A QUANTITATIVE ASSESSMENT OF MERCURY PARTITIONING AND TRANSFORMATIONS IN AN EXPERIMENTAL MARINE SYSTEM

by

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A quantitative assessment of partitioning and transformations of mercury in experimental marine systems

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ABSTRACT

Experiments are reported in which partitioning, transformation, uptake and clearance of mercury in the components of a marine model system are measured.

When mercuric chloride ($HgCl_2$) or methyl mercuric chloride (CH_3HgCl) were added to water, approximately 79 % was removed within 5 h, primarily by the sediments, and to a lesser extent by the periphyton and suspended particulates. The presence of the polychaete, Abarenicola pacifica , did not alter the total amount of mercury in the sediments, although it was distributed to greater depths.

In the absence of polychaetes, mercury was methylated or demethylated in the sediments within 2 days. The presence of Abarenicola pacifica prolonged the demethylation of CH_3HgCl by 20 days. Rates of methylation and demethylation were positively associated with bacterial biomass as measured by lipopolysaccharide (LPS) concentration. Mercury transformations occurred 2 to 4 times more slowly in low-bacteria inorganic sediments with low bacterial biomass.

No significant differences were seen in the uptake rate of $HgCl_2$ and CH_3HgCl by polychaetes. However, there were significant differences in the uptake rate of the two compounds by different body tissues and in the clearance rate of $HgCl_2$ and CH_3HgCl from individual polychaete and the body tissues. Methyl

mercuric chloride may persist in the polychaete body 14 times longer than mercuric chloride.

Methylation of $HgCl_2$ results in a longer retention time for mercury in the highest trophic level of the experimental marine system, and the demethylation results in a shorter retention time.

ACKNOWLEDGEMENTS

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I. Introduction

Mercury is naturally occurring and widely distributed in lakes and oceans. Cinnabar (HgS), the most common form of mercury originating from natural sources (Vostal 1972), has an extremely low solubility (.002 ng/mL) in water, and occurs mainly in a relatively immobile form in aquatic sediments (Hem 1970). In some areas, there have been recent local increases in mercury concentrations resulting from the discharge of mercury-laden wastes into receiving waters. While these additions may have been curtailed as a result of abatement programs, high concentrations of mercury compounds remain in aquatic sediments for extended periods after termination of the discharges. The fate and transformation of these compounds is of interest biologically and of concern environmentally.

Mercury in runoff waters and industrial effluents usually occurs in the +2 oxidation state, either in the ionic form (Hg^{++}) or in a complex, such as $HgCl_2$, $HgCl_4^{=}$, or CH_3HgCl. It is rarely in the +0 oxidation state (the volatile native metal) or in the +1 oxidation state, which is unstable in water.

The principal form of mercury in seawater is $HgCl_2$ (Wood pers. comm.). Aquatic organisms can incorporate $HgCl_2$ or transform it into methyl mercuric chloride (CH_3HgCl), which is

rarely discharged into the aquatic environment from industrial sources and seldom occurs dissolved in water. However, CH_3HgCl usually represents a high proportion of the total mercury found in fish and invertebrate tissue, and to a lesser extent, in the sediments (Eganhouse and Young 1978; Windom et al. 1976; Johnels 1971). This suggests that the well-known transformations of inorganic mercury to the organic form and vice versa, which occur in nature, are biologically mediated.

The purpose of this study was to investigate the fate of $HgCl_2$ and CH_3HgCl in a simulated marine benthic environment. The sedentary polychaete, Abarenicola pacifica, which lives in the marine sediments, and its associated micro-flora and fauna was chosen as the experimental system. I wanted to determine rates of mercury transformation and to assess the role of the sediments, polychaetes and micro-flora in these transformations. The factors which determine partitioning of mercury within the system were also evaluated.

II. Materials and Methods

The polychaetes (Abarenicola pacifica) used in this study were obtained from Maplewood mudflats on the north shore of Burrard Inlet. The polychaetes were collected in January 1977, April 1977, July 1978, September 1978, and January 1979. They ranged from 2.4 to 4.3 cm in length and from 0.57 to 1.29 g in wet weight. Before and during experiments, they were maintained in the laboratory in plastic garbage containers (70-L size) which were one-third filled with sediments (usually obtained from the Maplewood mudflats) over which 0.4 m of oxygenated and cooled (10 - 13 °C) seawater (18 - 23 ‰ salinity) was circulated at 2 L/min.

All experiments were initiated following 1 week of acclimation by the polychaetes to the system, At that time, $HgCl_2$ or CH_3HgCl mercury added to the water (1 mg/L Hg in 300 L water, for a total of 0.417 g $HgCl_2$ or 0.386 g CH_3HgCl) and the water continuously recirculated through the three experimental tanks (Fig. 1).

Partitioning Experiments

These studies were conducted from September to December 1978 by following the distribution with time of added mercury between water, suspended material in water and the sediments. Background concentrations were determined in water, suspended material and sediments. The system used in the partitioning experiments consisted of 3 tanks with polychaetes and natural sediments (Fig. 1). A control consisted of 3 tanks containing polychaetes and natural sediments, but no mercury was added. There were 25-30 polychaetes in the sediments of each tank. Water samples were collected and mercury concentrations determined 0.5, 1, 3 , 5 , 7 , and 12 h after $HgCl_2$ or CH_3HgCl mercury was introduced to the system.

Periphyton in the tanks was collected for mercury analysis at 1 h, 5 h, 24 h, 2 d, 4 d, 7 d,and 14 d for mercury analysis by scraping triplicate samples from tank walls in each tank with plastic weighing boats. Periphyton in the tygon tubing was obtained by removing 3 pieces of tubing, which was split and the periphyton removed with a plastic weighing boat.

Triplicate samples of suspended materials in the water were collected 8 h after the mercury was introduced to water and fractionated according to size using a series of pre-weighed Nucleopore filters. Background concentrations were determined on the filters. The adsorption of mercury on filters was determined

using filtered mercury-laden seawater. Each filter, and the material on it, was weighed (to constant weight) and retained for total mercury analysis. No attempt was made to differentiate between living and dead cells or the inorganic and organic fractions making up the suspended material.

The concentration of mercury in sediment was examined by collecting triplicate sediment samples at 0, 1, and 3 cm depths over a 2-week period following $HgCl_2$ or CH_3HgCl addition.

The total amount of mercury in the sediments was calculated by multiplying the concentration of mercury by the volume of the sediments. Sediment samples from each tank were collected at selected depths with a 50-ml syringe with a 1-cm dia aperture at its tip. The syringe, with plunger fully inserted, was lowered into the sediments to the desired depth whereupon the plunger was withdrawn until approximately 10 mL of material had been removed. After sampling, the syringe was placed upright on a stand for approximately 10 min to allow the sediments to settle. Any water was expelled from the syringe tip and the sediments divided into five aliquots of 1-2 g for mercury analysis. The total amount of mercury in the sediments wasa calculated by multiplying the mercury concentration by the respective sediment volume.

The polychaetes were sampled at the same time as the sediments. Depending on the weight of the individuals, 1-3 polychaetes were used in each sample.

The vertical distribution of mercury in sediments was examined by collecting triplicate sediment samples at 0, 1, 2, 3, 4, 6, 8, 10, 12, and 14 cm depths. Two systems were used in these experiments. Three tanks contained natural sediments, water and polychaetes, and 3 tanks contained only natural sediments and water. Dissolved oxygen in worm burrows and interstitial water was determined using a YSI oxygen meter or the spectrophotometric method of Duval et al. (1974).

Mercury Transformation Experiments

The transformation of mercury was examined by long-term (30 d) and short-term (48 h) experiments. The long-term experiments, carried out from February to June 1977, consisted of introducing $HgCl_2$ or CH_3HgCl to two separate systems, one with polychaetes and sediments, the other without polychaetes. Changes in concentrations of both forms in the sediments were monitored for 30 d. Triplicates from each tank were taken for mercury analysis.

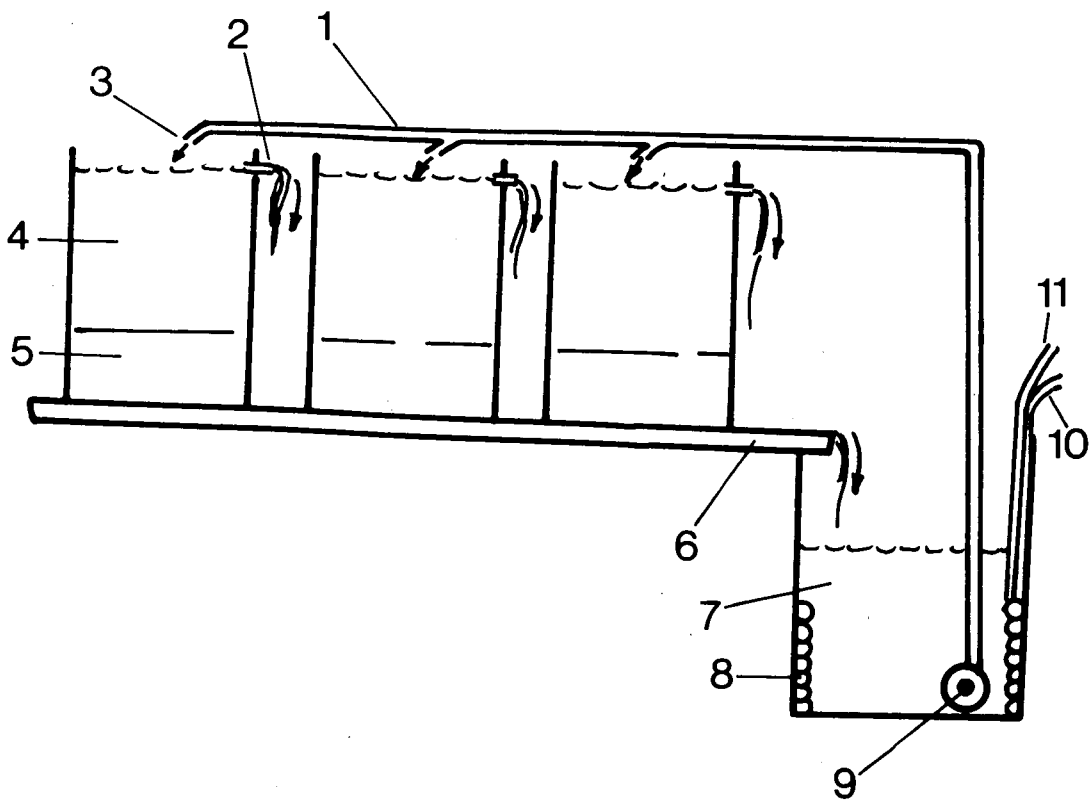
Short-term experiments were carried out in January and February 1979 to examine the role of bacteria in mercury transformations. Mercury ($HgCl_2$ or CH_3HgCl) was added to the water and concentrations of both forms in the water, sediments and polychaetes were determined 8 , 12 , 18 , 24 , 36 , and 48 h after the mercury was introduced. These experiments involved use

of three pairs of tanks. One pair contained natural sediments (including bacteria, fungi, meiofauna, and polychaetes) a second pair of tanks contained natural sediments but no polychaetes, while a third pair tanks contained polychaetes in previously dried inorganic sediments

The inorganic sediments, which were heated to 180 °C for 3 h before use, were obtained from a river bank archeological site near Hope, B.C. It was anticipated that the bacterial population in these sediments would be low and any transformations largely mediated by polychaetes. The intestinal contents of polychaetes were expelled prior to their introduction to furthur reduce bacterial abundance. This was accomplished by relaxing the animal in a magnesium sulfate/seawater solution and allowing it to defecate as it distended. The animals were acclimated in filtered seawater for 1 h before introduction to the inorganic sediments.

Fig. 1 Diagram of tanks, recirculating and cooling system used in these experiments.

(1). Interconnecting tygon tubing; (2). Tank outflow; (3). Water inflow to tank; (4). Large plastic tank containing sediments and seawater; (5). Sediments; (6). Collecting trough for water outflow; (7). Recirculation and cooling tank; (8). Tygon cooling coils; (9). Submersible pump for water circulation to experimental tanks; (10). Cooling water inflow from refrigeration unit; (11). Cooling water return to refrigeration unit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Bacterial biomass in sediments and water was estimated using the lipopolysaccharide (LPS) analysis of Watson et al.(1977). This method, specific for gram-negative bacteria, involves the reaction of Limulus amebocyte lysate (LAL) with a lipopolysaccharide found only in the walls of Gram-negative bacteria.

Pyrogen-free water was obtained from Abbott Laboratories for dilutions. All glassware was either obtained pyrogen-free (such as Corning disposable pipettes) or made pyrogen-free by heating to 180 °C for 3 h.

The spectrophotometric method of determining LPS was used rather than the tube inversion technique (Watson et al. 1977). A standard curve was determined with the Escherichia coli endotoxin standard obtained from Associates of Cape Cod Inc. Triplicate water and sediment samples were collected from each tank for LPS analysis 8, 24, and 48 h after mercury was introduced to the water. Water samples were taken with a 10 mL disposable syringe at the surface and mid-depth. Sediment samples were taken with a 5 mL syringe (modified to have a 0.5 cm aperture) at the 0, 1, 2, and 3 cm and an average calculated. Water and sediment samples were Gram-stained each time a sample was taken for biomass determination to determine if Gram-positive bacteria were present.

Uptake and Clearance Experiments

The uptake and clearance of $HgCl_2$ and CH_3HgCl by the polychaetes in sediments was monitored over 14 d and 30 d respectively. These experiments were conducted from June to Spetember 1978. Polychaetes used in uptake studies were acclimated to natural sediments (with back-ground mercury) for 14 d before mercury, at a concentration of 1 mg/L water, was introduced into the experimental system. A control system, consisting of a set of three tanks with a separate cooling/recirculating tank and pump, but no added mercury, was maintained throughout uptake and clearance experiments to determine background mercury concentrations and any lethal effects of added mercury. Sediments and polychaetes were taken at selected time intervals (0.3, 1, 2, 4, 7, and 14 d) for both $HgCl_2$ and CH_3HgCl analysis. This permitted account to be taken of changes in concentration due to transformation.

Clearance experiments involved removing animals from tanks in which they had been continuously exposed to mercury for 30 d and placing them in mercury-free system from which samples were taken at 0.3, 1, 2, 4, 7, 14, and 30 d.

Mercury Analyses

Total mercury concentration was determined using the cold vapour atomic absorption technique described by Thompson and McComas (1973) (with a few modifications). Water, tissue, and sediment samples used for determination of total mercury concentration were digested in 15 mL concentrated sulfuric-nitric acid (1:3) mixture for 1 h at 80 °C to oxidize all the mercury to the +2 oxidation state. The acid digest was cooled in an ice bath to room temperature, each sample transferred to a side-arm reaction flask, and brought, when necessary, to a volume of 50 mL with deionized water.

The reaction flasks were connected to the atomic absorption unit as sealed vessels, since the nitrogen stream was in the bypass mode (Fig. 2). Hydroxylamine hydrochloride (5 mL, 20 % w/v) and stannous chloride (2 mL, 20 % in 6N HCl) were added to the reaction flask by a syringe through the side-arm septum to reduce the mercury. After stirring for 1 min, the flask was purged with nitrogen at 300 mL/min, which passed the mercury through the absorption cell.

The absorption at 253.7 nm was measured in mv by a digital voltmeter and recorded on a Datex-equipped teletype. When the absorption signal returned to zero, the nitrogen stream was returned to the bypass mode and the sample flask disconnected. A calibration curve of at least 5 data points was established before any sample analysis took place. Blanks were run to

determine residual mercury levels in the reagents.

The atomic absorption unit consisted of a flow-through system with a 30 cm dual-path cell mounted on a Model 100 M Pharmacia Mercury Monitor (Fig. 2). Two drying tubes containing magnesium perchlorate were used to remove water vapours which interfere with the mercury absorption peak at 253.7 nm (Kopp et al. 1972). One was before the reference column, the other between the reaction flask and the sample column. The lower detection limit was 1 ng, and the upper limit was 125 ng. Dilution of test solutions before analysis permitted measurement of higher mercury concentrations.

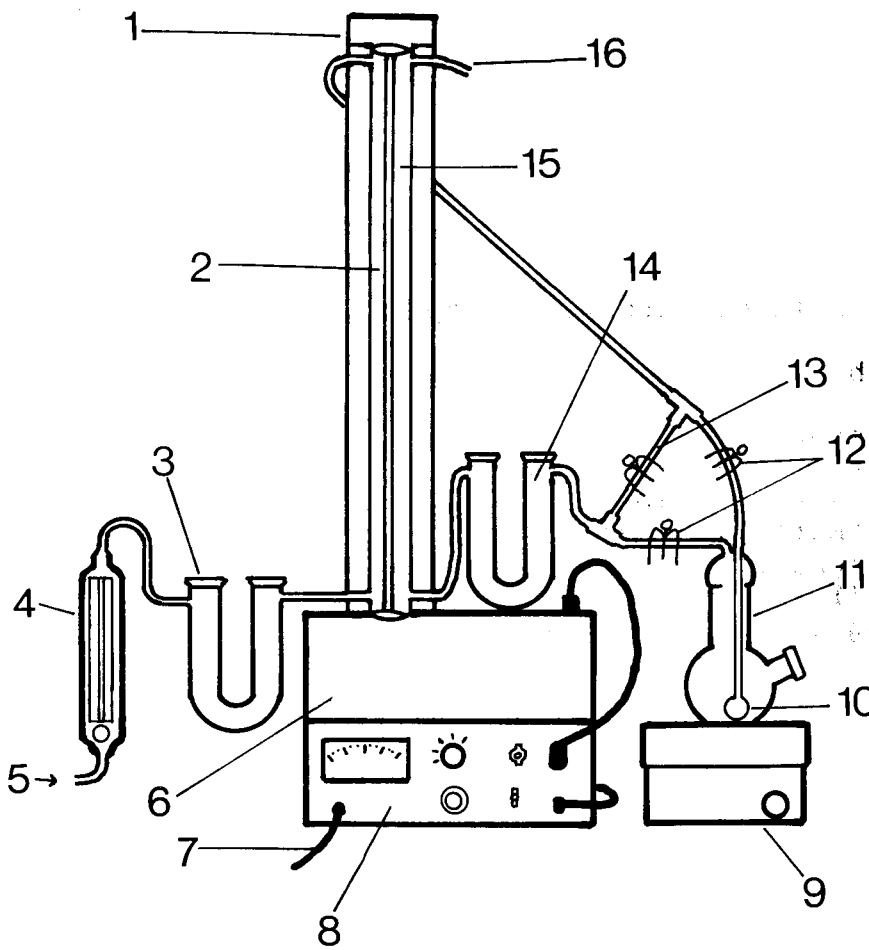
Monomethyl mercury concentrations in the water, sediments, and tissues were determined using the double benzene-cysteine extraction of Bisogni and Lawrence (1973) followed by acid digestion and atomic absorption spectrophotometry. Benzene was removed from the aqueous phase by purging with nitrogen for 20 min before acid digestion since benzene interferes with measured mercury absorption. Blanks were run to insure all the benzene was removed from the aqueous phase. The aqueous extract was digested in a sulfuric - nitric acid (1:3) mixture for 2 h at 95 °C. In addition to the acid mixture, $KMnO_4$ (1 mL, 5 % w/v) and $K_2S_2O_8$ (2 mL, 5 % w/v) was introduced to insure the complete oxidation of the organic mercury. The acid digest was then analyzed by cold - vapour atomic absorption spectrophotometry as described for total mercury. Blanks were run on distilled

deionized water. A background was determined on water, sediments and polychaetes. Mercuric chloride ($HgCl_2$) concentration was determined by subtracting concentrations of CH_3HgCl from total mercury.

All glassware used in mercury analyses was washed in hot soapy water, rinsed with distilled water, soaked in a 1:1 nitric acid-water bath overnight, rinsed in deionized water three times and oven dried. All chemicals used in analyses were reagent-grade low-mercury quality .

Figure 2 Atomic absorption spectrophotometer with long-path flow-through cell.

(1). Photodetector; (2). Reference column; (3). Drying tube; (4). Nitrogen flowmeter; (5). Nitrogen source; (6). Optical unit of atomic absorption spectrophotometer; (7). Signal cord to digital voltmeter and teletype; (8). Control unit of atomic absorption spectrophotometer; (9). Magnetic stirrer; (10). Airstone; (11). Reaction flask; (12). Clamps; (13). Bypass; (14). Drying tube; (15). Sample column; (16). Exhaust to scrubber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Data Analyses

Data from methylation, demethylation, uptake and clearance experiments were curvilinear with time, rapidly approaching an asymptote. This was similar to data decribed by Rigler (1973) and Lean (1973a,1973b) for phosphorous kinetics in lake water. Their data demonstrates phosphorous movement through at least two phases in lake water.

Data from methylation, demethylation, uptake and clearance experiments were plotted on semi-log paper, resulting (clearance data excepted) in a relationship similar to the data depicted in Fig. 3. This curvilinear relationship suggests a movement of mercury compounds through more than one phase in the experimental system. The data from these experiments is the result of movement of mercury through the system (ie. more than one phase). I had to determine a rate of movement through the initial phase (ie. methylation, demethylation, uptake or clearance) from data which was the result of movement through the system.

The initial slope in Fig. 3 is a measure of mercury movement through the initial phase before any mercury in the other phase is returned to the original phase. Rigg's (1963)

method of slope determination derives a time constant by taking the reciprocal of the initial slope, which represents movement through the initial phase. The time constant is the time required to process the total amount of compound in the system through a particular phase. In transformation studies, this constant is the time required to methylate all the $HgCl_2$ or demethylate all the CH_3HgCl in the system before the mercury is retransformed to its original state. I named this constant the methylation time or demethylation time. In uptake studies, the constant is the time required for sediment mercury uptake by the polychaete before clearance begins from the polychaete, returning mercury to the sediments. In this case, I named the constant the uptake time.

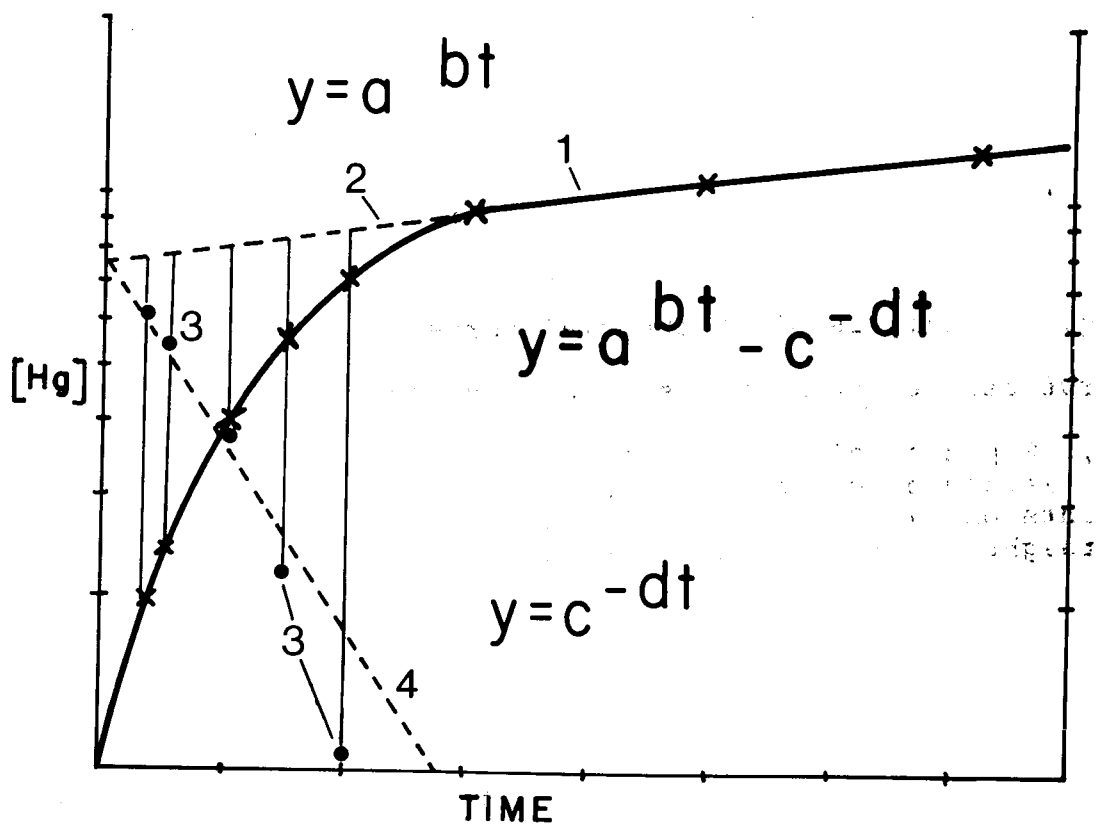
Data from the methylation, demethylation, uptake and clearance experiments were analyzed using Rigg's (1963) graphical method of slope determination and a packaged non-linear derivative-free regression program (BMDPAR). The method involved fitting a straight line to the plateau of the curve (Fig. 3). The points on the curve were subtracted from the corresponding points on the fitted line. The differences were plotted and another straight line was fitted to these points. The equations of the two straight lines, which were actually exponential curves, were added together resulting in the equation of the illustrated curve.(Fig. 3) The second exponential term is the reciprocal of the initial slope of the

curve.

The terms of the equation were used as the initial parameters in determining a derivative-free non-linear regression using the BMDPAR computer program. This program calculates the equation of the curve more precisely than Rigg's (1963) method. The output of this program gives the asymptotic standard deviations of the new calculated parameters as well as the standard deviations of the predicted points.

Fig. 3. Analysis of exponential curve illustrative of type of curve derived from uptake, clearance and transformation data.

(1). Exponential curve to be analyzed; (2). Straight line fitted to plateau of curve; (3). Points calculated by subtracting points on exponential curve from corresponding points on fitted straight line; (4). Straight line fitted to calculated points


y=a bt
1
2
3
[Hg]
y=a bt - c -dt
y=c -dt
3
4
TIME

III. Results

Partitioning Experiments

The distribution of $HgCl_2$ and CH_3HgCl (in the experimental system) is shown in Table 1. Results are expressed as a percentage of the total quantity of mercury initially added to the water of the system (0.417 g $HgCl_2$, 0.386 g CH_3HgCl), which is equivalent to 1 mg/L. Both forms of mercury were rapidly taken out of solution, mainly by the sediments. Only small quantities of mercury (< 20 ng/mL) were detectable in water 7 h after its introduction to the experimental tanks (Fig. 4). A relatively high percentage of $HgCl_2$ and CH_3HgCl (19 and 17 % respectively) was unaccounted for after the first 5 h, but this decreased to <10 % after 4 d and 1 % by 14 d (Table 1).

Seston retained only 2 and 3 % of $HgCl_2$ and CH_3HgCl respectively 8 h after mercury was introduced to water. The distribution of mercury on those particles making up the seston was a function of particle size. Mercuric chloride was apparently concentrated on small particles (Table 2). Methyl mercuric chloride was not determined on particles < 10 μ dia. On particle > 10 μ dia, CH_3HgCl concentration was 1.78 μg/g. It was

assumed the seston was evenly distributed through the experimental system, as the flushing time was approximately 40 minutes in the experimental tanks.

The periphyton on the tank walls and interconnecting tygon tubing also concentrated a small percentage of the total mercury (Table 1).

Mercury added to the system was found primarily near the surface (< 4 cm) of the sediments in which no polychaetes were present. However, it was more deeply distributed (to 14 cm) when polychaetes were present (Table 3). Sediment dissolved oxygen concentration paralleled that of mercury (Table 3).

Table 1

Distribution of mercury (% total amount introduced) measured as total mercury in the experimental system following addition of 1 mg/L in water at t = 0 h.

Mercuric chloride ($HgCl_2$)

Time	Water	Periphyton	Sediments	Worms	Unaccounted
1 h	93	<1	1	-	5
5 h	3	1	77	<1	19
1 d	1	5	83	1	10
4 d	<1	1	84	7	8
7 d	<1	<1	89	9	2
14 d	<1	<1	90	9	1

Methyl mercuric chloride (CH_3HgCl)

1 h	93	<1	1	-	6
5 h	3	1	79	<1	17
1 d	2	4	86	<1	10
4 d	<1	4	87	3	6
7 d	<1	3	89	7	1
14 d	<1	3	89	7	1

Table 2

Mercury concentration on seston

at t=8 h following addition of

1 mg/L Hg++ to water (0.417 g $HgCl_2$ to 300 L water)

Particle dia μ	$HgCl_2$ conc. μg/g	Particle conc. in water mg/L
.5 - 1	2.10	0.057
1 - 5	1.95	0.091
5 - 10	1.69	0.049
>10	1.55	0.153

Table 3

Vertical distribution of sediment total mercury and dissolved oxygen 14 d after addition of 1 mg/L $HgCl_2$ (0.417 g $HgCl_2$ to 300 L water) in presence and absence of polychaetes.

Depth (cm)	% Mercury Burden		Dissolved oxygen (ppm)	
	No Animals	Animals	No Animals	Animals
Surface	30	20	4.1	3.1
1	29	16	0.5	1.3
2	21	13	0	0.8
3	9	10	0	0.5
4	5	10	0	0.3
6	3	9	0	0.3
8	2	9	0	0.2
10	1	0	0	0.2
12	0	5	0	0
14	0	3	0	0

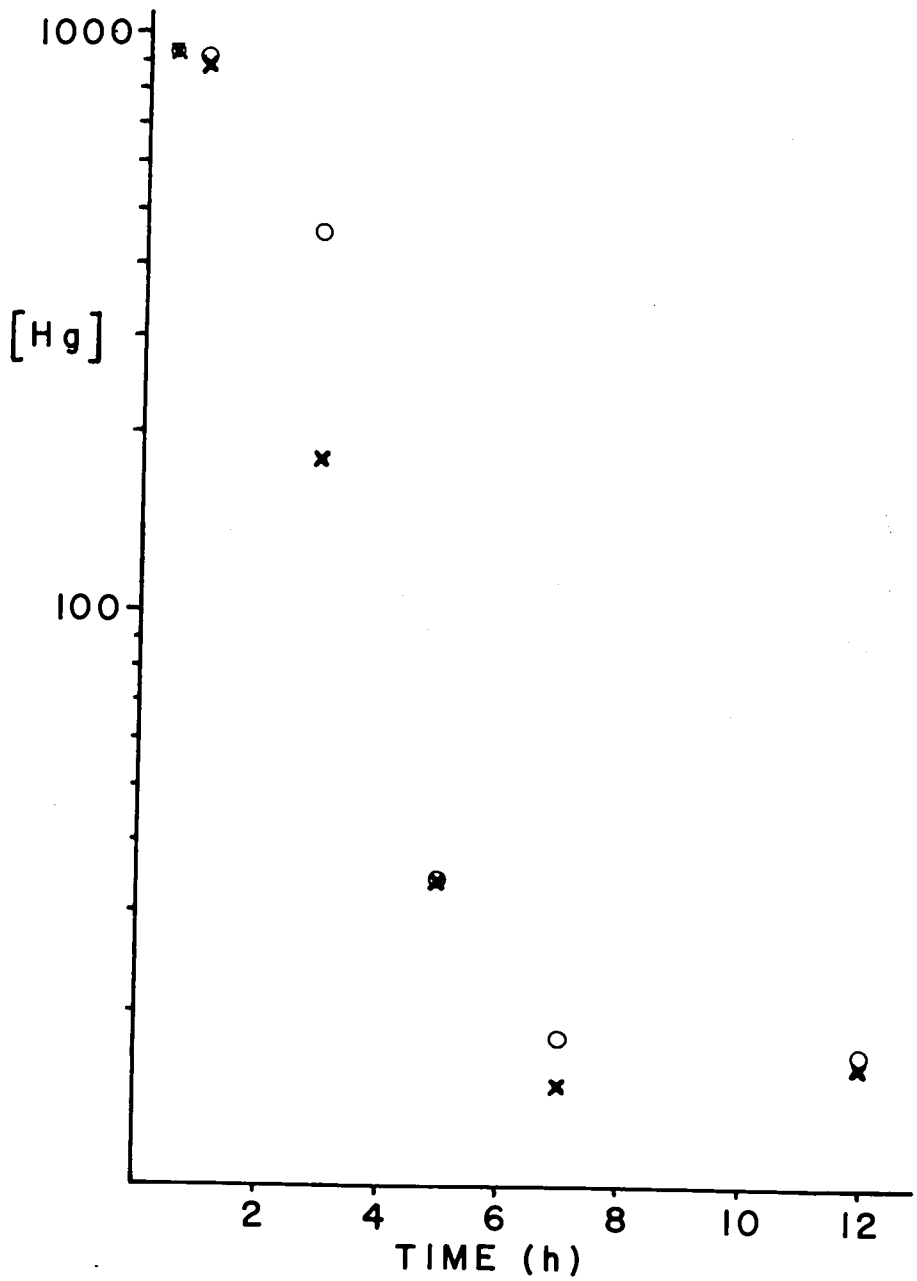
Fig. 4. Mercuric chloride and methyl mercuric chloride remaining in seawater (12 °C, 19 ‰ salinity) as a function of time after addition.

A total of 0.417 g $HgCl_2$ or 0.386 g CH_3HgCl was added to 300 L water (1 mg/L water).

Values are means of 3 replicates.

(x) $HgCl_2$

(O) CH_3HgCl


1000
[Hg]
100
2
4
6
8
10
12
TIME (h)

Mercury Transformation Experiments

The production of CH_3HgCl and $HgCl_2$ in sediments (due to methylation of $HgCl_2$ and demethylation of CH_3HgCl respectively) in the presence and absence of polychaetes are shown in Fig. 5 and 6 respectively. The reciprocal of the initial slope of these curves yields the transformation time. Resultant values are presented in Table 4.

Table 4

Transformation time (days) of mercury in the sediments in presence and absence of polychaetes calculated from 30-d experiments.
Mean initial conc. of $HgCl_2$ and CH_3HgCl were 890.0 ng/g and 950.0 ng/g respectively.

	Methylation	Demethylation
Polychaetes present	* not calculated	21.45 ± 3.88
Polychaetes absent	1.33 ± 4.40	1.71 ± 15.32

* Methylation of $HgCl_2$ data exhibited considerable scatter (Fig. 5) and calculation of transformation times were not warranted.

Fig. 5. Methylation of $HgCl_2$ in natural marine sediments in the presence and absence of polychaetes over 30 d.

Mean initial $HgCl_2$ concentration in sediments (surface) was 890 ng/g

Water temperature was 12-13 °C and salinity was 21-23 ‰

(●) Polychaetes present

(■) Polychaetes absent

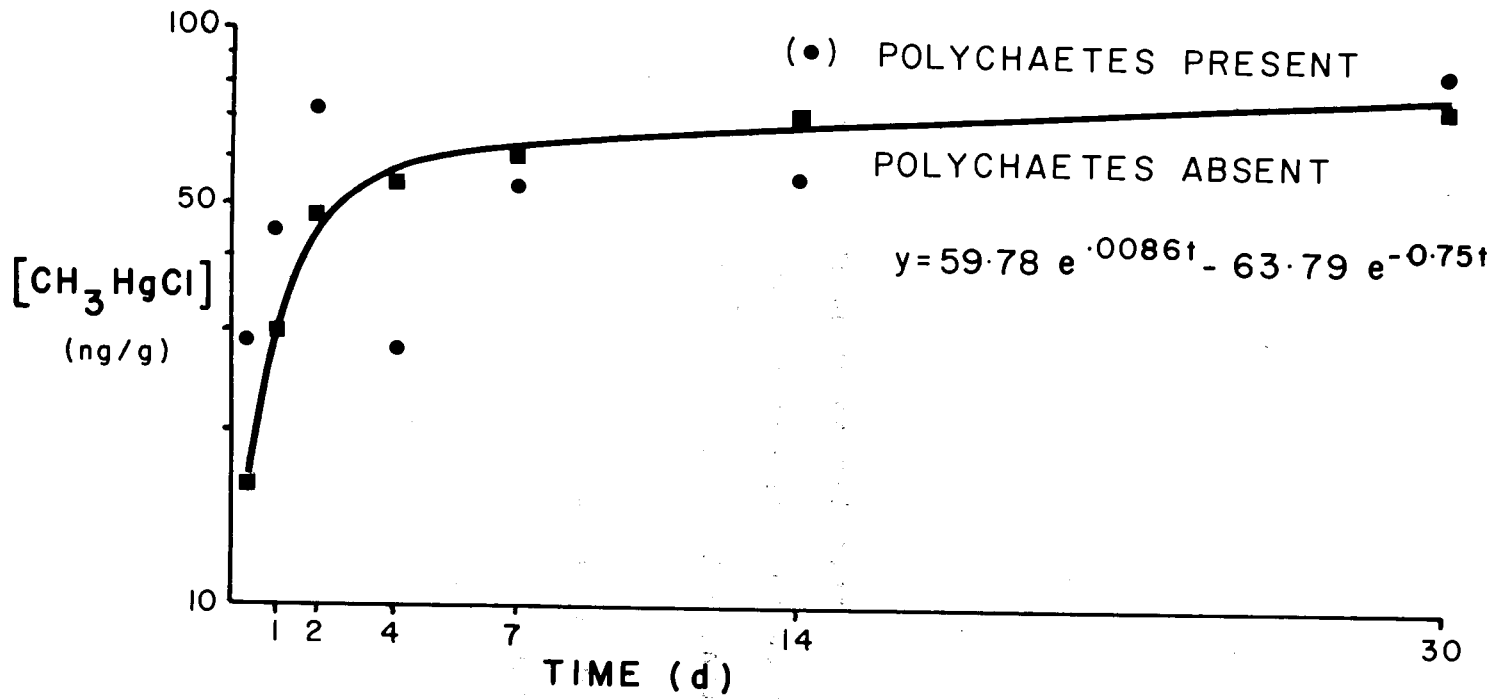
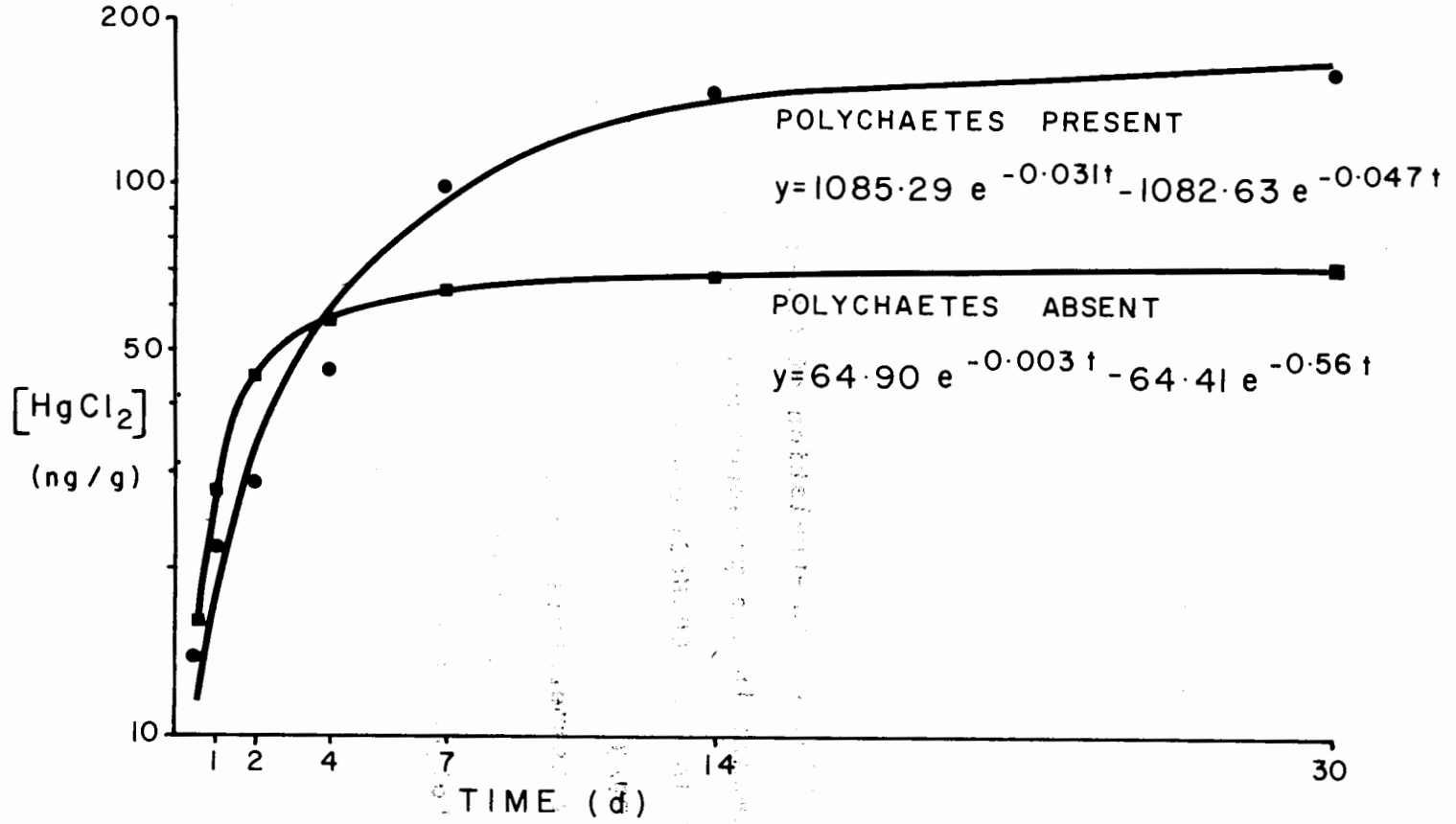

100
50
10
[CH3HgCl]
(ng/g)
(•) POLYCHAETES PRESENT
POLYCHAETES ABSENT
y=59·78 e ·0086t - 63·79 e -0·75t
1 2 4 7 14 30
TIME (d)

Fig. 6. Demethylation of CH_3HgCl in natural marine sediments in the presence and absence of polychaetes over 30 d. Mean initial CH_3HgCl concentration in sediments (surface) was 950 ng/g

Water temperature was 12-13 °C and salinity was 21-23 ‰

(●) Polychaetes present

(■) Polychaetes absent


200
100
50
10
[HgCl2]
(ng/g)
1
2
4
7
14
30
TIME (d)
POLYCHAETES PRESENT
y=1085·29 e -0·031t -1082·63 e -0·047 t
POLYCHAETES ABSENT
y=64·90 e -0·003 t -64·41 e -0·56 t

The data from the short-term (48 h) transformation experiments are shown in Fig. 7 and 8. The transformation times were calculated in the same manner as described for the long-term experiments, and are presented in Table 5. Methylation and demethylation are consistently more rapid in the natural sediments than in the inorganic sediments. The data were reduced to transformation times at an initial concentration of 100 ng/g mercury (Table 5). Polychaetes had little impact on transformation times in these 48 h experiments.

Bacterial biomass in each experiment is listed in Table 6. There were significant differences in bacterial biomass between inorganic and natural sediments as well as in the natural sediments with and without polychaetes. The transformation times (Table 5) are positively correlated with bacterial biomass. The relationships of biomass (Y) to transformation times (X) are:

$Y = 1.80 \times 10^7 - 3.32 \times 10^6 X$ $\quad r = .99$ $\quad$ Methylation

$Y = 1.37 \times 10^7 - 2.97 \times 10^6 X$ $\quad r = .97$ $\quad$ Demethylation

Table 5

Transformation time (days) for mercury in various substrates.

Mean initial surface sediment mercury concentration in brackets(ng/g)

	Methylation	Demethylation
Natural sediments (no polychaetes)	1.45 ± 2.63 (157)	1.65 ± 4.70 (121)
Natural sediments (with polychaetes)	1.49 ± 0.73 (142)	2.22 ± 3.61 (119)
Inorganic sediments (with polychaetes)	7.09 ± 1.92 (131)	6.58 ± 0.82 (145)

Transformation times as expressed in terms of mean initial sediment mercury concentrations of 100 ng/g

Natural sediments (no polychaetes)	0.92 ± 1.68	1.36 ± 3.88
Natural sediments (with polychaetes)	1.05 ± 0.51	1.87 ± 3.03
Inorganic sediments (with polychaetes)	5.41 ± 1.47	4.54 ± 0.57

Fig. 7. Methylation of mercuric chloride ($HgCl_2$) in various substrates following addition of 1 mg/L $HgCl_2$ to overlying water.

(.)Natural sediments

(x)Natural sediments with polychaetes

(o)Inorganic sediments with polychaetes

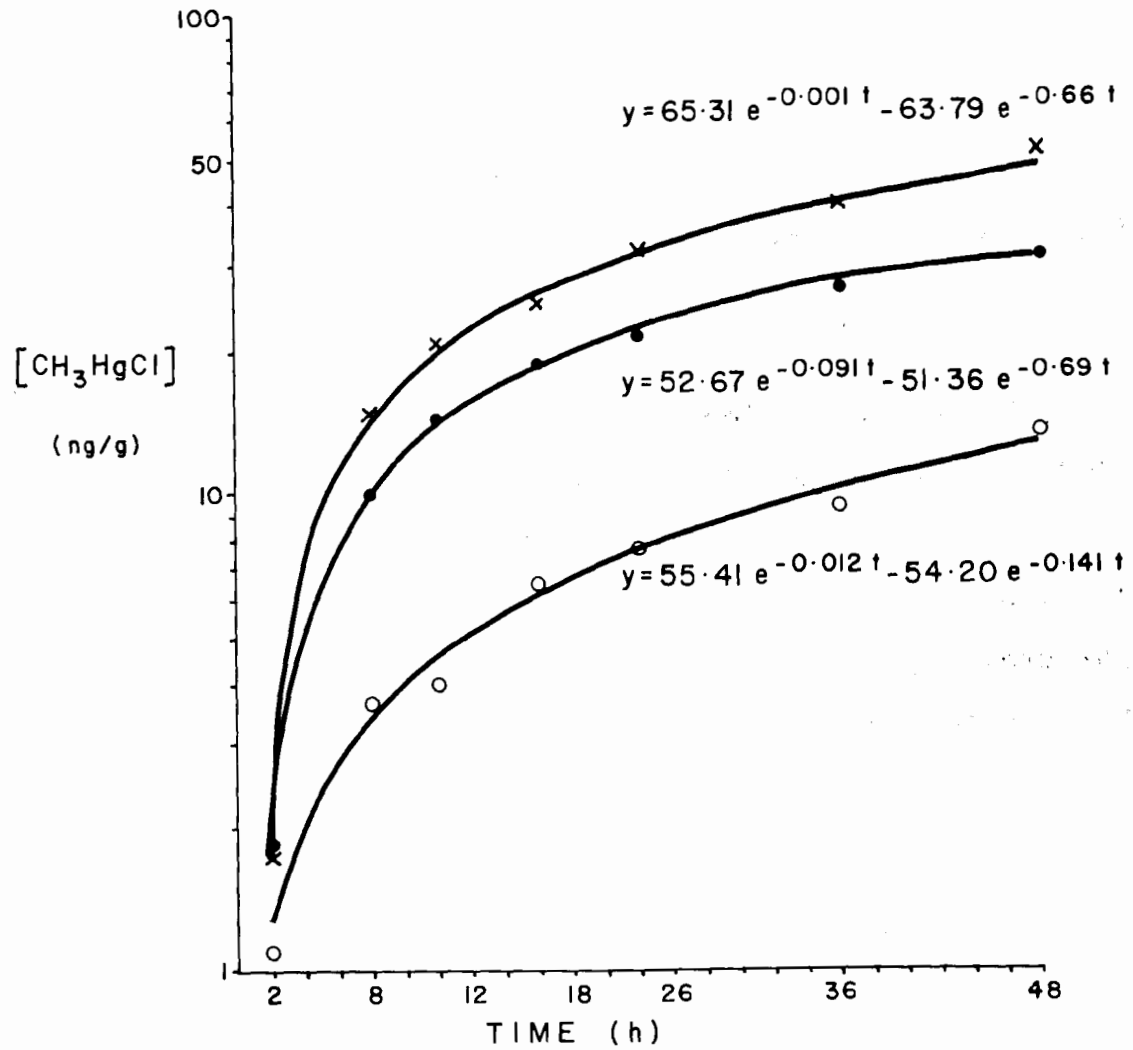

100
50
10
1
[CH3HgCl]
(ng/g)
y = 65·31 e -0·001 t - 63·79 e -0·66 t
y = 52·67 e -0·091 t - 51·36 e -0·69 t
y = 55·41 e -0·012 t - 54·20 e -0·141 t
2
8
12
18
26
36
48
TIME (h)

Fig. 8. Demethylation of methyl mercuric chloride (CH_3HgCl) in various substrates following addition of 1 mg/L CH_3HgCl to overlying water.

(.)Natural sediments

(x)Natural sediments with polychaetes

(o)Inorganic sediments with polychaetes

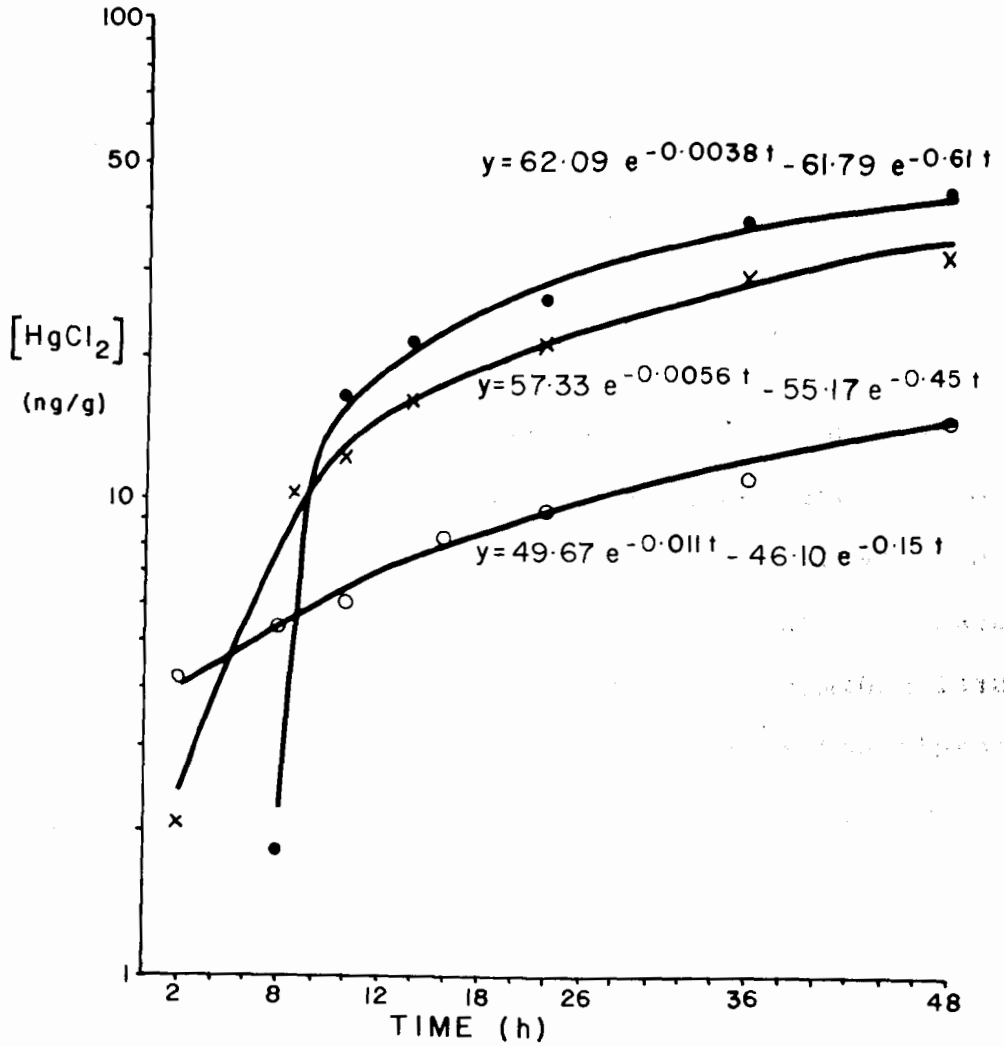

100
50
10
1
[HgCl2]
(ng/g)
y=62·09 e-0·0038 t - 61·79 e-0·61 t
y=57·33 e-0·0056 t - 55·17 e-0·45 t
y=49·67 e-0·011 t - 46·10 e-0·15 t
2
8
12
18
26
36
48
TIME (h)

Table 6

Mean bacterial biomass (fg/ml) at conclusion of 48-h mercury transformation experiments

	Methylation	Demethylation
Natural sediments (no polychaetes)	1.37×10^{7} $\pm 7.0 \times 10^{5}$	8.79×10^{6} $\pm 1.44 \times 10^{4}$
Natural sediments (with polychaetes)	1.58×10^{7} $\pm 9.8 \times 10^{5}$	9.08×10^{6} $\pm 6.24 \times 10^{4}$
Inorganic sediments (with polychaetes)	1.06×10^{3} $\pm 5.6 \times 10^{1}$	9.2×10^{2} $\pm 4.8 \times 10^{1}$

Uptake and Clearance Experiments

The uptake of $HgCl_2$ and CH_3HgCl by the polychaete and its various body tissues are shown in Fig. 9 and 10 respectively. Uptake times were calculated in the same manner as the transformation times and are presented in Table 7. Each polychaete sample had 2-3 individuals. The uptake time for $HgCl_2$ and CH_3HgCl by whole polychaetes are very similar. However, there are significant differences in the uptake time of individual body tissues. Blood takes up CH_3HgCl more rapidly than $HgCl_2$, while muscle tissue takes up $HgCl_2$ more rapidly than CH_3HgCl.

The clearance of $HgCl_2$ from the polychaete and its various body tissues is shown in Figure 11. Clearance times differ for each body tissue (Table 8). Fig. 12 depicts the relationship of clearance of CH_3HgCl from the polychaete body tissues with time. They are similar for each body tissue, but differ significantly from the $HgCl_2$ clearance curves. The mean sediment $HgCl_2$ concentration was 21 ng/g at the end of the clearance experiments. No detectable CH_3HgCl was found in the sediments.

Fig. 9.

Uptake of $HgCl_2$ (ng/g tissue) by polychaete body tissues from sediments following addition 1 mg/L $HgCl_2$ to water.
Curves were calculated with BMDPAR non-linear regression program.

1. Whole body
2. Alimentary canal
3. Blood
4. Muscle tissue

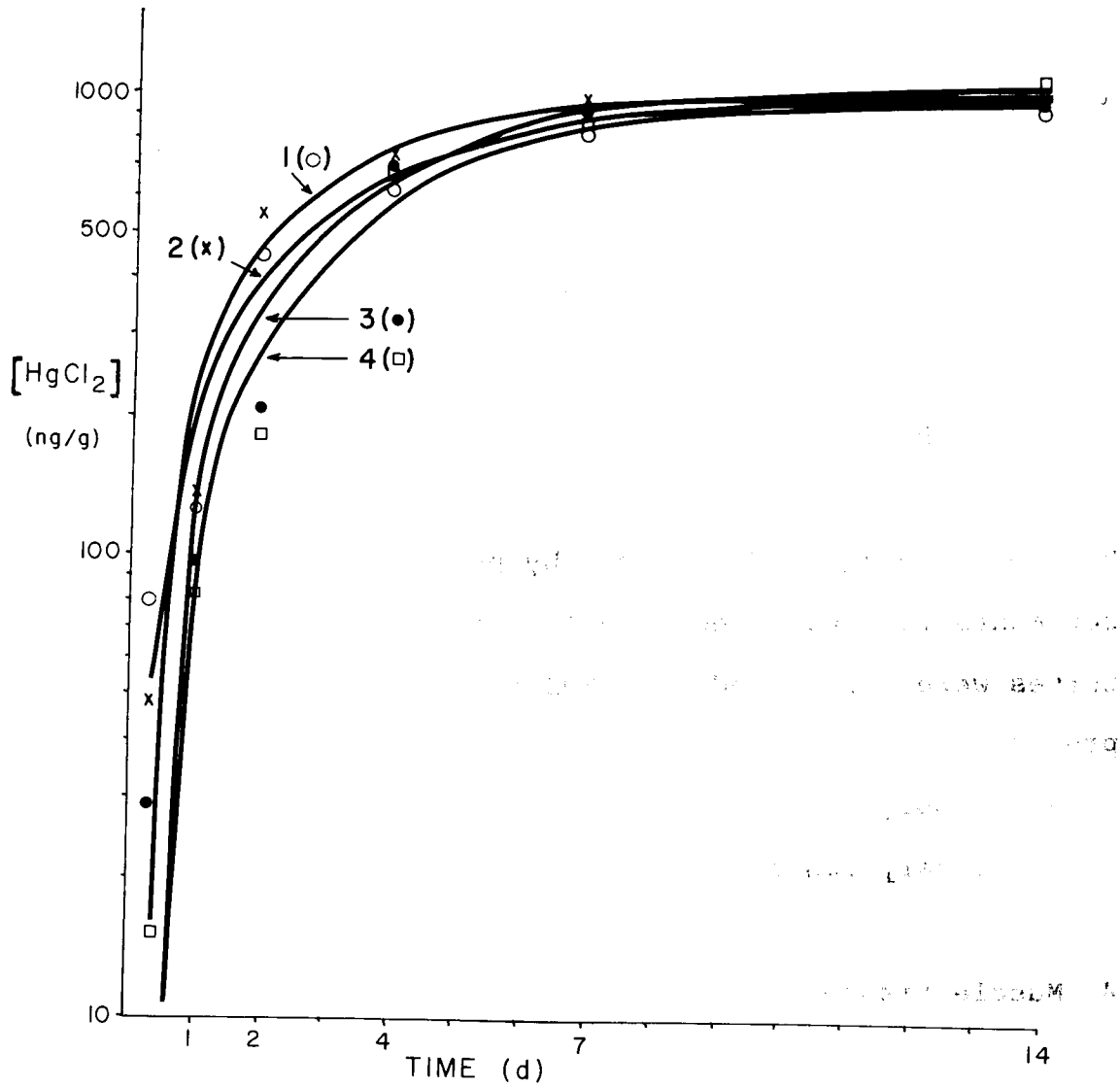

1000
500
100
10
[HgCl2]
(ng/g)
1 (○)
2 (x)
3 (●)
4 (□)
1
2
4
7
14
TIME (d)

Fig. 10.

Uptake of CH_3HgCl (ng/g tissue) by polychaete body tissues from sediments following addition 1 mg/L CH_3HgCl to water. Curves were calculated with BMDPAR non-linear regression program.

1. Whole body
2. Alimentary canal
3. Blood
4. Muscle tissue

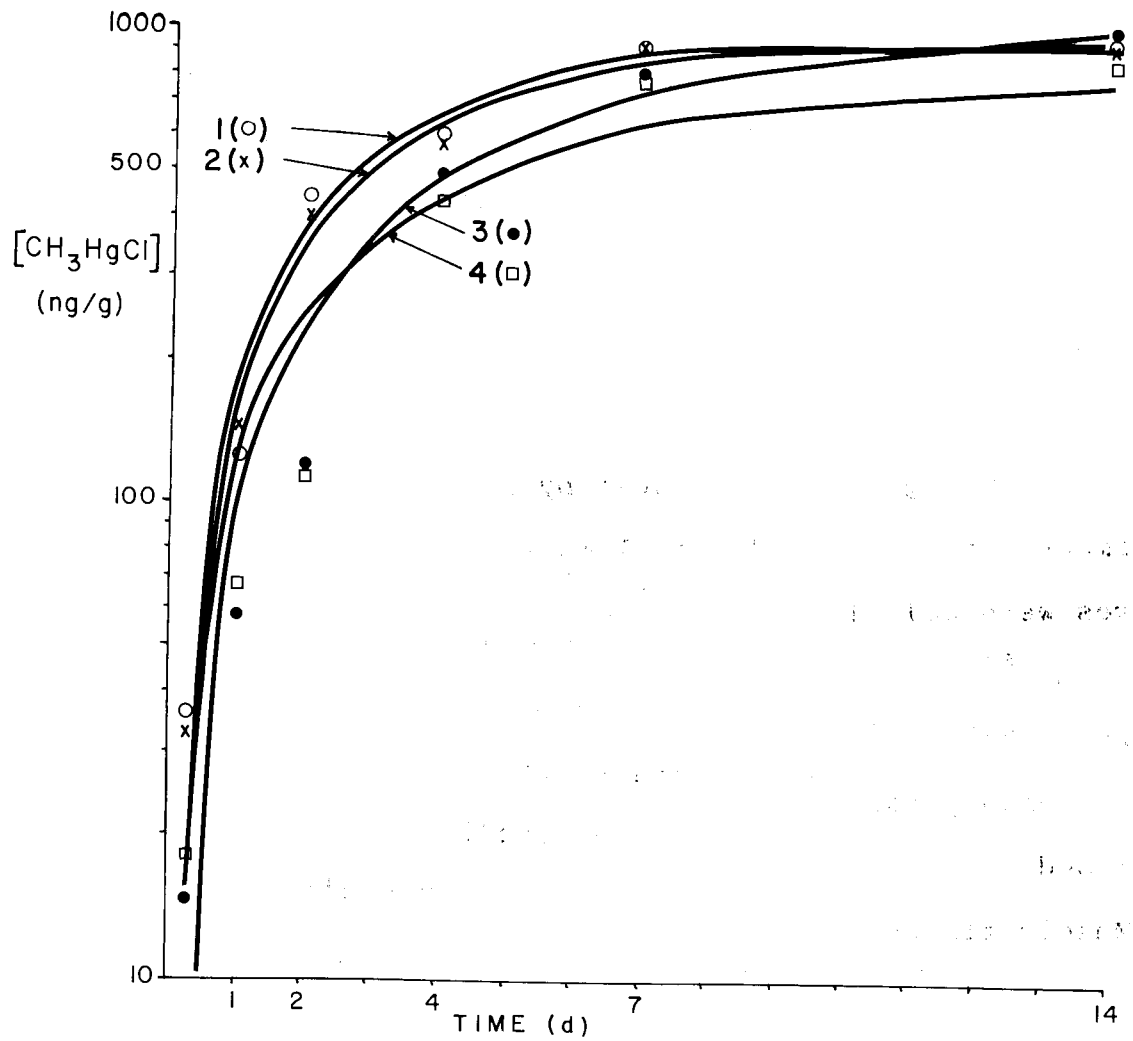

1000
500
100
10
[CH3HgCl]
(ng/g)
1(○)
2(x)
3(●)
4(□)
1
2
4
7
14
TIME (d)

Table 7

Uptake time (days) of $HgCl_2$ and CH_3HgCl by the polychaete *Abarenicola pacifica* from sediments.

(a) Expressed in terms of uptake of 927 ng/g $HgCl_2$ and 941 ng/g CH_3HgCl concentration in sediments.

	$HgCl_2$	CH_3HgCl
Whole body	10.75 ± 97.26	10.33 ± 92.59
Alimentary canal	4.17 ± 3.73	10.68 ± 47.46
Blood	12.42 ± 3.13	6.57 ± 1.02
Muscle tissue	8.48 ± 1.67	12.61 ± 0.88

(b) Expressed in terms of uptake of 1000 ng/g $HgCl_2$ and CH_3HgCl concentration in sediments.

	$HgCl_2$	CH_3HgCl
Whole body	11.60 ± 104.9	10.98 ± 98.40
Alimentary canal	4.50 ± 4.02	11.35 ± 50.37
Blood	13.40 ± 3.37	6.98 ± 1.06
Muscle tissue	9.15 ± 1.80	13.40 ± 0.935

Table 8

Clearance time (days) of $HgCl_2$ and CH_3HgCl in the polychaete Abarenicola pacifica

(a) Expressed in terms of clearance of 935 ng/g $HgCl_2$ and CH_3HgCl concentration in polychaete body.

	$HgCl_2$	CH_3HgCl
Whole body	71.4	1000.0
Alimentary canal	7.9 ± 31.55	1428.6
Blood	2.21 ± 2.90	909.1
Muscle tissue	90.9	1250.0

(b) Expressed in terms of clearance of 1000 ng/g $HgCl_2$ and CH_3HgCl concentration in polychaete body.

	$HgCl_2$	CH_3HgCl
Whole body	76.4	1069.5
Alimentary canal	8.5 ± 33.76	1527.9
Blood	2.4 ± 3.11	972.3
Muscle tissue	97.2	1336.9

Fig. 11. Clearance of $HgCl_2$ from a polychaete and its component tissues maintained in a Hg-free system for 30 *d*. Mean initial $HgCl_2$ concentration was 935 ng/g in polychaete body.

1. Whole body
2. Alimentary canal
3. Blood
4. Muscle tissue

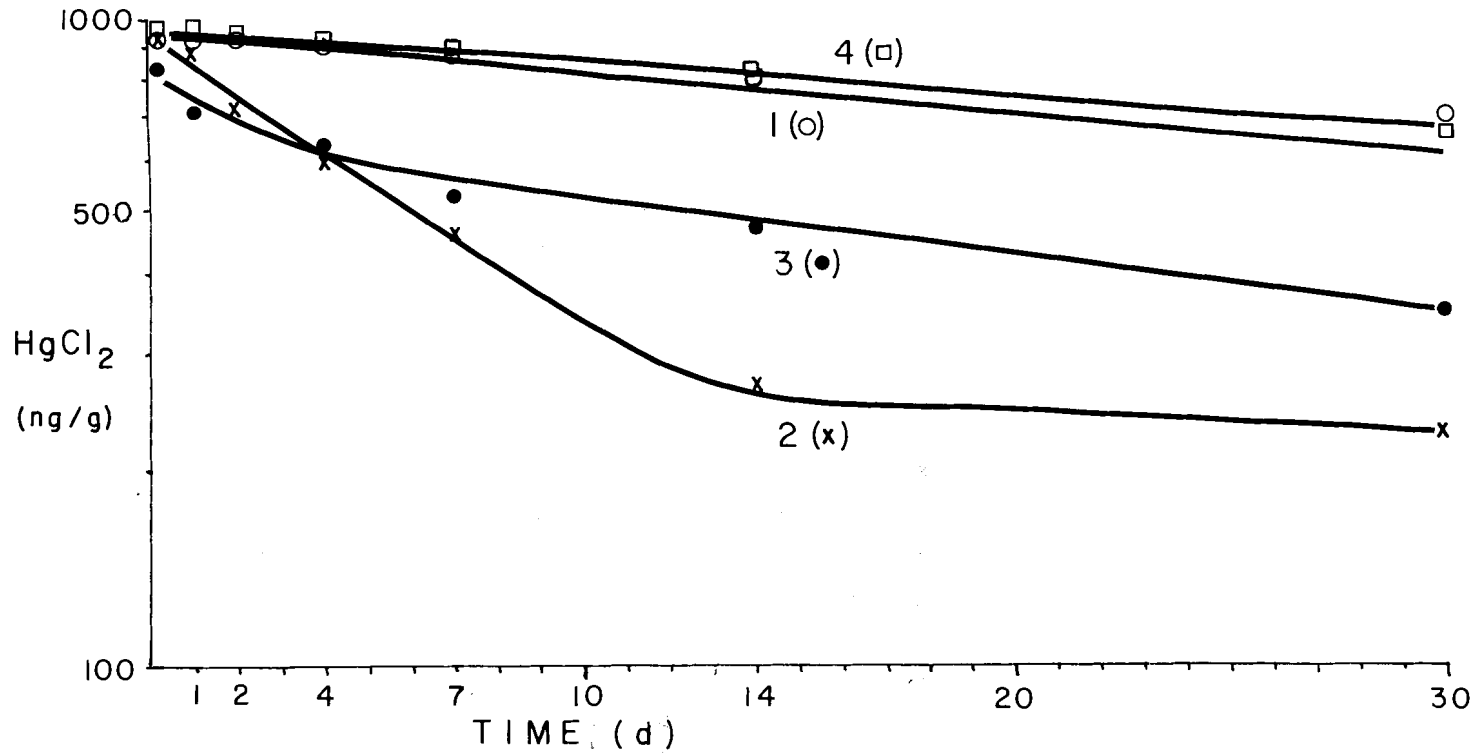
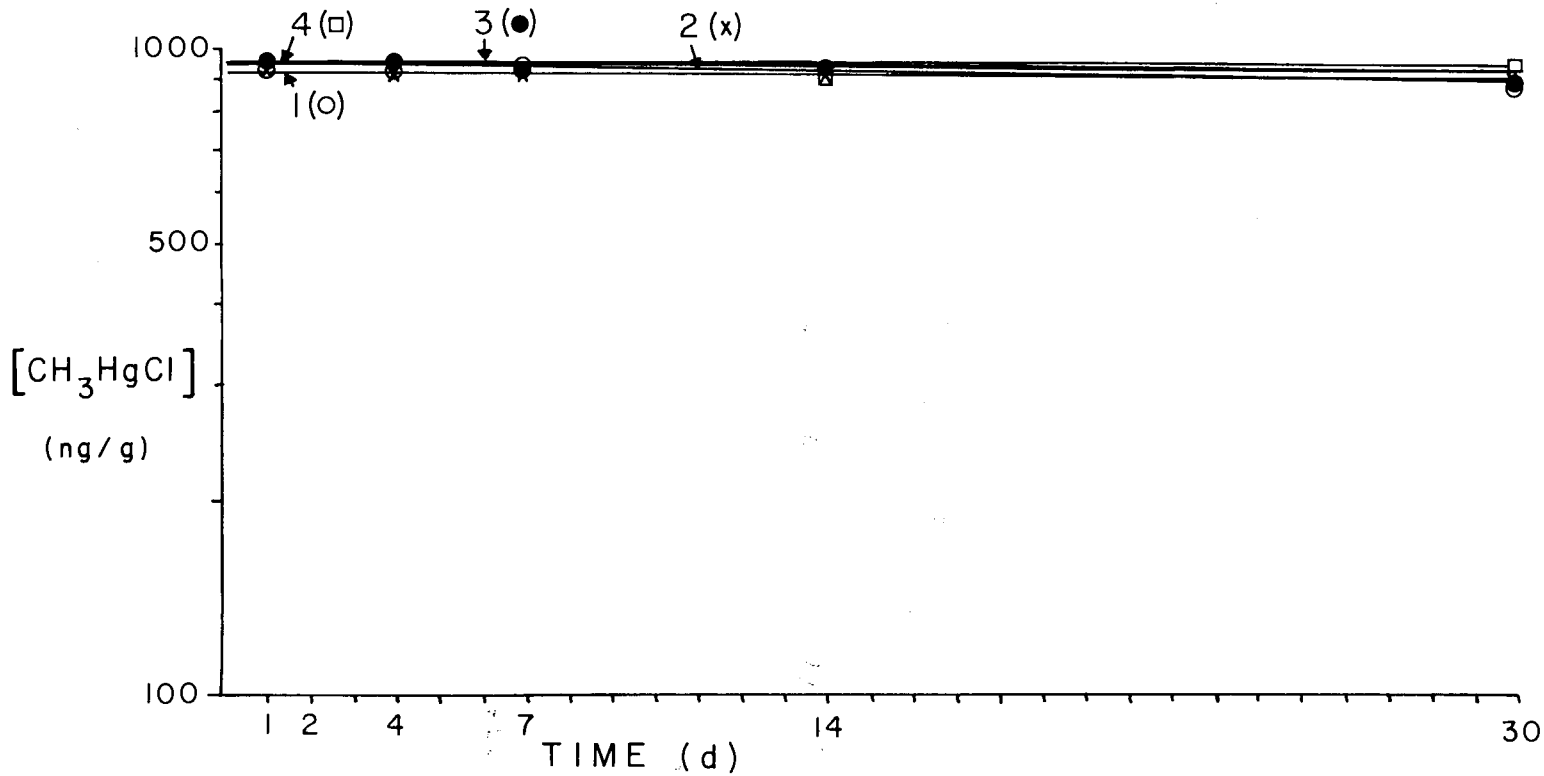

1000
500
100
HgCl2
(ng/g)
4 (□)
1 (○)
3 (●)
2 (x)
1 2 4 7 10 14 20 30
TIME (d)

Fig. 12. Clearance of CH_3HgCl from a polychaete and its component tissues maintained in a Hg-free system for 30 d. Mean initial CH_3HgCl concentration was 935 ng/g in polychaete body.

1. Whole body
2. Alimentary canal
3. Blood
4. Muscle tissue


4 (□)
3 (●)
2 (x)
1 (○)
1000
500
100
[CH3HgCl]
(ng/g)
1 2 4 7 14 30
TIME (d)

IV. Discussion

Partitioning Experiments

In the partitioning, transformation and uptake experiments the addition of $HgCl_2$ and CH_3HgCl to water resulted in a rapid uptake by the sediments. Data from the partitioning experiments show a high percentage of $HgCl_2$ and CH_3HgCl (77 % and 79 % respectively) added to water was taken up by the sediments within 5 h (Table 1), with trace amounts remaining in water 12 h after addition (Fig. 4). The mercury concentration in water 12 h after addition is comparable to mercury concentrations in industrial effluents (Cranston and Buckley 1972).

The mercury concentration on particles (Table 2) was similar to that found in nature by Cranston and Buckley (1972) and Cranston (1976), but the particle concentration in water an order of magnitude lower than their data (0.197 mg/L for particles <10 μ dia and 0.153 mg/L for particles >10 μ dia). The net effect was a small amount of mercury (2 % $HgCl_2$, 3 % CH_3HgCl) added to water was taken up by seston. In natural systems, mercury uptake by suspended particulates is the most important sedimentation mechanism for mercury compounds (D'Itri

1972).

The large amount of mercury rapidly taken up by the sediments (Table 1) suggests a competition situation for mercury exists between sediments and other components, such as periphyton and seston, in the experimental system. This situation was also seen by Ramamoorthy et al. (1977) in river sediments. There are no previous data on the partitioning of mercury in experimental marine systems. However, previous work in a natural system (Cranston and Buckley 1972) showed mercury concentrations in estuarine sediments were 3-4 orders of magnitude higher than mercury concentrations in water and suspended particulates.

The presence of Abarenicola pacifica did not alter the total amount of mercury taken up by the sediments from water (99 % $HgCl_2$, 98 % CH_3HgCl 14 d after addition to water), but resulted in a deeper distribution to a depth of 14 cm (Table 3). A t-test on mercury concentrations below 3 cm showed significantly ($p>.05$) higher mercury concentration in tanks containing polychaetes. Sediment turnover by Abarenicola pacifica has been shown to be 3.4-3.8 g per day (Hobson 1967). This results in mixing of sediments by polychaetes with population densities (25-30 ind./tank) used in these experiments.

Dissolved oxygen concentration paralleled mercury concentration (Table 3). Hylleberg (1975) found Abarenicola

pacifica increases dissolved oxygen in sediment interstitial water.

The presence of Abarenicola pacifica has been shown by Hylleberg (1975) to alter redox potentials in sediments, by creating an oxidized zone in reducing sediments, and to alter sediment grain size by aggregating particles. This results in coarser sediments with larger intersitia. His study showed the presence of the animal stimulated bacterial growth in sediments surrounding the worm burrow by increasing the organic content of the sediments.

It is well known that in reducing sediments, mercury is immobilized by the sulfide ion (Gavis and Ferguson 1972; Hem 1970). However, in oxidizing conditions, mercury is released slowly from sediments to water (Gavis and Ferguson 1972; Kudo 1976) with mercury concentration in interstitial water 3-4 orders of magnitude lower than sediment concentration (Kudo 1976). The release of mercury from sediments to interstitial water has been shown to be positively correlated with dissolved organic carbon (Lindberg and Harriss 1974).

Data from the partitioning experiments indicate mercury is rapidly adsorbed by sediments. Data from these experiments and Hylleberg (1975) suggest the presence of Abarenicola pacifica alters sediment conditions which could lead to the mobilization of mercury in reducing sediments.

Mercury Transformation Experiments

In the 14-d experiments, the demethylation of sediment CH_3HgCl was significantly ($p \geq .05$) longer in the presence of polychaetes in comparison to demethylation without polychaetes (Table 4). Hylleberg (1975) demonstrated the presence of polychaetes stimulated bacterial growth, and data from partitioning experiments showed the presence of polychaetes resulted in a deeper distribution of mercury in sediments. The combined effect of polychaetes in sediments may be an increased bacterial population taking up smaller amounts of CH_3HgCl per cell. Mason et al. (1979) demonstrated bacterial demethylation rate was dependent on substrate concentration. The possible decrease in CH_3HgCl concentration per cell could be the cause of the prolonged demethylation time in comparison to demethylation without polychaetes. However, significantly ($p \geq .05$) more CH_3HgCl was demethylated after 14 d in the presence of polychaetes (Fig. 6).

There were no significant differences in methylation and demethylation times without polychaetes (Table 4). The methylation time in sediments with polychaetes was not calculated, as I felt an attempt to calculate a curve and methylation time from the data in Fig. 5 would not be warranted. However, data from Table 5 do not suggest major differences in methylation time with and without polychaetes.

Results from 48-h experiments show no significant differences between methylation and demethylation times in each experimental system (Table 5). In these short-duration experiments, the polychaetes were not likely able to sufficently alter sediment conditions, and affect the transformation time, as was seen in the 30-d experiments. However, there were significant differences in transformation time between the inorganic and natural sediments with and without polychaetes. Mercury transformations in sediments were likely bacterially mediated, as methylation and demethylation times were positively correlated ($r=.99$, $r=.97$ respectively) with bacterial biomass.

Bacterial biomass (Table 6) was measured using the LPS system of Watson et al. (1977). The LPS method only determines biomass of Gram-negative bacteria. Gram stains indicated only Gram-negative bacteria were present in the water, sediments and polychaetes during the experiments.

There are several reported cases of bacteria which are capable of methylating (Wood et al. 1968; Shin and Krenkel 1976; Berdicevsky et al. 1979) and demethylating mercury (Spangler et al. 1973; Mason et al. 1979; Shariat et al. 1979). It is not known whether the same bacteria both methylate and demethylate mercury in this system, although some Pseudomonas species are capable of both processes (Spangler et al. 1973; Mason et al. 1979).

A comparison of data from Table 4 and 5 must take into consideration the difference in initial concentrations between the two experiments. The data can be converted to transformation rates by dividing the initial concentration by the respective transformation time. The data may also be reduced to transformation times for a common initial concentration, as was done in the bottom half of Table 5. A comparison of converted data from Table 4 and 5 will show a much faster transformation time (or rate, depending on which data conversion is utilized) in Table 4, indicating the rate of transformation is dependent on mercury concentration. This has been shown by Berdicevsky et al. (1979) and Mason et al. (1979).

Results of these experiments indicate $HgCl_2$ methylation and CH_3HgCl demethylation in sediments is dependent on bacterial biomass and mercury concentration.

Uptake and Clearance Experiments

Close examination of the uptake data depicted in Fig. 9 and 10 shows a lag time of approximately 5 h before there was measurable uptake of $HgCl_2$ and CH_3HgCl by polychaetes. Partitioning experiment data indicated that a sediment uptake of a large percentage of mercury occurs within 5 h of mercury addition to water (Table 1). This suggests that $HgCl_2$ and

CH_3HgCl are taken up by the polychaete from sediment rather than water.

The accumulation of $HgCl_2$ in the alimentary canal is more rapid than in the other measured body tissues (Fig. 9, Table 7), whereas the accumulation of CH_3HgCl in blood was significantly more rapid than in the other measured body tissues (Table 7).

My results suggest $HgCl_2$ uptake by polychaetes was primarily by ingestion. This is supported by data from other work which indicates mercury is found on sediment grains (Cranston and Buckley 1972) or bacteria (Ramamoorthy et al. 1977) ingested by Abarenicola pacifica (Hylleberg 1975).

A different uptake mechanism for CH_3HgCl likely exists, as uptake of CH_3HgCl is more rapid in blood and the polychaete whole body than $HgCl_2$ uptake in the same compartments (Table 7). The uptake of CH_3HgCl by absorption through the body wall has been demonstrated by Medeiros et al. (1980) in an errant polychaete. The increased solubility of CH_3HgCl over $HgCl_2$ in water and lipids allow CH_3HgCl to penetrate cell walls more readily than $HgCl_2$ (Gavis and Ferguson 1972).

Mercury methylation has been shown to be a release mechanism of mercury from sediments to water (Shin and Krenkel 1976; Windom et al. 1976; Berdicevsky et al. 1979). However, CH_3HgCl is rarely found dissolved in water, as it is rapidly taken up by organisms.

Data from previous work on partitioning of CH_3HgCl in sediments and my results on CH_3HgCl uptake by Abarenicola pacifica suggest a difference in $HgCl_2$ and CH_3HgCl uptake mechanisms in the animal. Uptake of CH_3HgCl through the gills is indicated by the rapid CH_3HgCl uptake by the blood in comparison to other measured body tissues. Some CH_3HgCl is likely taken up by ingestion, as previously suggested, but not to the same extent as $HgCl_2$ uptake.

The exponential relationship between $HgCl_2$ concentration and time seen in the clearance of $HgCl_2$ from the alimentary canal and blood (Fig. 11) indicates an equilibrium of $HgCl_2$ between phases. There is likely an equilibrium between sediment $HgCl_2$ and gut $HgCl_2$, and an equilibrium between blood $HgCl_2$ and gut $HgCl_2$, as $HgCl_2$ is released from the gut to sediments and from the blood to the gut. The slow clearance of the $HgCl_2$ from the polychaete whole body is due to the retention of $HgCl_2$ by the muscle tissue (Table 8).

Clearance of CH_3HgCl from the polychaete is remarkably slower than $HgCl_2$ clearance, with CH_3HgCl remaining in the body 14 times longer. There was no significant ($p \geq .01$) difference between the slopes in Fig. 12 and a slope=0. The calculated values for clearance times (Table 8) are approximate, as the data were collected over 30 d. The retention of CH_3HgCl by the polychaete could be due to the presence of a metallothionein in the polychaete, but this was not verified.

This suggests the methylation of $HgCl_2$ results in a longer retention time for mercury in the biota of the experimental system, and demethylation of CH_3HgCl results in a shorter retention time.

While mercury methylation has been shown to be a release mechanism of mercury from sediments in nature (Windom et al. 1976), CH_3HgCl is rapidly taken up by Abarenicola pacifica and retained 14 times longer than $HgCl_2$. This results in the prolonged retention of mercury in its most toxic form in a marine food chain.

Literature Cited

Berdicevsky,I.,H.Shoyerman,and S.Yannai. 1979. Formation of methylmercury in the marine sediment under in vitro conditions.Environ. Res. 20:325-334

Bisogni,J.J. and A.W.Lawrence. 1973. Determination of submicrogram quantities of monomethyl mercury in aquatic samples.Environ. Sci. and Technology 8:850-852

Cranston,R.E.,and D.E.Buckley. 1972. Mercury pathways in a river and estuary. Environ.Sci.Tech. 6:274-278

Cranston,R.E. 1976. Accumulation and distribution of total mercury in estuarine sediments. Estuarine and Coastal Marine Science. 4:695-700

D'Itri,F.M. 1972. The Environmental Mercury Problem CRC Press.Chemical Rubber Co.,Ohio

Duval, W.S., P.Brockington, M.S. vonMelville, and G.H.Geen. 1974. Spectrophotometric determination of dissolved oxygen in water. J.Fish.Res. Board Can. 31:1529-1530

Eganhouse,R.P. and D.R.Young. 1978. Total and organic mercury in benthic organisms near a major submarine wastewater outfall system. Bull.Environ. Contam.Toxicol. 19:758-766

Gavis,J. and Ferguson,J.F. 1972. The cycling of mercury through the environment. Water Research. 6:989-1008

Hem,J.D. 1970. Chemical behaviour of mercury in aqueous media. U.S. Geol. Prof. Paper 713.

Hobson,K.D. 1967. The feeding and ecology of two north Pacific Abarenicola species(Arenicolidae,Polychaeta). Biol.Bull. 133:343-354

Hylleberg,J. 1975. Selective feeding by Abarenicola pacifica with note on Abarenicola vagabunda and a concept of gardening in lugworms, Ophelia 14:113-137

Johnels,A.G. 1971. Mercury:Observed levels and their dynamics in the environment:Results from Sweden; in Proceedings of the Royal Society of Canada Special Symposium on Mercury in Man's Environment. Ottawa. Feb.15-16, 1971.

Kopp,J.F.,M.C.Longbottom,and L.B.Lobring. 1972. Cold vapour method for determining mercury. J. Amer. Waste Water Assn. xxx:20-25

Kudo,A. 1976. Mercury transfer from bed sediments to freshwater fish. J.Environ.Qual.5:427-430

Lean,D. 1973a. Phosphorous dynamics in lakewater. Science.179:678-680

Lean,D. 1973b. Phosphorous movement between biologically important compartments. J.Fish.Res.Board Can. 30:1525-1536

Lindberg,S.E.,and R.C.Harriss. 1974. Mercury-organic matter associations in estuarine sediments and interstitial water. Environ.Sci.Tech. 8:459-462

Mason,J.W.,A.C.Anderson,and M.Shariat. 1979. Rate of demethylation of methylmercuric chloride by Enterobacter aerogenes and Serratia marcescens Bull. Environ. Contam. Toxicol. 20:262-268

Medeiros,D.M.,L.L.Cadwell,and R.L.Preston. 1980. A possible physiological uptake mechanism of methyl mercury by the marine bloodworm (Glycera dibranchiata). Bull. Environ. Contam. Toxicol.24:97-101

Ramamoorthy,S., S.Springthorpe,and D.J.Kushner. 1977. Competition for mercury between river sediment and bacteria. Bull. Environ. Contam. Toxicol.17:505-511

Riggs,D.S. 1963. The Mathematical Approach to Biological Problems: A Critical Primer. The Williams and Wilkins Co., Baltimore

Rigler,F.H. 1973. A dynamic view of phosphorous cycle in lakes, in Environmental Phosphorous Handbook. E. Griffiths et al. (ed). John Wiley and Sons. Toronto

Shariat,M.,A.C.Anderson and J.W.Mason. 1979. Screening of common bacteria capable of demethylation of methylmercuric chloride.Bull.Environ.Contam. Toxicol. 20:255-261

Shin, E.B. and P.A.Krenkel. 1976. Mercury uptake by fish and biomethylation mechanisms. J.Water Poll.Control Fed. 48:473-501

Spangler,W.J., J.L.Spigarelli,J.M.Rose, R.S.Flippin, and H.M.Miller. 1973. Degradation of methylmercury by bacteria isolated from environmental samples. Appl.Microbiol. 25:488-493

Thompson,J.A.J. and F.T.McComas. 1973. Distribution of mercury in the sediments of Howe Sound, British Columbia. Fish. Res. Board Technical Report No.396

Vostal,J. 1972. Transport and transformation of mercury in nature and possible routes of exposure, in Mercury in the Environment L. Friberg and J. Vostal (ed). CRC Press. Cleveland

Watson,S.W., T.J.Novitsky,H.L.Quinby and F.W.Valois. 1977. Determination of bacterial number and biomass in the marine environment. Applied and Environmental Microbiolgy. 33:940-946

Windom,H.,W.Gardner, J.Stephens and F.Taylor. 1976. The role of methylmercury in a salt marsh ecosystem. Estuarine and Coastal Marine Science. 4:579-583.

Wood.J.M.,F.S.Kennedy,and C.G.Rosen. 1968. Synthesis of methyl-mercury compounds by extracts of a methanogenic bacterium. Nature 220:173-174