jars with parafilm and caps to exclude air. After approximately an hour the jars were reopened, and the change in oxygen content of the water was determined. All measurements were made with a YSI Model 54 oxygen meter. At the termination of the experiment the clams were sacrificed, and dry weights of the soft tissues were determined, so that respiration rates could be expressed as mg of oxygen consumed per gram of clam per hour.

The animals in this series of experiments were acclimated at 20 C for four weeks and all heart and respiration rate measurements were made at that temperature.

RESULTS

1. Field Data

Clams, water and wet sediments from Mad Dog and Minnedosa Lakes showed concentrations of mercury only 5 - 12% of those measured in Clay Lake (Table 2). Analysis of variance performed on data obtained from homogenates of Anodonta grandis, Lampsilis radiata, and Lasmigona complanata from Minnedosa and Clay Lakes supported this observation, revealing highly significant ($P < 0.005$) differences in mercury levels due to lake sampled. Differences due to clam species were also highly significant, A. grandis concentrating less mercury than either Lampsilis radiata or Lasmigona complanata.

The mercury level of 0.01 µg/l found in Minnedosa Lake water is within the range of 0.01 - 0.05 µg/l mercury given by Dall'Aglio (1968) for waters uninfluenced by mineralization or contamination, while the 0.2 µg/l measured in Clay Lake is slightly higher than the 0.1 µg/l found by Stock and Cucuel (1934) in the Rhine River near industrial effluents.

The sediment mercury levels measured in Clay Lake are low compared with the values reported by Armstrong and Hamilton (1973) for that lake. However, they found a

TABLE 2. Mercury concentrations of water, sediment and clams from three lakes. Values are given as mg Hg/kg wet weight, except where noted.

Substance analyzed	Year	Lakes		
		Mad Dog	Minnedosa	Clay
A. grandis (foot)	1971	0.06(9.7%)[1]	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[2]	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

[1]Values in parentheses are Hg levels expressed as a percentage of the mercury concentration of the same substance from Clay Lake.

[2]Water [Hg] is given as µg Hg/l.

significant correlation between mercury concentration and clay content of the sediments. The sediments sampled for the analyses reported here contained a high proportion of sand, which may account for the lower mercury levels.

The mercury content of A. grandis from Clay Lake decreased significantly ($P < 0.025$) from 1971 to 1972 (Table 3). However, since the two groups of animals were collected from slightly different locations in the lake, this decrease is difficult to interpret.

No significant correlation between mercury content of foot muscle and length or weight of A. grandis was observed (Figure 1).

2. Factors Affecting Uptake and Release of Mercury by Clams

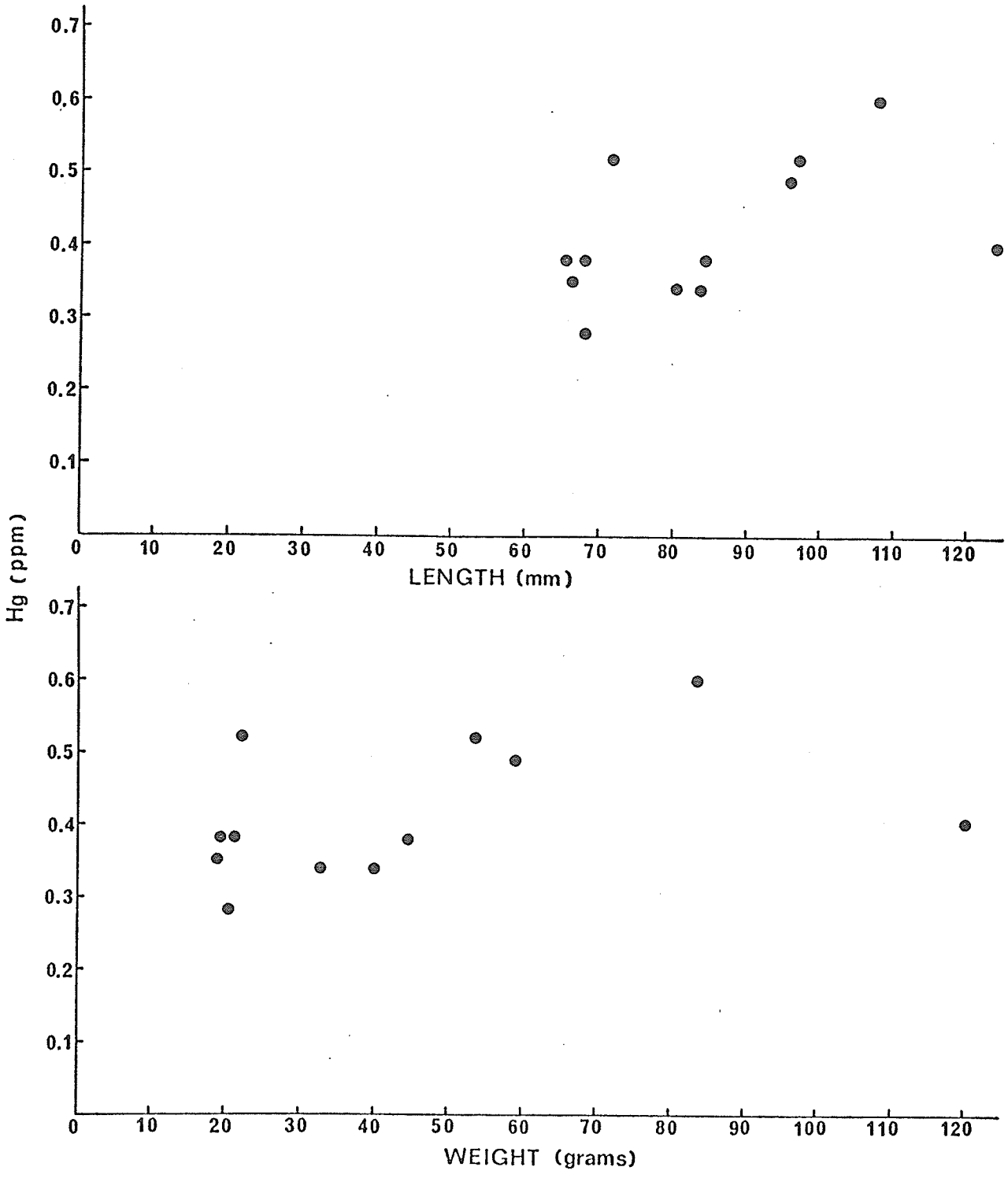
Factorial analysis of variance showed the mercury compound used to be the most significant source of variation in uptake of mercury by clam foot ($P < 0.01$), with the concentration of mercury in the water also being significant ($P < 0.05$). Differences in temperature at which the experiment was run did not contribute significantly to differences in mercury uptake, nor were any of the interactions statistically significant (Appendix I).

If there is only one mechanism of release, mercury loss should be represented by a straight line on a semi-log scale. Thus, the release data for all treatment combinations

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 mg Hg/kg wet weight. The number of clams in each sample is given in parentheses.

Organ	Year Clams Collected	
	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)	--

FIGURE 1. The relationship between mercury concentration in the foot muscle of Anodonta grandis from Clay Lake and the wet weight and length of the animal.


Hg (ppm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
70
80
90
100
110
120
LENGTH (mm)
WEIGHT (grams)

were analyzed as linear regressions of log mercury concentration in the foot muscle against time. Covariance analysis revealed no significant differences in slope ($P = 0.14$) and yielded a pooled regression coefficient estimate of -0.037 which was significantly ($P < 0.01$) different from zero. However, separate covariance analyses of the data for the three compounds suggest that MMC may not have any significant release rate over the time period considered. Since overall differences in regression coefficients were shown to be non-significant, factorial analysis of covariance was used to test for differences in intercepts. Mercury compound, concentration and temperature, as well as the compound-concentration and compound-temperature interactions all proved highly significant ($P < 0.01$). Since differences in intercept imply differences in extent of mercury uptake over the entire 14-day uptake experiment, these results are in agreement with the uptake data for compound and concentration, but not for temperature or the interactions.

The initial mathematical model based on these data was that the rate of change of mercury concentration in the animal (C_A) is equal to the rate of uptake, which is proportional to the theoretical concentration in the water (C_W), minus the release rate, which is proportional to the concentration in the animal. In differential form this is expressed

$$\frac{dC_A}{dt} = q\ C_W - r\ C_A \qquad (1)$$

and in integrated form, it is

$$C_A = \frac{q}{r}\ C_W\ (1 - e^{-rt}). \qquad (2)$$

Since the null hypothesis that the release rates of all three mercury compounds are equal could not be rejected by covariance analysis, the pooled regression coefficient was used as the instantaneous release rate, r. For all three compounds r = 0.037 per day. Initially it was assumed that the uptake rate would be constant for a given compound. However, fitting the model to the data revealed that q decreased with increasing mercury concentration in the water (Figure 2). The actual relationship was found to be

$$q = aC_W^{-0.586}\ , \qquad (3)$$

where a depends upon mercury compound and (in the case of PMA) temperature. Values of a for each compound-temperature combination are given in Table 4. Uptake and release curves were generated from the model and are shown in relation to the points obtained experimentally (Figure 3). Hereafter, this model (equations 1-3) will be called Model 1.

Half-time of elimination of all 3 mercury compounds from clam foot, as calculated from the model, is 18.9 days, with a 95% confidence interval of 12.7 to 36.9 days.

FIGURE 2. Calculated uptake rates (q) of mercuric chloride, phenylmercuric acetate and methylmercuric chloride as a function of mercury concentration in the water (C_W). Mercury concentration is given in milligrams per liter (parts per million).

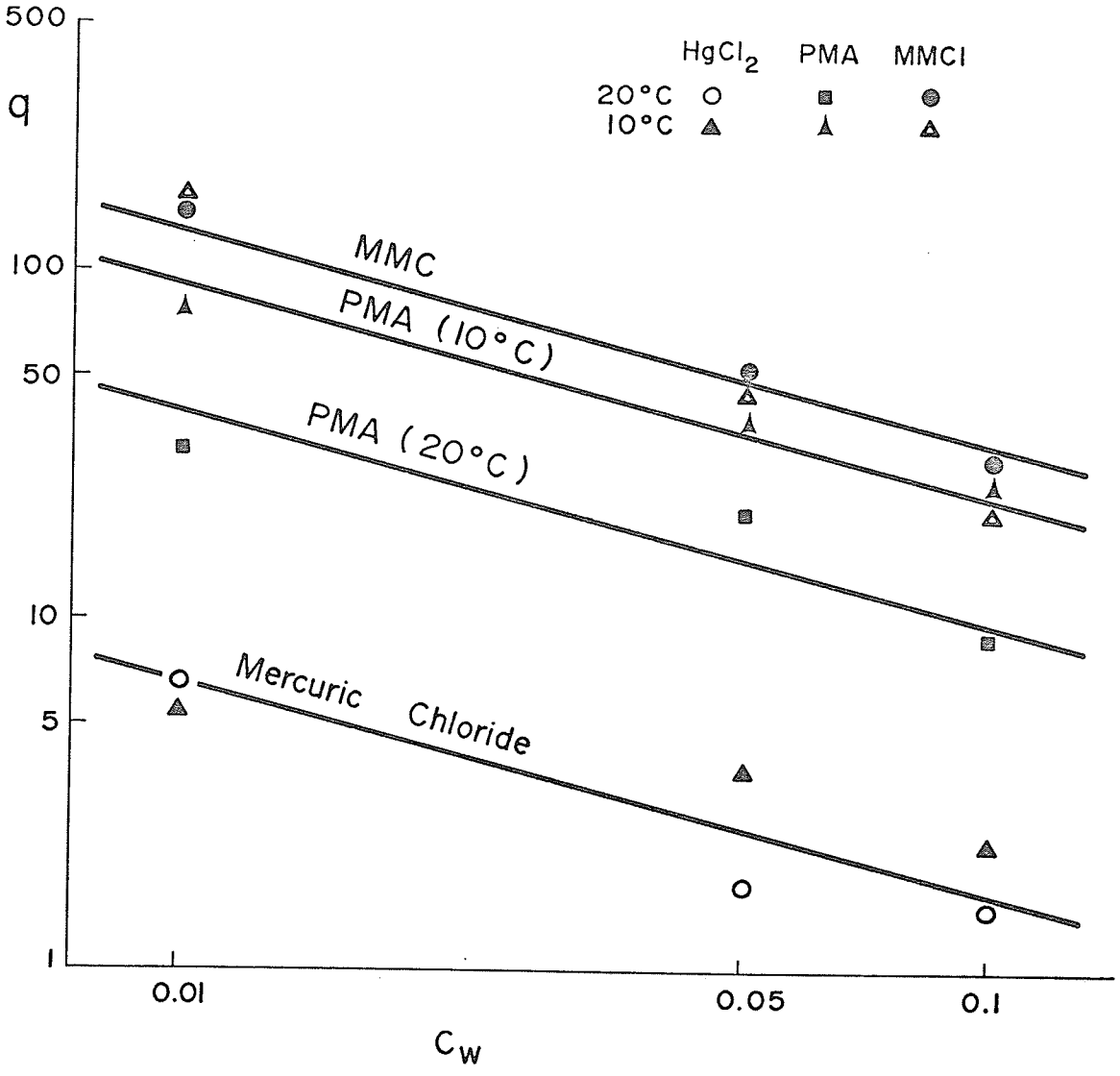
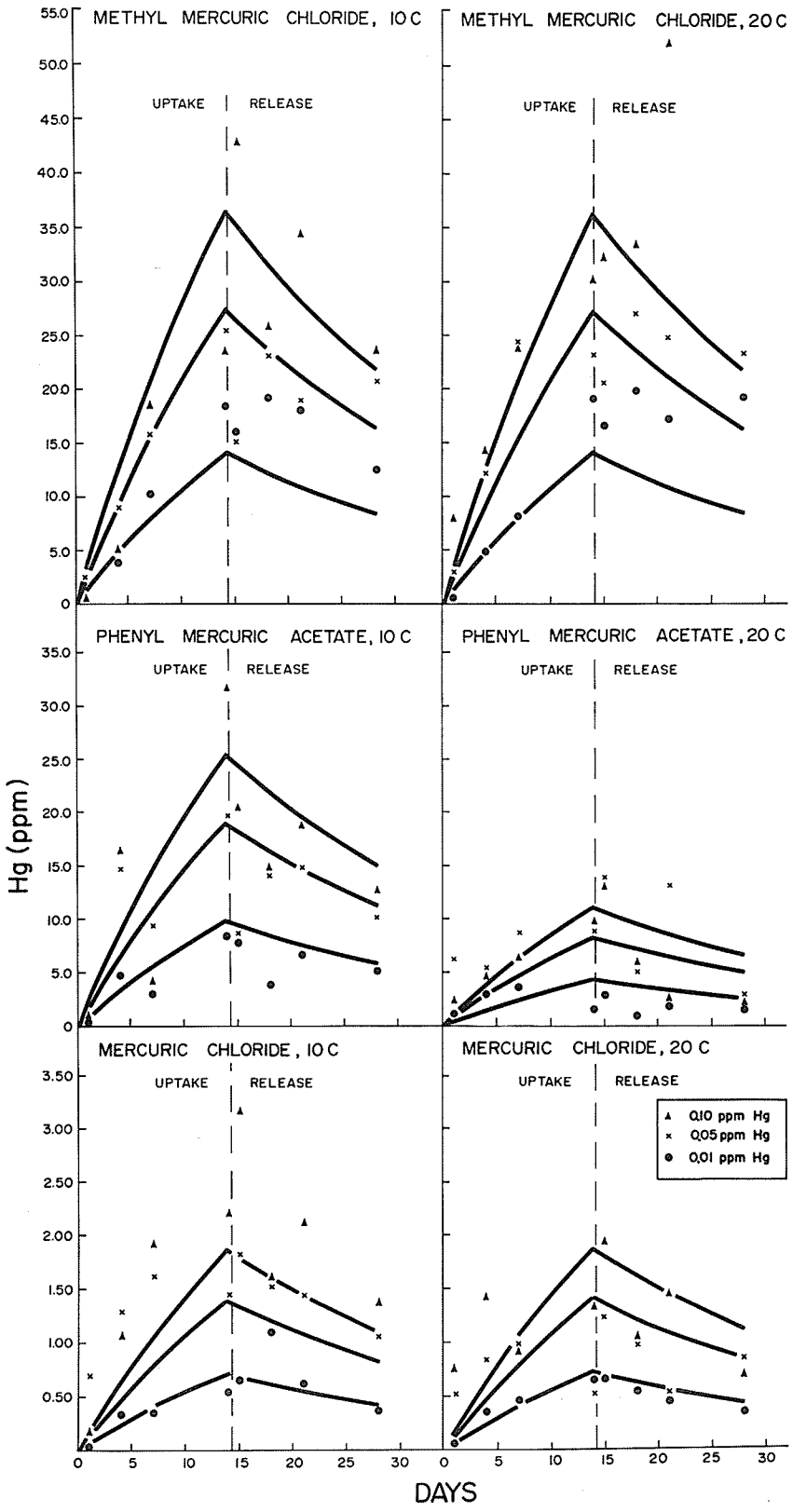

500
q
100
50
10
5
1
HgCl2
PMA
MMCl
20°C
10°C
MMC
PMA (10°C)
PMA (20°C)
Mercuric Chloride
0.01
0.05
0.1
Cw

TABLE 4. Values of a used to calculate q where $q = aC_W^{-0.586}$ (Model 1).

Compound	Temperature	a
$HgCl_2$	10 C	0.443
$HgCl_2$	20 C	0.443
PMA	10 C	6.02
PMA	20 C	2.63
MMC	10 C	8.64
MMC	20 C	8.64

FIGURE 3. Uptake and release of three forms of mercury at varying concentrations and temperatures. Lines represent the theoretical curve for each compound-concentration-temperature combination based on Model 1. Points are the actual values obtained experimentally.

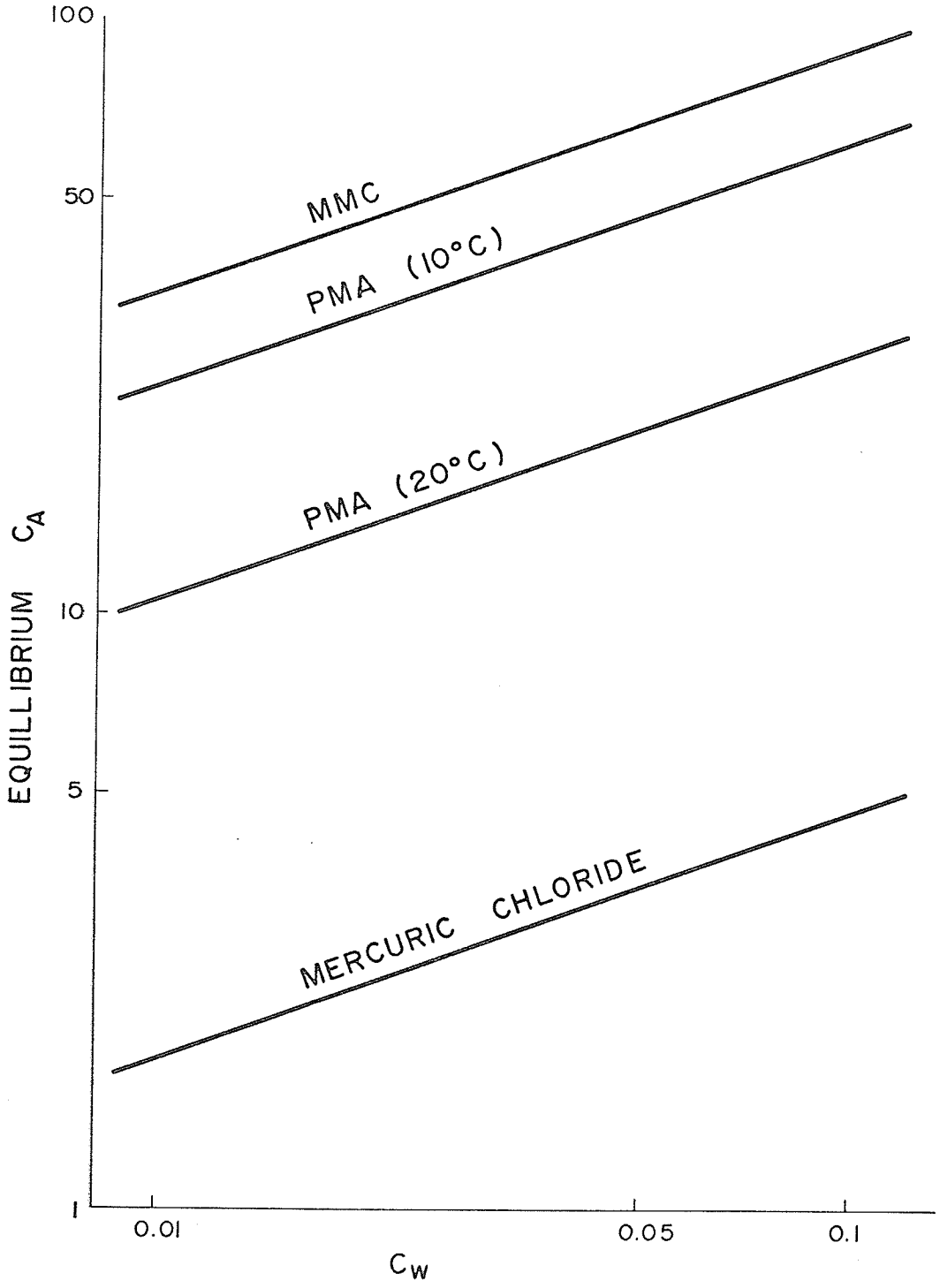

METHYL MERCURIC CHLORIDE, 10 C
METHYL MERCURIC CHLORIDE, 20 C
PHENYL MERCURIC ACETATE, 10 C
PHENYL MERCURIC ACETATE, 20 C
MERCURIC CHLORIDE, 10 C
MERCURIC CHLORIDE, 20 C
UPTAKE
RELEASE
Hg (ppm)
DAYS
0.10 ppm Hg
0.05 ppm Hg
0.01 ppm Hg

Expected equilibrium concentrations were calculated for each compound and for both temperatures for PMA and are given in Figure 4. If it is assumed that the mercury levels in clams collected from natural waters have reached equilibrium, then Figure 4 can be used to predict environmental mercury levels.

When the data obtained from homogenates of clams exposed to MMC were treated statistically in the manner described for the previous experiment, both the length of exposure and the concentration were found to influence uptake significantly ($P < 0.01$), but temperature influence was not significant. Release rates (Day 21-Day 49) 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 (Appendix II).

According to the model of change in mercury concentration in clams, if the release rate were zero, then uptake would be described by a simple linear regression with a slope equal to qC_W. This equation implies infinite 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. Curvilinear regression analysis testing for the contribution of X^2 revealed no significant deviation from a straight line regression. Thus, saturation of binding sites for mercury apparently was not approached

FIGURE 4. Theoretical equilibrium concentrations of the three mercury compounds as a function of mercury concentration in the water. Mercury concentrations are given in parts per million.


100
50
10
5
1
EQUILLIBRIUM C_A
MMC
PMA (10°C)
PMA (20°C)
MERCURIC CHLORIDE
0.01
0.05
0.1
C_W

during this experiment.

Although no significant release of MMC could be demonstrated statistically, it may be of interest, for reasons which will be considered later, to base a model on the release rate calculated from these data. The pooled release rate as estimated by covariance analysis, r = -0.003 per day, was substituted into Equation 2 to obtain a value for q. Since, in this case, no statistical evidence suggested that q was not a constant, an average over all four temperature-concentration combinations was used to determine that the 95% confidence limits of q = 26.179 ± 17.764. The values of q obtained by regression analysis of the uptake data ($q_{0.001}$ = 34.0; $q_{0.01}$ = 17.04) fall within this confidence interval. Thus, the model for MMC becomes

$$\frac{dC_A}{dt} = 26.179\ C_W - 0.003\ C_A \qquad (4)$$

Uptake and release curves generated from this model are compared with those obtained through regression and covariance analysis in Figure 5. At equilibrium

$$26.179\ C_W = 0.003\ C_A$$

and the predicted equilibrium concentration of mercury in the animal is obtained from the simple regression

$$C_{A_{eq}} = 8756\ C_W \qquad (5)$$

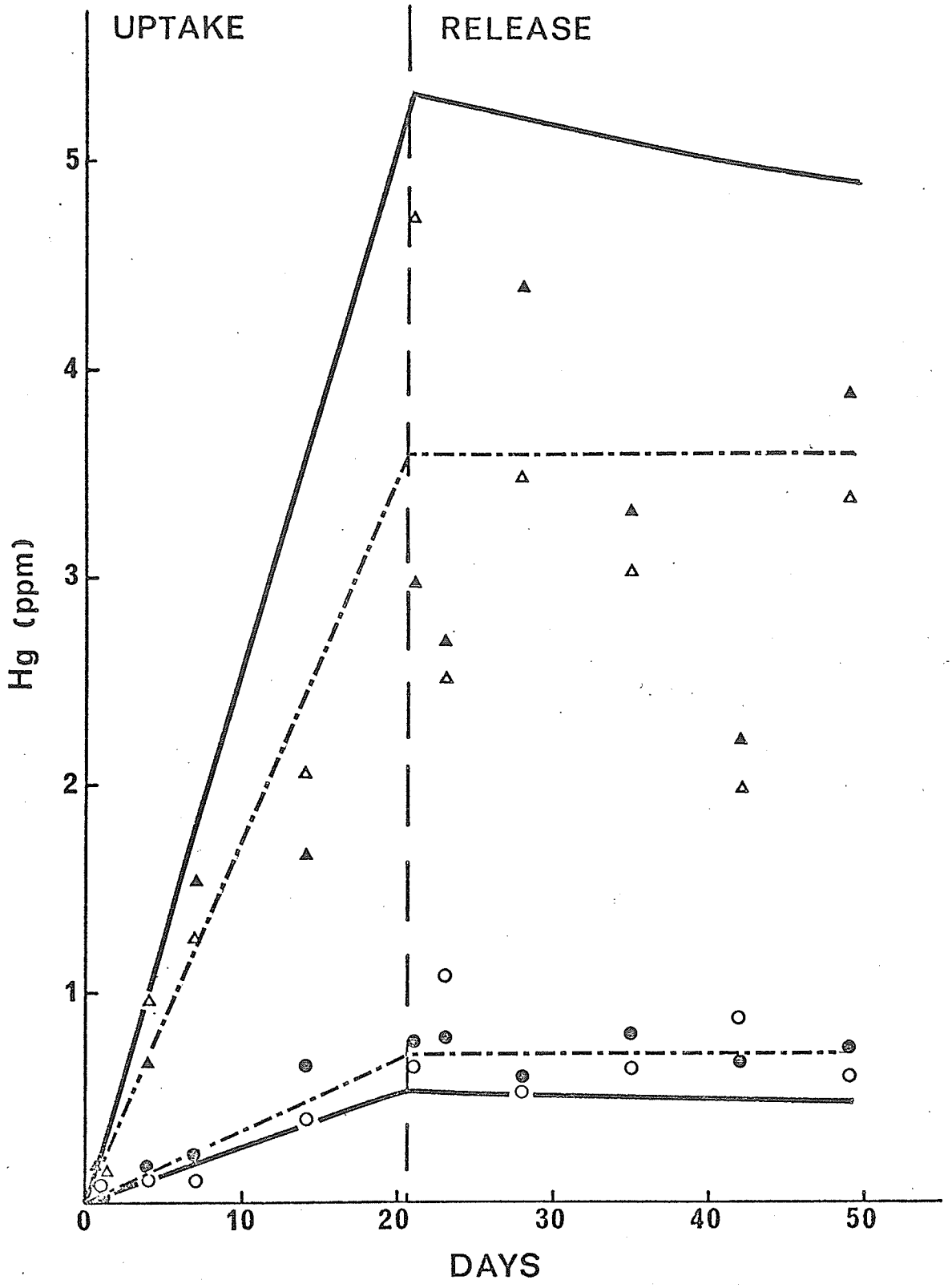
FIGURE 5. Uptake and release of methylmercuric chloride at two concentrations and two temperatures. Solid lines represent the theoretical curve for each concentration based on Model 2. Broken lines are the curves obtained through regression and covariance analyses. Points are the actual values obtained experimentally.

○ 0.001 mg Hg/l, 12 C

● 0.001 mg Hg/l, 20 C

△ 0.01 mg Hg/l, 12 C

▲ 0.01 mg Hg/l, 20 C


UPTAKE
RELEASE
Hg (ppm)
5
4
3
2
1
0
10
20
30
40
50
DAYS

The coefficient 8756 may be considered a concentration factor. Johnels et al. (1967) found mercury concentrations in pike from contaminated areas 3000 times or more that of water mercury concentrations, while in uptake experiments Hannerz (1968) obtained concentration factors for methylmercuric hydroxide of 3570 and 3480 for Planorbis sp. and Lymnaea stagnalis, respectively.

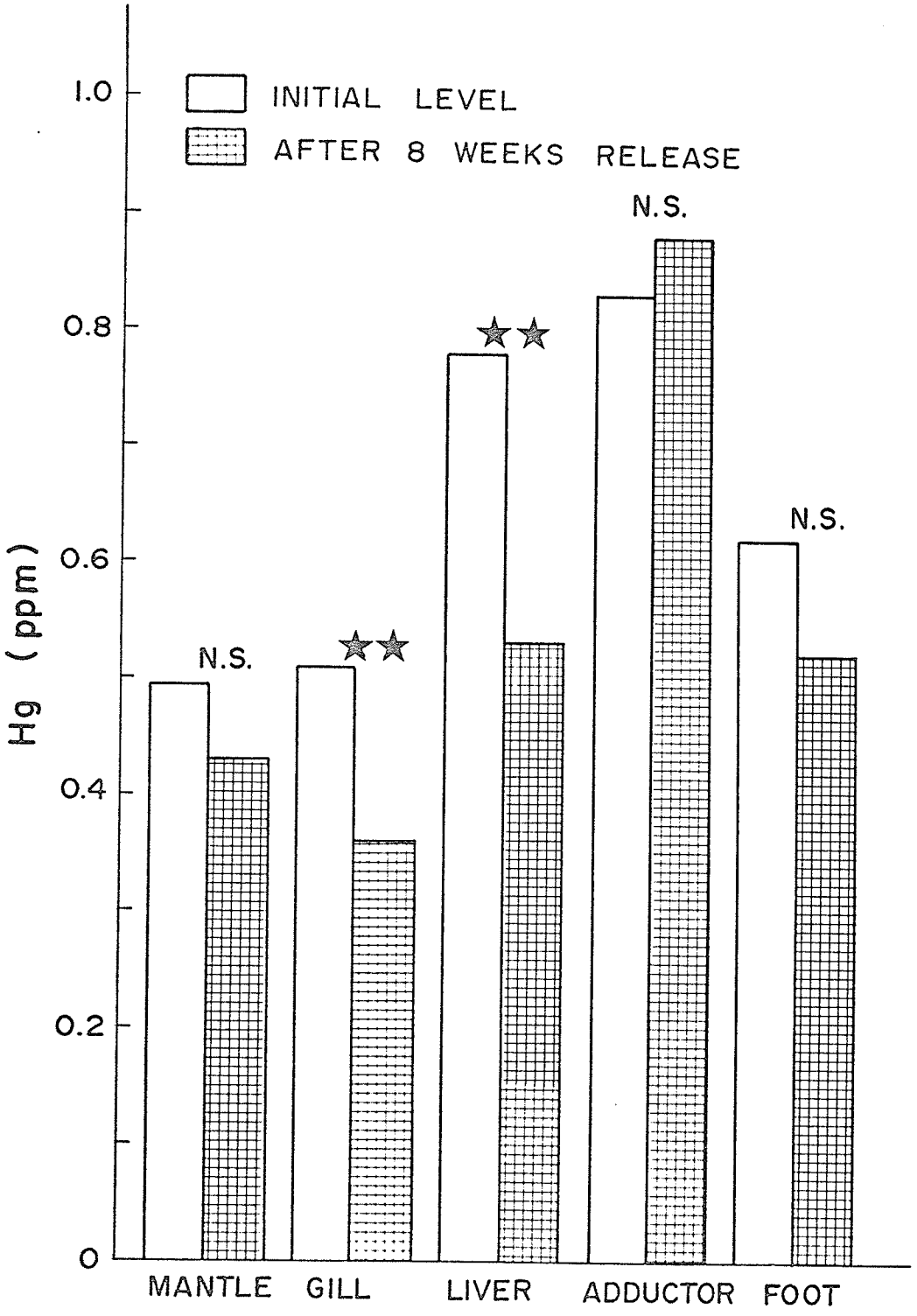
The model described above (equations 4-5) will be referred to as Model 2.

3. Uptake and Release of Mercury by Various Organs

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

Observation of contaminated clams allowed to release mercury in the laboratory suggested that elimination of the metal varies among tissues (Figure 6). For example, although initially the mercury concentration of adductor muscle was not significantly different from that of liver and foot, after the animals had been held in clean water for eight weeks, adductor muscle contained significantly more mercury per unit weight ($P < 0.01$) than liver or foot.

FIGURE 6. Change in mean mercury concentration in several tissues of Anodonta grandis from Clay Lake after an eight-week period in uncontaminated water.


1.0
0.8
0.6
0.4
0.2
0
Hg (ppm)
INITIAL LEVEL
AFTER 8 WEEKS RELEASE
N.S.
**
**
N.S.
N.S.
MANTLE
GILL
LIVER
ADDUCTOR
FOOT

While 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. Changes in tissue mercury levels may be more complicated than Figure 6 implies. Figure 7 suggests that the release curve of mercury concentration of foot muscle cannot be described by a simple log-linear regression.

When the change in the mercury concentration of foot, liver and whole clam was observed at six weekly intervals, split plot analysis of variance revealed no decrease in mercury concentration (Figure 8; Appendix III). Throughout the experiment, foot and liver contained significantly ($P < 0.05$) more mercury per unit weight than the whole animal. Individual regression analyses revealed no change with time in the per cent of the body burden of mercury concentrated in liver, foot or adductor muscle. A significant increase ($P < 0.01$) in the mercury concentration of the animals was observed between the time of collection and the fourth day they were held in the laboratory, but this pattern did not continue. The increase in mercury concentration (but not necessarily total mercury content) could be due to initial weight loss, since the clams were not fed in the laboratory.

Laboratory studies of uptake and release of mercury by various tissues revealed a complex pattern of mercury transfer (Figure 9, Appendix IV). Split plot analysis of

FIGURE 7. Changes in mercury concentration in foot muscle of Anodonta grandis from Clay Lake over an eight-week period in uncontaminated water.

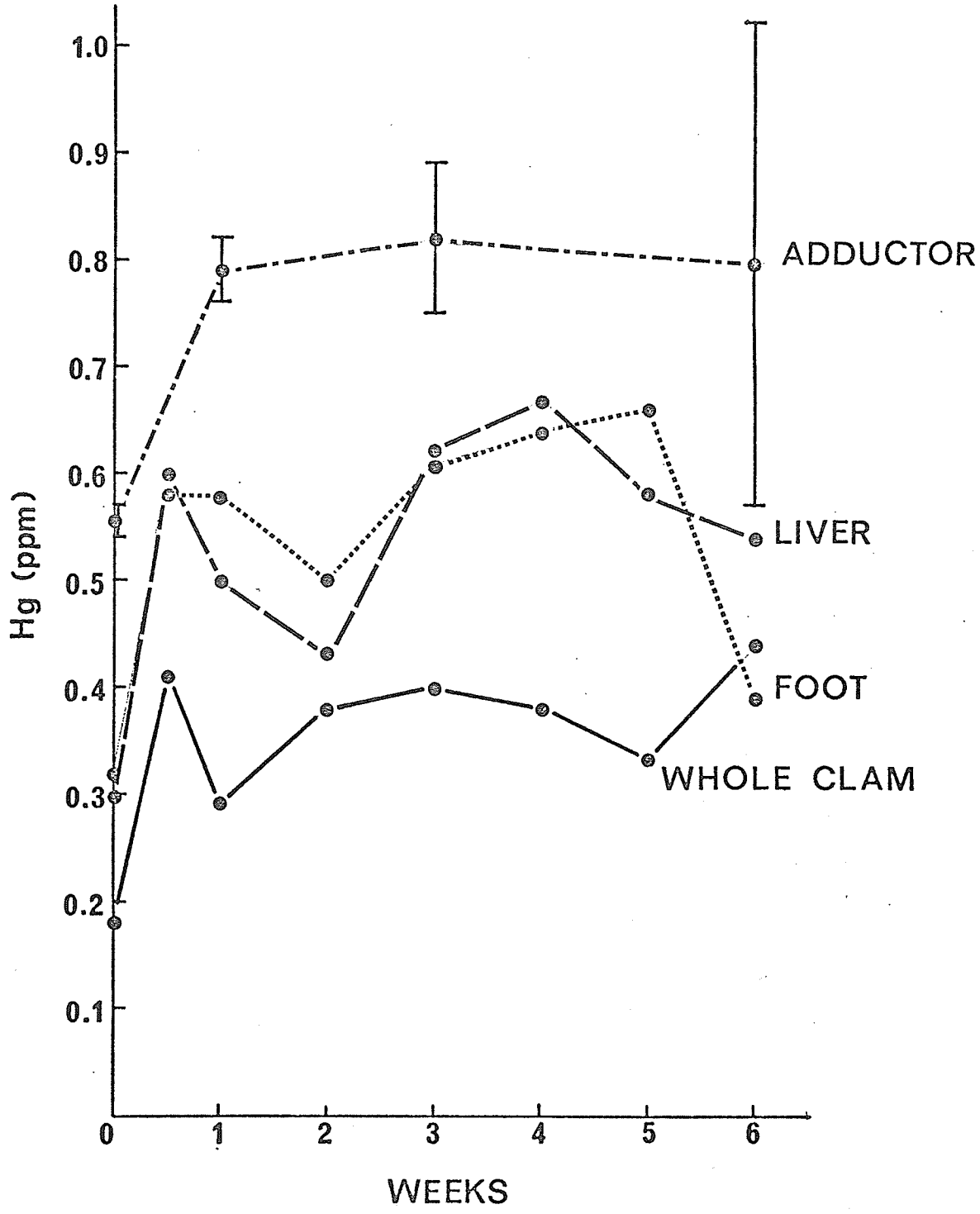

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Hg (ppm)
0
1
2
3
4
5
6
WEEKS
ADDUCTOR
LIVER
FOOT
WHOLE CLAM

FIGURE 8. Changes in mercury concentration of whole clam and several organs of Anodonta grandis from Clay Lake over a six-week period in uncontaminated water. Bars represent standard errors of adductor muscle mercury levels. The standard error for all other values, as estimated by analysis of variance, is 0.07 ppm.

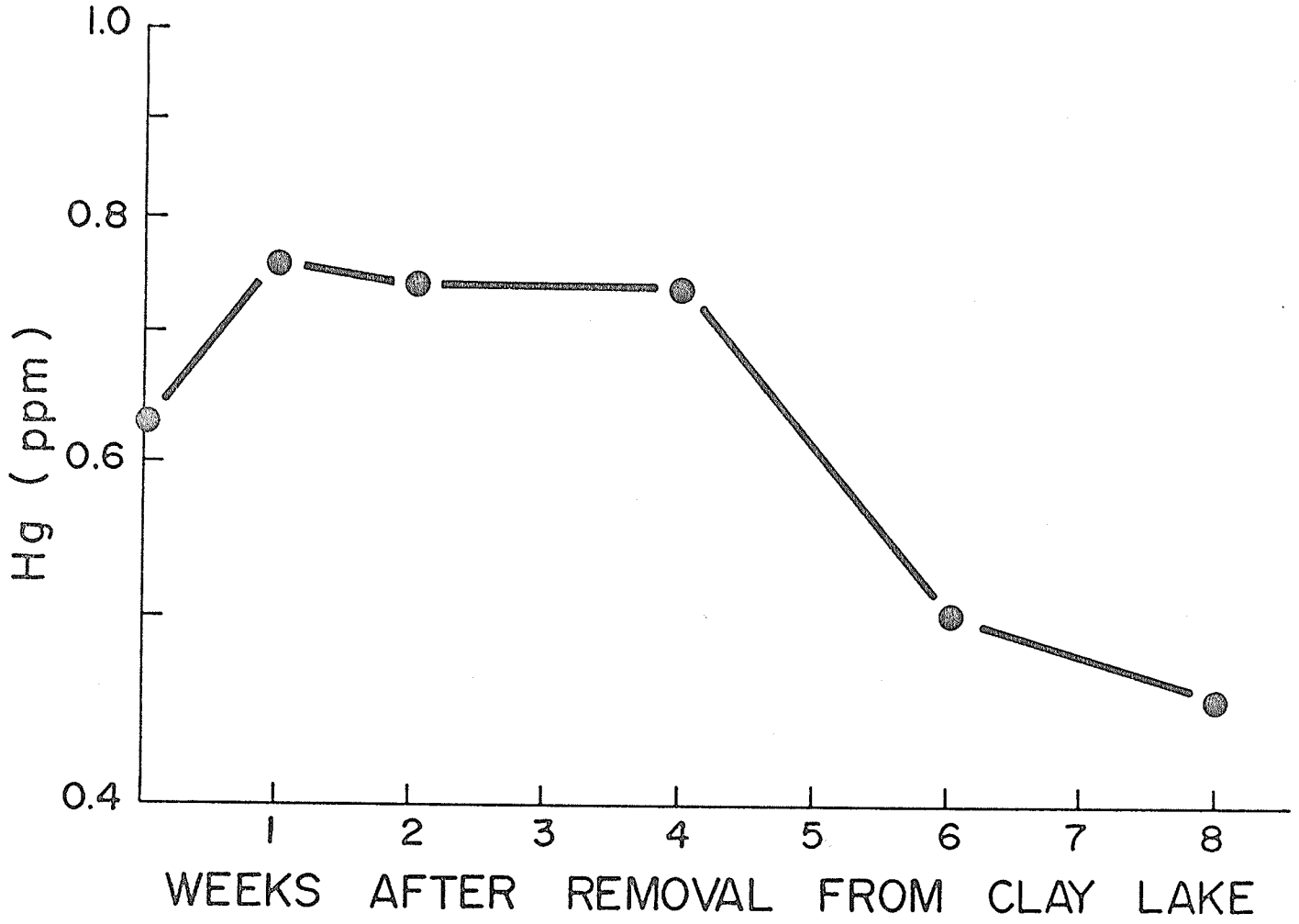
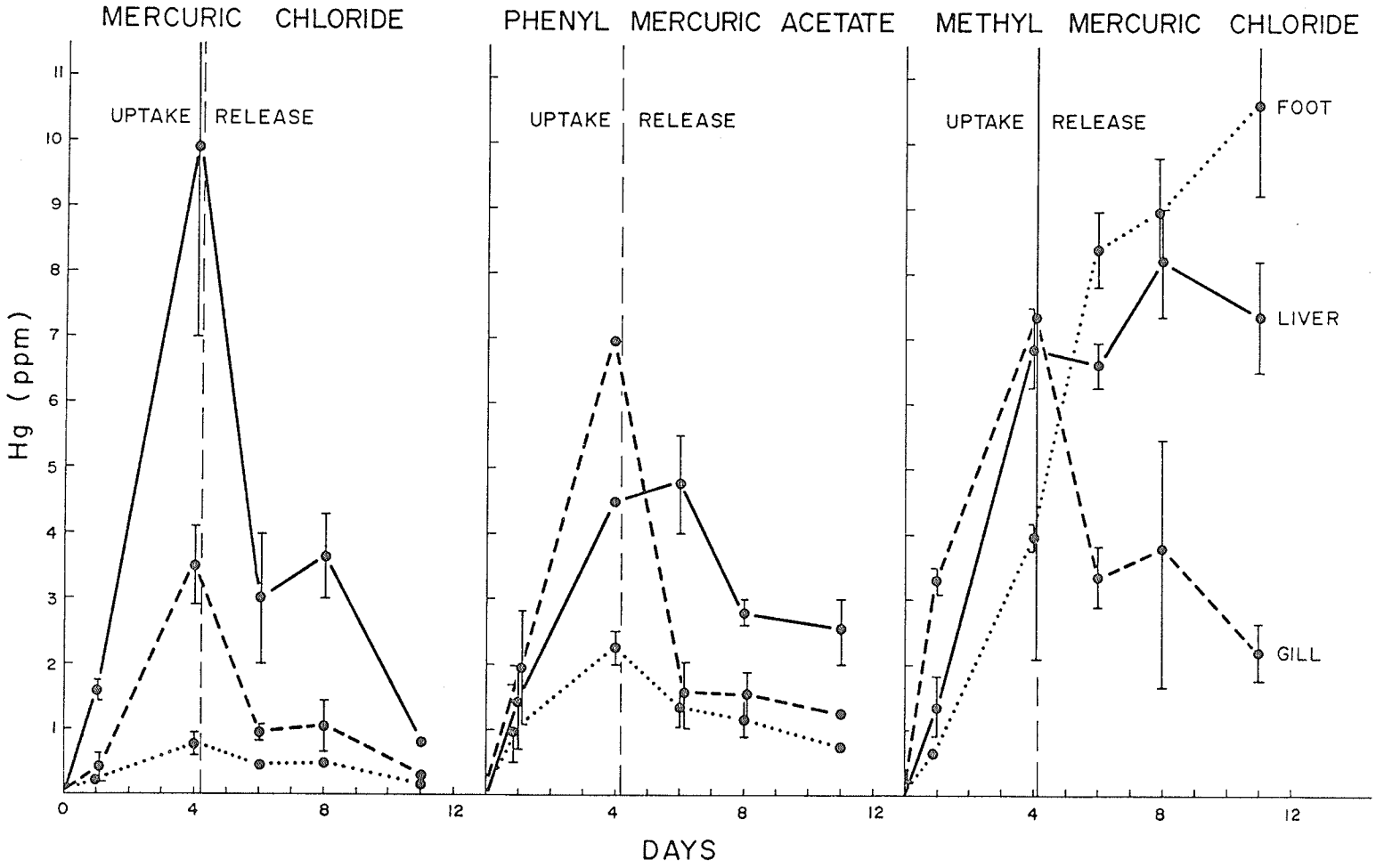

1.0
0.8
0.6
0.4
Hg (ppm)
1
2
3
4
5
6
7
8
WEEKS AFTER REMOVAL FROM CLAY LAKE

FIGURE 9. Uptake and release of three mercury compounds by Anodonta grandis gill, foot and liver at 20 C. Mercury concentration in the water during the uptake portion of the experiment was 0.05 mg/l. Bars represent standard errors.


MERCURIC CHLORIDE
PHENYL MERCURIC ACETATE
METHYL MERCURIC CHLORIDE
UPTAKE
RELEASE
FOOT
LIVER
GILL
Hg (ppm)
DAYS
0
4
8
12
1
2
3
4
5
6
7
8
9
10
11

variance demonstrated highly significant ($P < 0.01$) differences in mercury concentration over time (i.e., uptake occurred) and among organs, with liver and gill concentrating more mercury per unit weight than foot muscle. A significant interaction between time and organ was present, since initially the organs contained approximately equal concentrations of mercury, but as uptake proceeded differences among organs appeared. During the four-day exposure, the form in which the mercury was administered ($HgCl_2$, PMA or MMC) made no statistically significant contribution to uptake ($P > 0.25$).

The decrease in mercury concentration after transfer to clean water, however, was highly dependent ($P < 0.01$) upon the compound to which the animals had been exposed, with the order of retention of compounds being MMC > PMA > $HgCl_2$. Individual organs also varied considerably 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 9 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.

When the final day of the uptake experiment was included in the analysis of variance of the release data

(Uptake Day 4 = Release Day 0), significant differences ($P < 0.01$) in the mercury levels measured at different times were observed, and Day 0 was significantly ($P < 0.01$) different from the remainder of the release period. When day four of the uptake experiment was excluded from the analysis, time was not a significant factor ($0.10 < P < 0.25$). Apparently there is a change in the slope of the release curve, with rapid loss of mercury during the first two days after exposure ends, followed by a period of much slower release or no release of the remaining mercury.

4. Sublethal Effects of Mercury

The mortality rate of clams involved in the heart rate study was high. Of the eight animals in each treatment, three in the control group, four in the low level mercury (0.001 mg/l) group, and seven in the high level mercury (0.01 mg/l) group died during the one-week exposure period. Although more deaths occurred in the group exposed to 0.01 mg Hg/l, the differences in mortality rates were not statistically significant. The deaths probably were related to the holes in the clams shells rather than to any toxic effects of the mercury. Changes in heart rates of the survivors following treatment did not differ significantly among groups (Table 5).

Analysis of variance performed on changes in respiration rates revealed significant ($P < 0.05$) among-group

TABLE 5. Mean changes in heart and respiration rates of A. grandis following exposure to methylmercuric chloride for seven days. Numbers in parentheses represent the number of clams on which each mean was calculated.

	Heart Rate Change (beats/minute)	Respiration Rate Change (mg O_2 consumed/g dry wt/hr)
Error Mean Square	21.4145	0.0146
Control	+ 1.46 (5)	- 0.050 (10)
0.001 mg Hg/l	- 2.35 (4)	+ 0.036 (10)
0.01 mg Hg/l	- 0.50 (1)	- 0.108 (10)

differences. Paired t-tests comparing changes in respiration rates of individual clams indicated a significant ($P < 0.025$) decrease of 0.108 mg oxygen consumed/g animal weight/hr in the 0.01 mg/l exposure group. There was no significant change in either the 0.001 mg/l exposure group or the control group (Table 5; Appendix V).

DISCUSSION

1. General Factors Affecting Uptake and Release of Mercury

The concentration of mercury in clam tissue depends on the compound to which the animals have been exposed, but the reason for compound-dependent differences is not clear. 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 they are released. However, the experiment including gill and liver with foot muscle 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. If either of these conclusions is entirely correct, the second seems the more reasonable.

Similar release rates of different types of mercury compounds conflict with reports in the literature that methyl mercury binds more tightly to tissue than inorganic forms (Brown and Kulkarni, 1967) and in mammals is excreted more slowly than PMA (Gage, 1964) or inorganic mercury (Friberg, 1959; Berlin and Ullberg, 1963b; Takahashi and Hirayama, 1971). The major compound-dependent differences in mercury retention observed in the multi-organ experiment

apparently are due largely to a rapid loss of unbound or weakly bound mercury from the tissues during the first 48 hours or less following exposure, followed by a slower release of the remaining mercury. This type of release pattern was observed for zinc in Mytilus galloprovincialis by Keckes et al. (1968), who suggest that the original high rate represents the removal of absorbed ions, while the lower rate represents loss of metal that has bound more or less irreversibly with the tissue. Since the calculation of release rates of the three mercury compounds from foot muscle was based on mercury levels measured at least 24 hours after exposure, initial differences in release may have been obscured. Compound-dependent differences, if any, of the slower release of bound mercury may not have been detected due to the short duration of the release experiment, high among-clam variation in mercury concentration, or departure from simple exponential release because of lags and inter-organ transfer of mercury. There may also be real differences in rates of uptake of the various compounds which were not detected in the different organs due to the small number of samples included in the uptake experiment.

Norseth (1971) and Gardiner (1972) have suggested that the elimination rate of methylated mercury is dose-dependent, but the present data do not confirm this

observation for short term release.

Pringle et al. (1968) have reported that different environmental concentrations of metals will result in different uptake rates and different concentrations attained in the animal, depending upon the duration of exposure. These observations are consistent with the experimental results I obtained for all three mercury compounds. They have also observed that temperature is closely related to uptake rate and concentration level obtained for a given metal. However, the results of the present experiments do not support this observation. No significant differences in uptake rates or concentrations attained were observed at different temperatures except in the case of PMA, which was concentrated to a greater extent at 10 C than at 20 C. Lack of temperature dependence of uptake rates implies 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). A passive process might account for similar instantaneous rates of uptake of different mercury compounds, although different sizes and structures of molecules would not be expected to diffuse through biological membranes at the same rate. Accumulation of

mercury apparently depends on the strength and degree of binding, which is consistent with the formation of stable complexes.

The lower uptake of PMA at 20 C was unexpected and is difficult to explain. Since the experiment involving temperature effects considered uptake of PMA by foot muscle only, it is possible that temperature influenced the rate of transfer to or from that organ rather than the overall uptake of the compound. 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 high 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.

Although the amount of mercury accumulated by clams increases with increasing mercury concentration in the water, the proportion of mercury extracted from the water decreases (as indicated in Model 1 by decreasing q with increasing C_W). Pringle et al. (1968) report that the rate of uptake of a metal is limited by exchange at the cell membrane or by bulk phase diffusion, with the former being limiting at low external concentrations of the metal and the latter limiting at high external concentrations. If mercury is accumulated

in clam tissue by diffusion followed by the formation of stable complexes, complex formation may be equated with Pringle's "bulk phase diffusion". In other words, at low water mercury concentration, mercury diffuses into clam cells and is rapidly bound to the tissue. Thus, the equilibrium between mercury in the water and mercury in the cells is shifted in favor of further diffusion into the cells. As the water mercury concentration increases, more mercury is able to diffuse into the cells, and competition for binding sites arises, resulting in higher concentrations of unbound mercury in the cells and shifting the equilibrium to limit further diffusion of mercury into the animal. Thus, the rapidity with which mercury can bind to the tissue limits the rate of uptake. In Model 1, the term b is a measure of this limiting factor.

In Model 2 it could not be verified that q was not a constant. However, there was some suggestion ($0.05 < P < 0.10$) that q decreased with increasing C_W. Had there been more than four estimates of q on which to base the statistical test, significance might have been established. On the other hand, Model 2 was based on the behavior of MMC which is highly soluble in the lipid component of cell membranes and thus can diffuse readily throughout the body (Hughes, 1957). Diffusion away from the site of uptake could also shift the equilibrium in favor of further diffusion from the water into the animal,

and if this secondary diffusion were rapid enough, the rate of uptake of mercury from water might not be limited, at least at the concentrations considered.

Variations in release rates at different temperatures should have been observed, as the release of bound mercury should involve temperature-dependent biochemical processes. Jarvenpaa et al. (1970) have demonstrated considerably longer half-life of mercury in pike and flounder held at 1 C than in the same species held at 13-19 C. In all cases these half-lives were in excess of one hundred days and ranged as high as 780 days. If the half-life of bound mercury in clam tissue at any temperature falls within this range, as the separate investigation of MMC suggests, temperature-dependent differences would be difficult to detect in experiments of 14 to 28 days duration.

2. Models of Mercury Uptake and Release

A few models of mercury uptake and release by various organisms have already been described. Hannerz (1968) suggests that accumulation of mercury by fish is the result of simultaneous uptake and elimination, with uptake rate higher than elimination rate. As uptake decreases, elimination rate increases, eventually resulting in a steady state. The models derived here also predict a steady state but provide constants for instantaneous uptake and elimination rates. Ulfvarson's (1962) model for mercury

uptake and release in rats is generally the same as my basic model. His model is

$$x = \frac{\delta}{\kappa} (1 - e^{-\kappa t})$$

where

x = the mercury concentration in the animal

κ = a proportional constant relating excretion of mercury per unit time to the total body concentration at each moment

δ = the constant dosage of mercury per unit time

The relationship between uptake by clams and the water concentration of mercury, qC_W, is equal to Ulfvarson's δ. Ulfvarson assumes a distribution equilibrium among organs which implies a constant ratio x_i/x, where x_i = the concentration in any organ "i". He gives this ratio the value q_i and describes the model for any given organ

$$x_i = q_i \frac{\delta}{\kappa} (1 - e^{-\kappa t}).$$

Berglund and Berlin (1969) agree that if transfer of mercury among organs occurs freely at a rate which is rapid when compared with the excretion rate, an equilibrium among compartments would be reached; Ulfvarson's equation should hold, and a sample of mercury in a single tissue should provide an index of the body burden. If Ulfvarson and Berglund and Berlin are correct, then the Model 1 could be used to calculate a reasonable estimate of C_W.

When the mean value of mercury measured in foot muscle of A. grandis from Clay Lake, 0.40 mg/kg, is substituted into Model 1, the model predicts that the water concentration of mercury is 0.0001µg/l, but the actual value of mercury measured in Clay Lake water is 0.2 µg/l. Obviously this model does not provide an accurate prediction.

It appears that the flaw in Model 1 lies in the assumption of a constant relation between the mercury concentration of foot muscle and that of the whole clam. Figure 9 suggests that the mercury concentration of foot muscle of clams which have been exposed to MMC increases for at least a week after exposure ends, while a separate study of MMC revealed no measurable change in total body concentration over this length of time. Model 1 assumes that, in practice, the animal acts as a single compartment concentrating and eliminating mercury. Norseth (1972) has found that biotransformation of methylated mercury results in time-dependent distribution changes which would indicate that a multi-compartment model is more correct. However, he feels that deviations from a single compartment model caused by biotransformation are too small to affect the overall experimental fit to the model proposed by Ulfvarson and Berglund and Berlin. This may be true for long term experiments, but when release rates for foot muscle are estimated over a two-week period, and during at least half of that time mercury levels may actually be increasing in that

organ, significant deviations from the proposed model must be expected.

A model of mercury uptake and release by whole clam should avoid inaccuracies caused by time lags in uptake and release or inter-organ transfers of mercury. If, in addition, the model were based solely on the behavior of MMC, the situation in natural waters might be approximated more closely, since mercury is methylated in lake sediments (Jensen and Jernelov, 1969) and subsequently released from the sediments by Anodonta (Jernelov, 1970); also methyl mercury is the form most commonly found in fish (Westoo, 1969; Zitkov et al., 1971; Kamps et al., 1972). Such a model is difficult to construct since it was impossible to demonstrate statistically that MMC is actually eliminated from whole clam.

However, it is reasonable to assume that some release does occur. If A. grandis were incapable of eliminating mercury, or if the release of the metal were as slow as Jarvenpaa et al. (1970) reported for pike, continued accumulation of mercury throughout the animals' lifetime would be expected, with larger, older clams containing higher mercury levels than their smaller conspecifics. A linear relationship between weight and mercury content of pike (Johnels et al., 1967; Johnels and Westermark, 1969) and a curvilinear relation between body length and mercury content of spiny dogfish (Forrester et al., 1972) have been

described. No relationship could be demonstrated for A. grandis. This pattern could, nevertheless, be observed without release of mercury if the growth rate of the clams were rapid enough that increase in tissue weight would offset the increasing amount of mercury obtained from the environment.

If clams took up mercury linearly at a rate qC_W, where the mean value of q = 25.52, animals exposed to the 0.2 µg/l of mercury measured in Clay Lake water would concentrate the 0.18 mg/kg observed in A. grandis in approximately 35 days. However, growth ring counts revealed that each of the animals on which the mean mercury concentration of 0.18 mg/kg was based was at least three years old, and records (Bligh, 1971) indicate that mercury contamination in Clay Lake had existed for several years prior to the time these measurements were made. Thus, if A. grandis took up mercury at the rate measured in the laboratory and were unable to release any of the metal, they would contain much higher mercury levels than were actually measured. Growth rate alone should not be high enough to account for the relatively low mercury concentrations observed.

The instantaneous release rate of 0.003 per day measured in the laboratory would produce a half-time of mercury in clam tissue of 233 days, which is not unreasonable. Miettinen et al. (1971) found the half-time of methyl mercury in Mytilus galloprovincialis to be over 1,000 days, and they

report that phylogenetically related species follow similar patterns of excretion of methylmercury. If such slow release of mercury actually occurs in A. grandis, an experiment of only 28 days duration would probably be too short to detect the statistical significance of the release rate.

If it is assumed that a release rate for methylated mercury does exist, then Model 2, as described in equations 4 and 5, should generally be valid, although the estimate of r may not be accurate. When the mean whole clam mercury values of A. grandis from Clay and Minnedosa Lakes (0.18 and 0.01 mg/kg, respectively) are substituted into Model 2, water mercury levels of 0.02 µg/l in Clay Lake and 0.001 µg/l in Minnedosa Lake are predicted. These estimates, while closer than the estimate provided by Model 1, are still consistently an order of magnitude lower than the measured values (Table 2).

The errors in predictions of both models may, in fact, be greater than they appear from the water mercury measurements presented here. Water near the lake bottom, to which clams are exposed, contains a higher concentration of suspended particles which potentially may adsorb large amounts of mercury. Thus, bottom water can contain higher mercury levels than the surface water which was used for mercury determinations.

The difference between predicted and observed mercury levels in lake water may be accounted for, in part,

by an inaccurate estimate of r, but only a higher instantaneous release rate would improve the predictions. If part of the measurable mercury in lake water were in a non-methylated form which would be excreted more quickly than methyl, or if part were in a form unavailable for uptake by clams, the model would underestimate the amount of mercury actually present. Mercury in the water is present as a macromolecule (Matsumura et al., 1972), and mercury in lakes may be adsorbed onto suspended organic or inorganic particulate matter or complexed with fulvates or humates (D'Itri et al., 1971). If mercury were adsorbed onto particles of a size rejected by filter-feeding clams or bound in a metallo-organic complex or macromolecule which the clams could not absorb, more mercury could be measured in the water than would be available to the animals. The water chemistry of a given lake may modify the uptake of mercury to create a deviation from the rate measured in deionized laboratory water. For example, Amend et al. (1969) report that the presence of chloride ions facilitates the absorption of ethylmercuric phosphate by rainbow trout. Bivalves take up mercury both from the water and through the food chain, but the excretion rate of mercury obtained from the food chain is slower than the excretion rate of that taken up directly (Yoshida et al., 1967). Such variation in the release rate of mercury would create deviations from the proposed model and reduce the reliability

of its predictions.

In general, it is difficult, based on laboratory data, to build a model of metal uptake and release which will be applicable to the natural situation. Kopfler and Mayer (1969) explain that it is difficult to determine the relationship between metals concentrated in oysters and in the water, since a water sample taken at any given time does not necessarily represent the water to which the animals have previously been exposed. Wolfe and Coburn (1970) have found that salinity has a greater influence on the accumulation of ^{137}Cs by estuarine clams in the laboratory than in the field, and Wolfe (1971) concludes that concentration factors obtained in the laboratory have little relevance to ^{137}Cs distribution in the environment.

3. The Roles of Various 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. Surface accumulation of mercury on the gills has been reported in fish (Hannerz, 1968). Korringa (1952) has stated that positive polyvalent ions may be taken up involuntarily by oysters by adhering to the mucous feeding sheets and thus being transferred to the gut where they may be absorbed. Since the mucous sheets of A. grandis pass across the gills, apparent mercury concentration in the gills could be attributed to

the presence of contaminated mucus. It is also possible that diffusion of mercury from the water occurs directly across the gills, as is suggested for fish (Jernelov and Lann, 1971), crabs (Corner, 1959) and mussels (Jones et al., 1972).

Jones et al. (1972), studying Mytilus edulis from the Tay region, have measured considerably higher mercury levels in the gills than in any other organ, which is in contrast with the observations of A. grandis from Clay Lake reported here. These elevated gill mercury levels may indicate that the Mytilus in Jones' study were concentrating mercury to a greater extent than were the A. grandis, either because of environmental or taxonomic differences. Pringle et al. (1968) have reported that various species of bivalves concentrate trace metals to different extents, and the present study has demonstrated significant differences in the mercury levels in three unionid species.

Differences in the role of the liver in the metabolism of different mercury compounds have been described. Phenylmercuric acetate shows pronounced accumulation in rat liver as compared with $HgCl_2$ (Berlin and Ullberg, 1963a), but since in the present experiment no compound-dependent differences in uptake of mercury by liver tissue could be demonstrated, the situation in clams may be different. However, there is some evidence of a lower release rate of PMA than of $HgCl_2$ from clam liver. Methylated mercury is metabolized by the liver of rats (Takahashi and Hirayama,

1971; Norseth and Clarkson, 1971). The sustained high level of MMC in the liver of Anodonta in the present laboratory study suggests similar importance of clam liver in the metabolism of MMC. It is probable that this constant mercury level represents a steady state between mercury transferred into the liver and metabolized mercury removed from the liver.

Only small amounts of $HgCl_2$ and PMA were accumulated in foot muscle during the one week exposure, and this apparently was excreted in clean water. However, the mercury concentration in the foot of animals exposed to MMC continued to increase after exposure had terminated. A similar situation is suggested by Jernelov and Lann (1971), who report that while inorganic mercury is accumulated in fish liver and subsequently excreted with little effect on muscle, methylmercury concentrated in the liver is not so rapidly excreted and subsequently accumulates in muscle. There are several possible explanations for this phenomenon. Jernelov and Lann 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. Transfer of mercury from some other organ into muscle could also account for continued accumulation. Biotransformation of methylmercury occurs in mammals, and the inorganic mercury thus released from one organ becomes available for redistribution to other organs (Norseth and

Clarkson, 1970). In rats, this redistribution conforms to the initial distribution of $HgCl_2$ (Norseth and Clarkson, 1970), which does not accumulate in muscle, but biotransformation of mercury varies among species, which may explain taxonomic differences in organ distribution (Norseth, 1971).

If methylated mercury in clams shows delayed accumulation in foot muscle simply because of the slow metabolic rate of that organ, an equilibrium should eventually be reached and no further transfer should occur. Such an equilibrium would be expected in clams collected from a contaminated lake, but Figure 7 suggests that some transfer of mercury into foot muscle occurs in clams removed from Clay Lake. However, since the clams were not fed, loss of weight rather than an increase in the amount of mercury present could have caused the observed increase in mercury concentration.

4. Sublethal Effects of Mercury

Exposure to 0.01 mg/l of MMC significantly depressed the respiration of A. grandis. This is in accordance with previous reports that mercury compounds affect respiration of both organisms and tissues. Mercuric chloride depresses the overall respiration of crustacean larvae (Corner and Sparrow, 1956; DeCoursey and Vernberg, 1972) as well as the cellular respiration of cultured mouse L-cells (Li and Traxler, 1972), while phenylmercuric hydroxide inhibits

mitochondrial respiration and the oxygen consumption of liver slices in vitro (Hell and Lindahl, 1971).

Oxygen consumption may be affected through a general depression of metabolism as would result from inhibition of necessary enzymes. Mercury is reported to block the function of numerous enzymes both in vitro (Jackim et al., 1970) and in vivo (Clarkson, 1968).

In aquatic organisms respiration may be depressed by metals which damage the gills. Collapse of oxygen uptake and gill damage has been reported in fish poisoned with zinc (Skidmore, 1964, 1970), while Burton et al. (1972) confirm that gill damage by zinc and other heavy metals modifies gas exchange and creates tissue hypoxia. Hypertrophy and hyperplasia of gill epithelial cells has been found in trout and salmon exposed to organic mercurials (Rucker and Amend, 1969). Phenylmercuric hydroxide causes decreased blood circulation in the secondary gill lamellae and detachment of epithelial cells resulting in inhibition of the respiration of isolated gill filaments (Lindahl and Hell, 1970).

Thus, the effect of MMC on respiration of A. grandis may be due to gill damage or to general metabolic depression through inhibition of enzymes or some other mechanism.

SUMMARY

1. Mercury concentrations were measured in three species of clams from natural waters and an attempt was made to relate them to water mercury levels. Clams were shown to concentrate mercury from the environment.

2. Uptake of mercury is influenced by the chemical form of mercury and the concentration in the water, but not by the water temperature. Model 1 of mercury uptake and release by foot muscle is

$$\frac{dC_A}{dt} = a\ C_W^{0.414} - 0.037\ C_A$$

where a depends upon the mercury compound to which the clams were exposed. Model 2, based on the uptake and release of MMC by homogenate of whole clam is

$$\frac{dC_A}{dt} = 26.179\ C_W - 0.003\ C_A$$

The former provides a better estimate of environmental mercury levels than the latter. Reasons for deviations of observed water mercury concentrations from predicted values are considered.

3. Different patterns of mercury uptake and release by various organs were observed, depending upon the

mercury compound to which the clams were exposed. A possible transfer of mercury among organs is discussed.

4. Sublethal exposure to MMC at 0.01 mg Hg/l was shown to depress respiration rates in clams by 0.108 mg O_2 consumed/g dry weight/hr but had no demonstrable effect on heart rates. Respiration rate may be inhibited by a general metabolic depression through blockage of enzymes or damage to the gills.

LITERATURE CITED

LITERATURE CITED

Amend, D. F., W. T. Yasutake, and R. Morgan. 1969. Some factors influencing susceptibility of rainbow trout to the acute toxicity of an ethyl mercury phosphate formation (Tisman). Trans. Am. Fisheries Soc. 98: 419-425.

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

Armstrong, F. A. J., and A. L. Hamilton. 1973. Pathways of mercury in a polluted N. W. Ontario Lake. In P. C. Singer (ed.), Metal-organic interactions in natural waters. Ann Arbor Scientific Publications, Ann Arbor. (In press).

Berg, W., A. Johnels, B. Sjostrand, and T. Westermark. 1966. Mercury content in feathers of Swedish birds from the past 100 years. Oikos 17: 71-83.

Berglund, F., and M. Berlin. 1969. Risk of methylmercury cumulation in man and mammals and the relation of body burden of methylmercury and toxic effects, pp. 258-273. In M. W. Miller and G. G. Berg (ed.), Chemical fallout: current research on persistent pesticides. Charles C. Thomas, Springfield, Illinois.

Berlin, M., and S. Ullberg. 1963a. Accumulation and retention of mercury in the mouse. II. An autoradiographic comparison of phenyl mercuric acetate with inorganic mercury. Arch. Environ. Health 6: 602-609.

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

Birkner, F. B., F. Hertsch, and R. Mohr. 1972. Preliminary investigation of mercury and gold levels in Chesapeake Bay oysters. A paper presented before the Division of Water, Air, and Waste Chemistry, Am. Chem. Soc., Boston. April, 1972.

Bligh, E. G. 1971. Mercury in Canadian fish. A paper presented at the Pacific Fisheries Technologists Conference, Victoria, B. C. March 22-24, 1971.

Borg, K., H. Wanntorp, K. Erne, and E. Hanke. 1966. Mercury poisoning in Swedish wildlife. J. Appl. Ecol. 3 (Suppl.): 171-172.

Brooks, R. R., and M. G. Rumsby. 1965. The biochemistry of trace element uptake by some New Zealand bivalves. Limnol. Oceanog. 10: 521-527.

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

Burton, D. T., A. H. Jones, and J. Cairns, Jr. 1972. Acute zinc toxicity to rainbow trout (Salmo gairdneri): confirmation of the hypothesis that death is related to tissue hypoxia. J. Fish. Res. Bd. Canada 29: 1463-1466.

Butler, P. A. 1969. The significance of DDT residues in estuarine fauna, pp. 205-220. In M. W. Miller and G. G. Berg (ed.), Chemical fallout: current research on persistent pesticides. Charles C. Thomas, Springfield, Ill.

Chau, Y. K., and H. Saitoh. 1970. Determination of sub-microgram quantities of mercury in lake waters. Environ. Sci. Technol. 4: 839-841.

Clarkson, T. W. 1968. Biochemical aspects of mercury poisoning. J. Occup. Med. 10: 351-355.

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

Corner, E. D. S., and B. W. Sparrow. 1956. The modes of action of toxic agents. I. Observations on the poisoning of certain crustaceans by copper and mercury. J. Marine Biol. Assoc. U. K. 35: 531-548.

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

Dall'Aglio, M. 1968. The abundance of mercury in 300 natural water samples from Tuscany and Latium, pp. 1065-1081. In L. H. Ahrens (ed.), Origin and distribution of the elements. Permagon Press, Oxford.

DeCoursey, P. J., and W. B. Vernberg. 1972. Effect of mercury on survival, metabolism and behaviour of larval Uca pugilator (Brachyura). Oikos 23: 241-247.

D'Itri, F. M., C. S. Annett, and A. W. Fast. 1971. Comparison of mercury levels in an oligotrophic and a eutrophic lake. Marine Technol. Sci. J. 5(6): 10-14.

Evans, R. J., J. D. Bails, and F. M. D'Itri. 1972. Mercury levels in muscle tissues of preserved museum fish. Environ. Sci. Technol. 6: 901-905.

Fimreite, N. 1970. Mercury uses in Canada and their possible hazards as sources of contamination. Environ. Pollut. 1970 (1): 119-131.

Fimreite, N., R. W. Fyfe, and J. A. Keith. 1970. Mercury contamination of Canadian Prairie seed eaters and their avian predators. Can. Field-Nat. 84: 269-276.

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.

Forrester, C. R., K. S. Ketchen, and C. C. Wong. 1972. Mercury content of the spiny dogfish (Squalus acanthias) in the Strait of Georgia, British Columbia. J. Fish. Res. Bd. Canada 29: 1487-1490.

Friberg, L. 1959. Studies on the metabolism of mercuric chloride and methyl mercury dicyandiamide. Arch. Industr. Health 20: 42-49.

Gage, J. C. 1964. Distribution and excretion of methyl and phenyl mercury salts. Brit. J. Industr. Med. 21: 197-202.

Gardiner, E. E. 1972. Differences between ducks, pheasants, and chickens in tissue mercury retention, depletion, and tolerance to increasing levels of dietary mercury. Can. J. Anim. Sci. 52: 419-423.

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

Hell, C. E. B., and P. E. Lindahl. 1971. Effects of phenylmercuric hydroxide on energy metabolism of rat liver and brain. Oikos 22: 30-36.

Hughes, W. L. 1957. A physicochemical rationale for the biological activity of mercury and its compounds. Ann. New York Acad. Sci. 65: 454-460.

Irukayama, K., T. Kondo, F. Kai, and M. Fujiki. 1961. Studies on the origin and causative agent of Minamata disease. I. Organic mercury compounds in the fish and shellfish of Minamata Bay. Kumamoto Med. J. 14: 157-169.

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.

Jackim, E., J. M. Hamlin, and S. Sonis. 1970. Effects of metal poisoning on five liver enzymes in the killifish (Fundulus heteroclitus). J. Fish. Res. Bd. Canada 27: 383-390.

Jarvenpaa, T., M. Tillander, and J. K. Miettinen. 1970. Methylmercury: half-time of elimination in flounder, pike, and eel. Suomen Komistilehti, B, 43: 439-442.

Jensen, S., and A. Jernelov. 1969. Biological methylation of mercury in aquatic organisms. Nature 223: 753-754.

Jernelov, A. 1970. Release of methyl mercury from sediments with layers containing inorganic mercury at different depths. Limnol. Oceanog. 15: 958-960.

Jernelov, A., and H. Lann. 1971. Mercury accumulation in food chains. Oikos 22: 403-406.

Johnels, A. G., T. Westermark, W. Berg, P. I. Persson and B. Sjostrand. 1967. Pike (Esox lucius L.) and some aquatic organisms in Sweden as indicators of mercury contamination in the environment. Oikos 18: 323-333.

Johnels, A. G., and T. Westermark. 1969. Mercury contamination of the environment in Sweden, pp. 221-241. In M. W. Miller and G. G. Berg (ed.), Chemical fallout: current research on persistent pesticides. Charles C. Thomas, Springfield, Ill.

Jones, A. M., Y. Jones, and W. D. P. Stewart. 1972. Mercury in marine organisms of the Tay region. Nature 238: 164-165.

Kamps, L. R., R. Carr, and H. Miller. 1972. Total mercury-monomethyl mercury content of several species of fish. Bull. Environm. Contam. Toxicol. 8: 273-279.

Keckes, S., B. Ozretic, and M. Krajnovic. 1968. Loss of Zn^{65} in the mussel Mytilus galloprovincialis. Malacologia 7: 1-6.

Kopfler, F. C., and J. Mayer. 1969. Studies on trace metals in shellfish. Proc. Gulf and South Atlantic States Shellfish Sanitation Res. Conf. March 21-22, 1967, pp. 67-80.

Korringa, P. 1952. Recent advances in oyster biology. Quart. Rev. Biol. 27: 266-308.

Kurland, L. T., S. N. Faro, and H. Siedler. 1960. Minamata disease. The outbreak of a neurological disorder in Minamata, Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds. World Neurol. 1: 370-395.

Lee, R. F., R. Sauerheber, and A. A. Benson. 1972. Petroleum hydrocarbons--uptake and discharge by marine mussel Mytilus edulis. Science 177: 344-346.

Li, M. F., and G. S. Traxler. 1972. Tissue culture bioassay method for water pollution with special reference to mercuric chloride. J. Fish. Res. Bd. Canada 29: 501-505.

Lindahl, P. E., and C. E. B. Hell. 1970. Effects of short-term exposure of Leuciscus rutilus L. (Pisces) to phenylmercuric hydroxide. Oikos 21: 267-275.

Matida, Y., and H. Kumada. 1969. Distribution of mercury in water, bottom mud, and aquatic organisms of Minamata Bay, the River Agano, and other water bodies in Japan. Bull. Freshwater Fisheries Res. Lab. (Tokyo) 19: 73-93.

Matsumura, F., Y. Gotoh, and G. M. Boush. 1972. Factors influencing translocation and transformation of mercury in river sediments. Bull. Environm. Contam. Toxicol. 8: 267-272.

Miettinen, J. K., M. Heyraud, and S. Keckes. 1971. Mercury as a hydrospheric pollutant. II. Biological half-time of methylmercury in four Mediterranean species: a fish, a crab, and two molluscs. (Abstr.) FAO Fisheries Rep. No. 99, p. 171.

Norseth, T. 1971. Biotransformation of methyl mercuric salts in the mouse studied by specific determination of inorganic mercury. Acta Pharmacol. Toxicol. 29: 375-384.

Norseth, T. 1972. Biotransformation of methyl mercuric salts in the rat with chronic administration of methyl mercuric cysteine. Acta Pharmacol. Toxicol. 31: 138-148.

Norseth, T., and T. W. Clarkson. 1970. Studies on the biotransformation of ^{203}Hg-labeled methyl mercury chloride in rats. Arch. Environ. Health 21: 717-727.

Norseth, T., and T. W. Clarkson. 1971. Intestinal transport of ^{203}Hg labeled methyl mercury chloride. Arch. Environ. Health 22: 568-577.

Pillay, K. K. S., C. C. Thomas, Jr., J. A. Sondel, and C. M. Hyche. 1972. Mercury pollution of Lake Erie ecosphere. Environ. Res. 5: 172-181.

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.

Rucker, R. R., and D. F. Amend. 1969. Absorption and retention of organic mercurials by rainbow trout and chinook and sockeye salmon. Progr. Fish-Cult. 31: 197-201.

Seagram, H. L. 1970. Mercury in Great Lakes fish. Limnos 3(2): 3-10.

Skidmore, J. F. 1964. Toxicity of zinc compounds to aquatic animals, with special reference to fish. Quart. Rev. Biol. 39: 227-249.

Skidmore, J. F. 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J. Exp. Biol. 52: 481-494.

Stock, A., and F. Cucuel. 1934. Die Verbreitung des Quecksilbers. Naturwissenschaften 22: 390-393.

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.

Underdal, B., and T. Hastein. 1971. Mercury in fish and water from a river and a fjord in the Kragerø region, South Norway. Oikos 22: 101-105.

Uthe, J. F., and E. G. Bligh. 1971. Preliminary survey of heavy metal contamination of Canadian freshwater fish. J. Fish. Res. Bd. Canada 28: 786-788.

Vernberg, W. B., and J. O'Hara. 1972. Temperature-salinity stress and mercury uptake in the fiddler crab, Uca pugilator. J. Fish. Res. Bd. Canada 29: 1491-1494.

Westoo, G. 1969. Methylmercury compounds in animal foods, pp. 75-93. In M. W. Miller and G. G. Berg (ed.), Chemical fallout: current research on persistent pesticides. Charles C. Thomas, Springfield, Ill.

Wilbur, K. M. 1964. Shell formation and regeneration, pp. 243-282. In K. M. Wilbur and C. M. Yonge (ed.), Physiology of Mollusca, vol. 1. Academic Press, New York.

Wobeser, G. N., D. Nielson, R. H. Dunlop, and F. M. Atton. 1970. Mercury concentrations in tissues of fish from the Saskatchewan Riber. J. Fish. Res. Bd. Canada 27: 830-834.

Wolfe, D. A. 1971. Fallout cesium-137 in clams (Rangia cuneata) from the Neuse River estuary, North Carolina. Limnol. Oceanog. 16: 797-805.

Wolfe, D. A., and C. B. Coburn, Jr. 1970. Influence of salinity and temperature on the accumulation of cesium-137 by an estuarine clam under laboratory conditions. Health Phys. 18: 499-505.

Yoshida, T., T. Kawabata, and Y. Matsue. 1967. Transference mechanism of mercury in marine environments. Tokyo Univ. Fish. 53: 73-84.

Zitkov, V., B. J. Finlayson, D. J. Wildish, J. M. Anderson, and A. C. Kohler. 1971. Methylmercury in freshwater and marine fishes in New Brunswick, in the Bay of Fundy, and on the Nova Scotia Banks. J. Fish. Res. Bd. Canada 28: 1285-1291.

APPENDICES

APPENDIX I. Experimental data from initial Anodonta grandis uptake-release model experiments. Each level is based on analysis of foot muscle of one specimen, with 2 analytical replicates. C_W = concentration in water. Time is in days. Clam lengths average 69 mm, with standard deviation S = 4 mm.

Compound	C_W	Temp.	Uptake expt. times					Release expt. times			
			0	1	4	7	14	1	4	7	14
$HgCl_2$	0.01mg/l	20°C	.02	.06	.37	.46	.65	.66	.54	.42	.34
"	"	10	.02	<.02	.35	.36	.55	.66	1.11	.63	.38
"	0.05	20	<.02	.52	.83	.96	.52	1.24	1.00	.54	.84
"	"	10	.02	.71	1.30	1.62	1.44	1.83	1.52	1.46	1.07
"	0.1	20	.04	.76	1.43	.95	1.32	1.93	1.02	1.44	.67
"	"	10	.04	.17	1.06	1.92	2.20	3.16	1.60	2.12	1.47
PMA	0.01	20	.04	1.16	3.04	3.70	1.60	2.88	.88	1.80	1.62
"	"	10	.08	.49	4.80	3.62	8.40	7.83	3.86	6.71	5.18
"	0.05	20	.06	6.18	5.44	8.94	8.90	13.8	5.44	13.2	2.37
"	"	10	.08	.84	14.7	8.98	19.6	8.70	14.1	14.8	10.0

APPENDIX I (Continued)

Compound	C_W	Temp.	Uptake expt. times					Release expt. times			
			0	1	4	7	14	1	4	7	14
PMA	0.1 mg/l	20°C	.11	2.36	4.74	6.44	9.58	13.0	5.92	2.38	1.97
"	"	10	.07	.93	16.4	4.16	31.6	20.4	14.8	18.8	12.6
MMC	0.01	20	.06	.40	4.92	8.15	19.2	16.6	19.8	17.3	19.3
"	"	10	.10	.34	3.90	10.3	18.3	16.1	19.2	18.0	12.5
"	0.05	20	.08	2.75	12.2	24.2	23.1	20.5	27.2	24.8	23.4
"	"	10	.11	2.56	9.12	15.8	25.5	15.0	23.1	19.0	20.6
*"	0.1	20	.14	7.79	14.2	24.2	30.1	32.2	33.4	52.0	--
"	"	10	.06	.38	4.92	18.3	23.4	42.8	25.6	34.3	23.4

*All specimens in the MMC 0.1 mg/l 20°C Release expt. were behaving abnormally and dying. All had died before 14 days of the Release expt.

APPENDIX II. Experimental data from Anodonta grandis MMC uptake-release model experiment with each level based on one homogenized whole clam (soft tissues), with two analytical replicates. Time is in days. Clam lengths average 121.9 mm with standard deviation S = 9.0 mm.

Water [Hg]	Temp.	Uptake Times					Release Times				
		1	4	7	14	21	2	7	14	21	28
0.001 mg/l	12 C	0.04	0.12	0.10	0.39	0.65	1.08	0.53	0.65	0.88	0.64
"	20 C	0.05	0.16	0.22	0.66	0.76	0.78	0.60	0.82	0.68	0.74
0.01 mg/l	12 C	0.19	0.95	1.26	2.06	4.71	2.51	3.46	2.79	1.96	3.40
"	20 C	0.17	0.66	1.53	1.66	2.97	2.70	4.41	3.33	2.22	3.90

APPENDIX III. Change in mercury content in the organs of contaminated Anodonta grandis after removal from Clay Lake. Mercury levels are given for foot, liver, adductor muscle, and whole clam. Each value is based on two specimens with two analytical replicates. Time is given in days.

Organ	Release Times							
	0	4	7	14	21	28	35	42
Foot	0.32	0.58	0.58	0.50	0.61	0.64	0.66	0.39
Liver	0.30	0.60	0.50	0.43	0.62	0.67	0.58	0.54
Adductor	0.56	--	0.79	--	0.82	--	--	0.80
Whole Clam	0.18	0.41	0.29	0.38	0.40	0.38	0.33	0.44

APPENDIX IV. Uptake and release of mercury by different organs of Anodonta grandis. Clams were exposed to 3 compounds at a concentration of 0.05 mg/l as Hg at 20° C for 4 days, and then placed in uncontaminated running water for 4 days. Organs analyzed were gill, foot and liver. Time is in days. Each value is based on 2 specimens, with 2 analytical replicates.

Organs	Gill						Foot						Liver					
	Uptake			Release			Uptake			Release			Uptake			Release		
Times	0	1	4	1	4	7	0	1	4	1	4	7	0	1	4	1	4	7
$HgCl_2$	.06	.38	3.5	.94	1.1	.52	.07	.23	.78	.47	.49	.20	.06	1.6	9.9	3.0	3.7	.82
PMA	.11	2.0	6.9	1.6	1.5	1.2	.03	.96	2.3	1.4	1.2	.74	.06	1.4	4.5	4.8	2.8	2.6
MMC	.04	3.3	7.3	3.4	3.8	2.2	.03	.66	4.0	8.4	9.0	10.6	.07	1.4	6.8	6.6	8.2	7.3

APPENDIX V. Effect of one week exposure to MMC on respiration rates of Anodonta grandis. Pre- and Post-exposure rates are given for each clam. Respiration rates are given as mg O_2 consumed/g clam dry wt/ hr. Mean clam dry weight was 8.75 g with standard deviation S = 1.57 g.

Water [Hg]	Respiration Rates		
	Initial	Final	Difference
Control	0.341	0.398	+0.057
	0.426	0.399	-0.027
	0.343	0.391	+0.048
	0.197	0.161	-0.036
	0.268	0.039	-0.299
	0.286	0.358	+0.072
	0.316	0.060	-0.256
	0.250	0.257	+0.007
	0.271	0.253	-0.018
	0.193	0.080	-0.113
0.001 mg/l	0.256	0.345	+0.089
	0.526	0.467	-0.059
	0.173	0.229	+0.056
	0.389	0.434	+0.045
	0.116	0.224	+0.108
	0.341	0.449	+0.108
	0.368	0.329	-0.039
	0.273	0.309	+0.036
	0.303	0.067	-0.236
	0.166	0.417	+0.251

APPENDIX V (Continued)

Water [Hg]	Respiration Rates		
	Initial	Final	Difference
0.01 mg/l	0.213	0.110	-0.103
	0.335	0.062	-0.273
	0.191	0.062	-0.129
	0.031	0.074	+0.043
	0.235	0.285	+0.050
	0.299	0.269	-0.030
	0.279	0.247	-0.032
	0.349	0.053	-0.296
	0.346	0.175	-0.171
	0.424	0.281	-0.143