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-原 著-

Uptake and Fate of Inorganic Mercury in the Eastern Oyster, *Crassostrea virginica*

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이스턴 오이스터 *Crassostrea virginica* 에서 無機水銀의 攝取와 蓄積

조 정 현

뉴저지 채리힐 보건부

要 約

重金屬은 海水에서보다 海水中의 生物系에 그 濃度가 더 높은 것으로 알려져 있으며 水銀도 無機型으로 生物系에 存在하다가 生物活動으로 因해 알킬型으로 變한다. food chain이 微量金屬 蓄積의 主通路로 알려져 있으나 그외 몇가지 方法이 提示되고 있다. 特히 연체동물은 微量金屬의 蓄積現狀이 溫度, 폭로시간, 時間, 生理的 活動에 따라 相當히 달라진다. 또한 有機알킬 水銀이 無機水銀보다 比較的 낮은 毒性을 나타내지만 後者의 被害도 무시할 수는 없다. 굴(crassostrea virginica)은 鹽化水銀濃度가 比較的 낮은 狀態에서도 相當量의 蓄積現狀을 보여주었다. 두개의 compartment system을 連繫的인 狀態에서 보면 첫 compartment에서의 初期 蓄積은 可逆的이었으나 둘째 Compartment에서 測定된 損失率로 보아 非可逆的 蓄積現狀을 確認해 주었다. 뿐만 아니라 微量金屬이 아가미와 外部 筋肉에서 가장 높은 蓄積率을 보인것은 flow system의 regression model과 매우 흡사함을 암시하며 濃度가 낮은 狀態에서의 蓄積現狀에 重要한 의미를 부여했다.

Introduction

It is recognized that the concentration of metals is significantly higher in the marine biosphere than in the hydrosphere, yet the mechanisms through which trace metals enter the marine biosphere are not completely defined.

In the case of mercury, it was initially throught that concentrations present in the biosphere were in inorganic or phenyl forms as released in industrial wastes or fungicides. Later developments, however, have shown that mercury exists predominantly as alkyl compounds formed as a result of biological activity. (Jensen and Jernelov, 1969; Wood, *et al.*,

Further, while the food chain has been throught to be the primary route by which marine organisms concentrate trace metals, several alternate pathways have been suggested

for concentration, which include: 1) ingestion of suspended detritus containing the element, complexing of metals by coordinate linkages, 3) the incorporation of metal ions into physiologically important systems, and 4) uptake by ion exchange, as demonstrated on the mucous sheets of the oyster, (Goldwater, 1971; Brooks and Rumsby, 1965). Mollusks in the natural environment have been shown to vary in their accumulation of trace metals with temperature, duration of exposure, the species concerned, and physiological activities of the mollusks themselves all playing significant roles.

It is clear at this time that the alkyl mercury compounds are considerably more toxic than their inorganic and aryl counterparts; however, the hazard of the inorganic compounds cannot be neglected (Nordberg, *et al.*, 1969). Recent studies by Kopfler have shown rapid direct uptake of mercuric chloride from water by oysters to levels that are considered prohibitive in human foodstuffs (Kopfler, 1973).

Materials and methods

Eastern oysters (*Crassostrea virginica*) were obtained from cultivated reefs in Louisiana estuaries through commercial fishermen. Specimens were selected by size (height 8-12 cm, length 5-8 cm) and adapted to controlled laboratory conditions, (T-24°C, salinity 16°/oo ± ± 1°/oo, turbidity 1 Jackson Turbidity Unit (JTU), pH 8.3 ± 0.2 units, aerated synthetic sea water*) over a seven day period prior to study.

The uptake of mercury was observed in a series of six experiments held in a recirculating 340 liter filtering ($\geqslant$ 6 liters/oyster/hour) system composed of epoxy coated mild steel. Reagent grade mercuric chloride was used as the source of mercury in all experiments.

At intervals of 2, 4, 8, 16, 32, 64, 128, and 256 hours, nine specimens were removed and pooled for analysis.

Decontamination studies were performed with oysters remaining after each initial 256 hour uptake experiment. Five specimens were removed at periodic intervals following the onset of decontamination. Mercury levels in the experimental system were decreased to levels below the limit of detection within one hour of the onset of decontamination by disconnecting the mercury feed system and replacement of water and filter. The 256th hour of uptake was taken as zero hour of decontamination. Approximately eighty oysters were maintained in a separate control system, with equal filtration rates, throughout the duration of each experiment.

Analyses were performed in duplicate by the Hatch and Ott (1968) procedure on 1 mg aliquots of homogenized oyster tissue resulting from the pooling of the nine specimens described above. Water condensors were used in refluxing the digests (Mayer, 1970; McDuffie, 1971). Sample aliquots were kept in a flowing cool (18°C) water bath following digestion to minimize volatile losses.

Results and discussion

Oysters (*C. virginica*) were found to accu-

* Instant Ocean, Registered Trademark

mulate significant levels of mercury on exposure to relatively low concentrations of mercuric chloride. Average tissue concentrations after 2.56 hours exposure varied from 12.3 μg/gm in a 10 μg/lexposure to 44.12 μg/gm at 100 μg/l. Concentration ratios of 1230 at 10 μg/l, 923 at 40 μg/l, 455 at 80 μg/l and 441 at 100 μg/l were reached following 256 hours of exposure. In a separate extended study at 25 μg/l the final concentration of total mercury and concentration ratio at 512 hours (22d) of exposure were 47.2 μg/gm and 1880 respectively.

1. Accumulation

The accumulation of mercury was found to occur in two distinct phases similar to that reported for the uptake of ^{51}Cr by Hermione

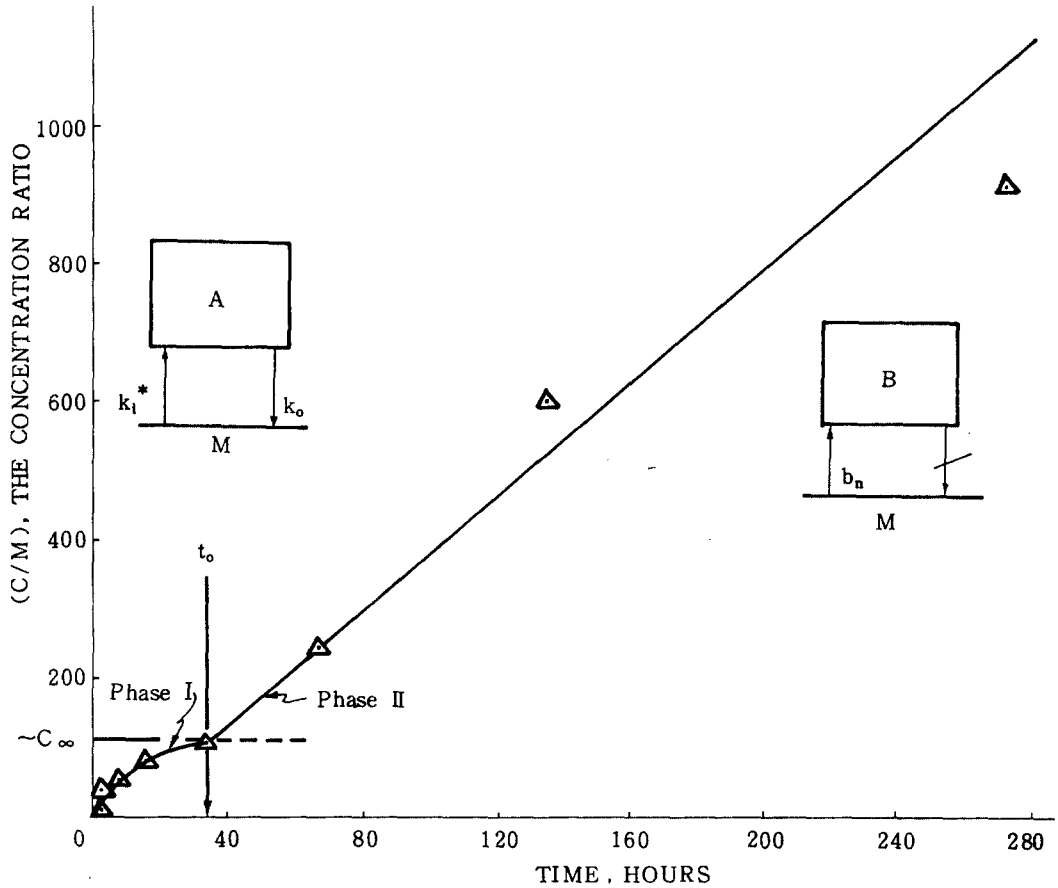

Fig. 1. The accumulation of mercury at 40μg/l by C. virginica. A=Model for Phase I representing a single compartment in exchange with a medium of constant concentration k_i^*, k_o (the influx & efflux constants after Ruzic 1972). B= Model for Phase II, representing a single compartment with the influx constant equal to the slope b_n and $k_o = 0$. t_o corresponds to the beginning of Phase II.

* t_o represents the time at which the phase changes.

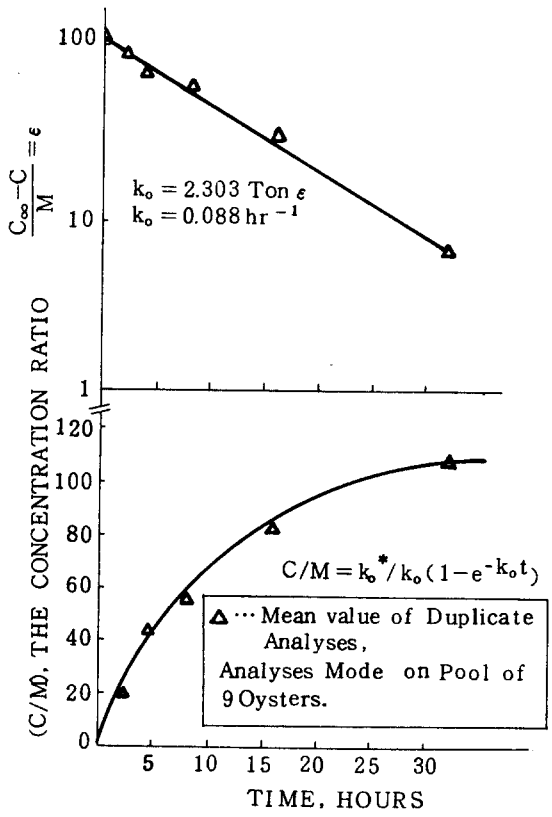

Fig. 2. The accumulation of mercury at 40 $\mu g/l$ by C. virginica during phase I, $t < t_o$, k_1^* = the influx constant, k_o = outflux constant, C_∞ = the estimated whole body concentration of mercury, $\mu g/gm$, for an infinite exposure during the reversible phase. C_∞ is estimated by least square regression of the logarithmic equation.

hystrix which was initially described by Chipman in 1966, and later interpreted by Ruzic (1972) as the first compartment of a sequential two compartment system. During the initial phase, accumulation may be described as a reversible first order process as shown in Figures 1 and 2.

Kinetic parameters may be obtained by graphic solution of the data as shown in Figure 2 and subsequent solution of the first order model for the estimated equilibrium position of the accumulation. Whole body concentrations calculated for the first phase of accumulation were found to agree closely with measured values (± 10%). During phase one there is apparently no depression of accumulation resulting from the action of mercury on the osyter and accumulation proceeds in proportion to the concentration of aqueous mercury. Examination of the data in Table 1 shows agreement of the kinetic constants (k_1^*, k_0) determined in each of the experiments; as would be expected in freely reversible accumulation of this form. While there is some variation in the experimental values found, these appear small ($0.088h^{-1} \sim 0.124h^{-1}$) with the exception of the data from the experiments at 25 $\mu g/l$.

Table 1: Comparison of influx and outflux constants developed for 10, 20, 25, 40, 80, and 100 $\mu g/l$ exposures to mercuric chloride.

Uptake Parameters	Mean Water Concentration of Mercury Exposed to Oysters, M, ($\mu g/l$)					
	10 $\mu g/l$	20 $\mu g/l$	25 $\mu g/l$	40 $\mu g/l$	80 $\mu g/l$	100 $\mu g/l$
k_o	$0.124\,hr^{-1}$	$0.099\,hr^{-1}$	$0.025\,hr^{-1}$	$0.088\,hr^{-1}$	$0.099\,hr^{-1}$	$0.104\,hr^{-1}$
k_1^*	$9.44\ hr^{-1}$	$9.90\ hr^{-1}$	$7.97\ hr^{-1}$	$10.68\ hr^{-1}$	$9.60\ hr^{-1}$	$10.36\ hr^{-1}$
C/M[a]	76	100	320	116	97	100
t_o	8	32	128	32	32	32

[a] C/M is the ratio of whole body concentration to aqueous concentration at the end of the reversible phase. C/M is assumed to approach the value C_∞/M as t approaches t_o.

Accumulation, however, shows a marked change in form as a second phase is entered just short of equilibrium in the initial uptake.

Examination of the data in Figure 3 shows the effect of increasing aqueous concentrations of mercury on the biological multiplication

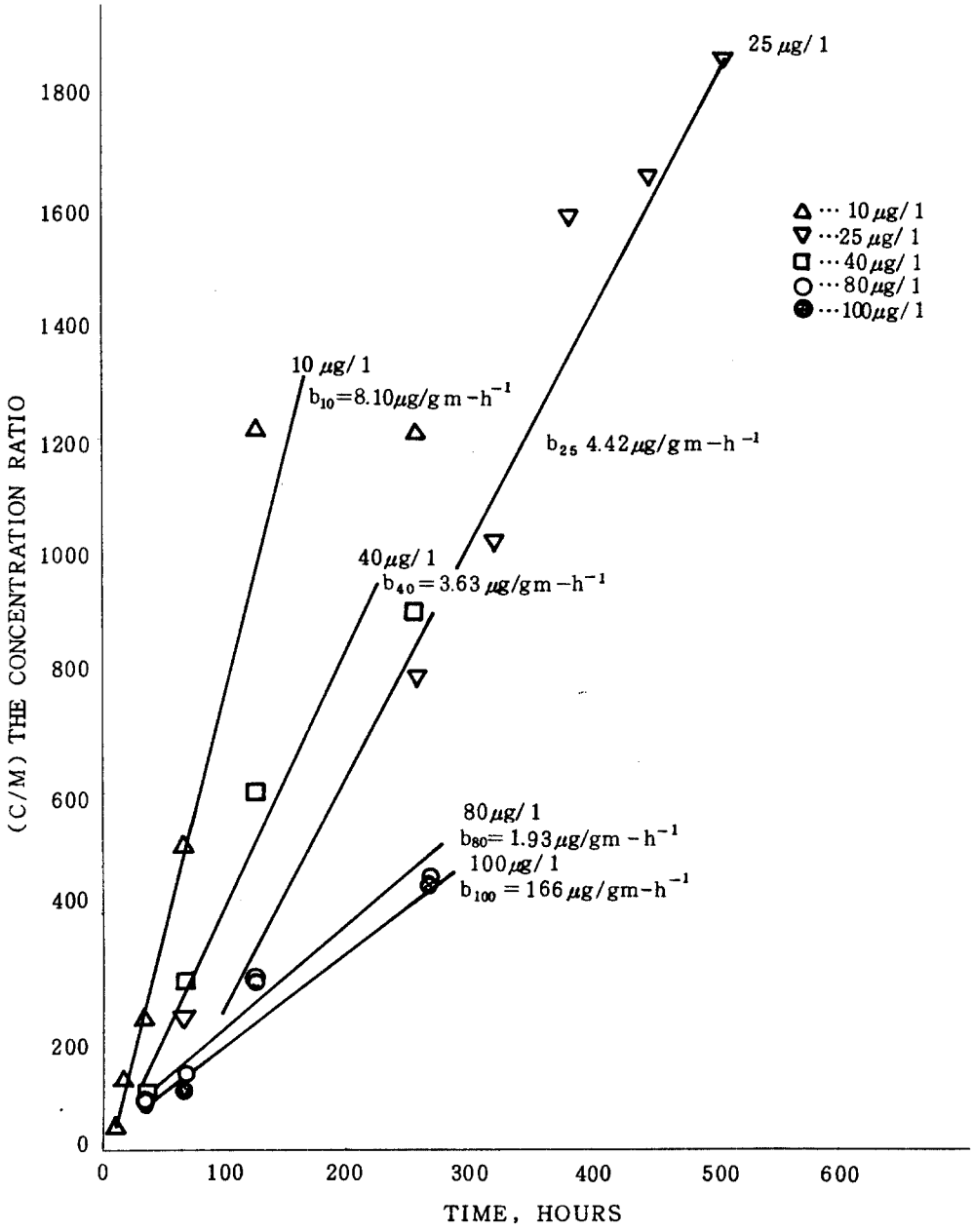

Fig. 3. Accumulation of mercury by C. virginica, Phase II, for $t > t_o$, b_n = slope of data at n μg/ I, $b_n \neq k_i^*$ of phase I.

Table 2: Mercury accumulation and concentration ratios during Phase I for oysters exposed to 10, 20, 25, 40, 80 and 100 μg/l mercuric chloride/sea water.

Time of Exposure, Hours	Mean Water Concentration of Mercury Exposed to Oysters, M, (μg/l)											
	10μg/l		20μg/l		25μg/l		40μg/l		80μg/l		100μg/l	
	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C(μg/kg)[a]	Conc Ratio (C/M)
0[b]	60	NA	ND	NA	ND	NA	ND	NA	ND	NA	150	NA
2	330	33	268	13	540	22	820	20	1,260	16	1,800	18
4	390	39	614	31	1,010	40	1,750	44	3,700	46	3,760	38
8	450	45	1,764	88	1,980	49	2,200	55	4,260	53	7,870	79
16	···	--	1,921	96	3,140	126	3,310	83	4,880	61	8,410	82
32	···	--	2,000	100	4,980	199	4,340	109	7,380	92	9,510	95
64	···	--	--	--	5,700	229	--	--	--	--	--	--
128	···	--	--	--	7,600	304	--	--	--	--	--	--

a Wet weight

b 0 hour sample was taken prior to addition of mercury and is considered the environmental level of mercury in oysters taken from Louisiana waters. Studies were performed between September 1972 and June 1973.

ND Not detectable.

NA Not applicable.

-- Data shown in phase II, Table 2

ratio or CR (C/M). With the exception of the data developed in the 25 μg/l experiment there is an ordered decrease in the concentration ratio at increased aqueous levels of mercury. Concentration ratios reached in our studies illustrate this relationship quite clearly; *e.g.* at 10 μg/l a CR of 1230 was reached after 256 hours (11 d) versus a CR of 441 for the same period at 100 μg/l. Slopes of the data shown in Figure 3 follow a logarithmic relationship with concentration of exposure which is amenable to simple curve fitting. Techniques for the concentration of mercury in the oyster with time. There is no discernible relationship between mercury level in the oyster and the change in form of accumulation (see Table 2). There is, however, an apparent relationship between the change in accumulation and the product of the concentration and duration of exposure – also borne out by the work of Kopfler (1974) and Galati (1974).

Comparison of the data in Table I with the investigations cited above is made in Figure 4. Data in that figure may be fit as either a rectilinear hyperbola, $(M-a)(t_0-b)=K$ where a, b, $(a=0,\ b=0)$, and K are constants or by an equation of the form $M=M_0e^{-kt}$. The importance of the relationship lies in the presence (or absence) of a concentration threshold below which accumulation will remain reversible over indefinite exposure. Statistical analysis of the data in Figure 4 suggests that the exponential model (no threshold) is the better fit of the two models shown; but neither model is of such good fit to the data as to define exactly the relation which exists. It is clear, however, that

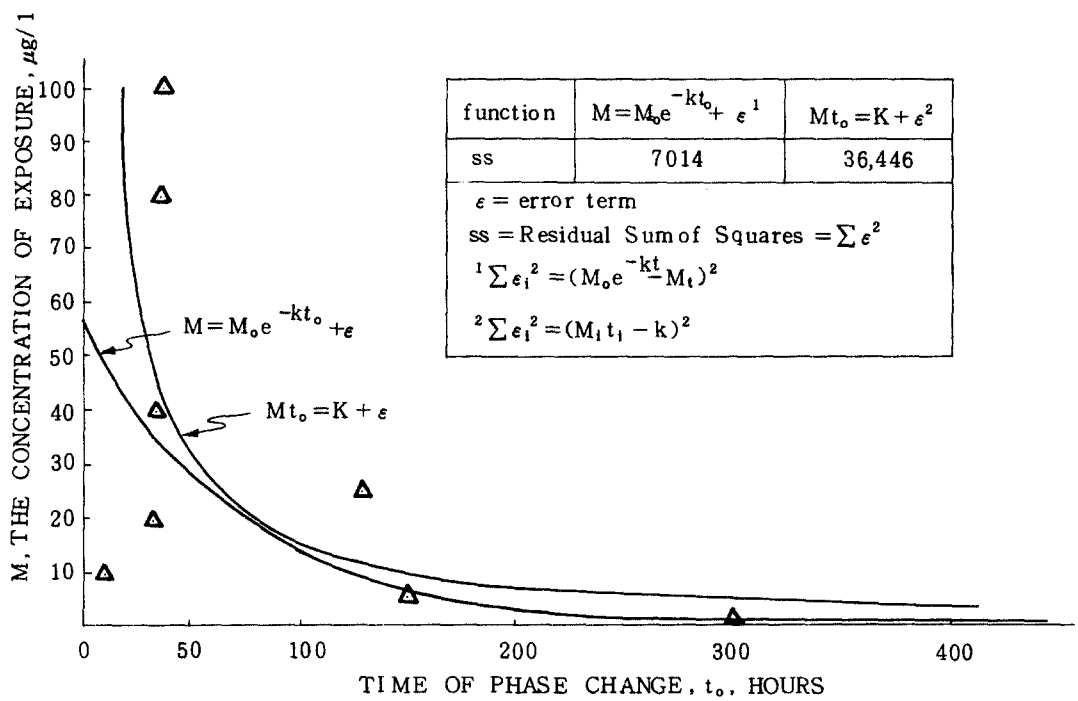

Fig. 4. The concentration of exposure versus duration of reversible accumulation.

if a threshold concentration does exist it is below 1 μg/l since aqueous concentrations of 1 μg/l result in a shift to linear, irreversible, accumulation after only 12 days of exposure (Figure 5) (Kopfler, 1974). At the other extreme, high concentrations of exposure (⩾ 40 μg/l) result in very short periods of reversible accumulation and are of much concern because of their potential to result in chronic mercury elevation in oysters exposed for brief periods.

Mercury concentrations reached in our experiments were well above the established limits for safe consumption as shown in Table 3

2. Excretion

Loss rates were determined to be negligible following entry into the second phase as shown in Figure 6. Examination of data in the loss studies following exposure to 80 μg/l Hg (as $HgCl_2$) shows a possibility of slight loss in that experiment – however, there was no statistical support that loss occurred at the level of confidence used in the study. Certainly, the possibility of eventual loss exists – however, it would appear from our data that the depuration processes currently envisioned do not allow adequate time for the decontamination of the accumulated burden once the switch to linear accumulation occurs.

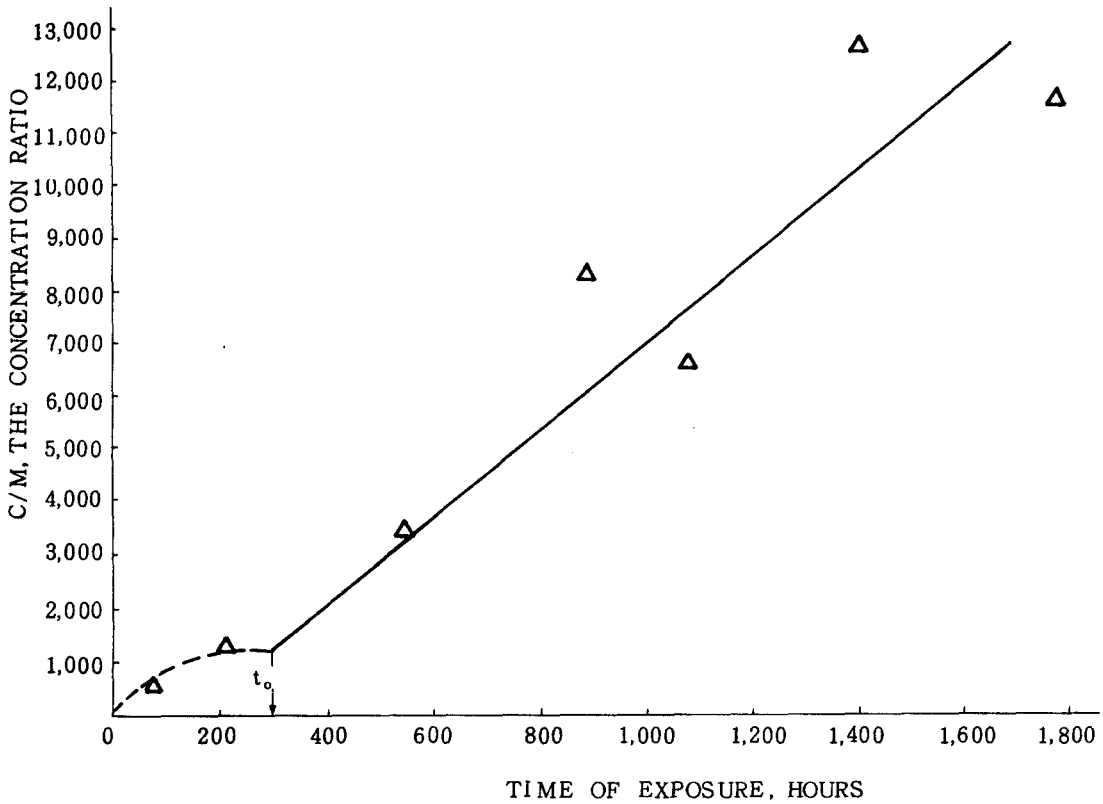

Fig. 5. The accumulation of mercury by C. virginica at 1μg/1, data from kopfler (1974).

Table 3 : Mercury accumulation and concentration ratios during phase II for oysters, exposed to 10, 25, 40, 80, and 100 μg/l mercuric chloride.

Time of Exposure, Hours	Mean Water Concentration of Mercury Exposed to Oysters, M, (μg/l)									
	10 μg/l		25 μg/l		40 μg/l		80 μg/l		100 μg/l	
	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)	Hg in Oysters, C (μg/kg)[a]	Conc. Ratio (C/M)
16	1,320	132	--	--	--	--	--	--	--	--
32[b]	2,300	230	--	--	4,340	83	4,880	92	8,410	84
64	5,200	520	--	--	9,950	249	10,740	134	10,550	106
128[b]	10,200	1,020	7,600	304	24,410	610	24,240	300	29,520	295
256	12,300	1,230	20,225	809	36,940	924	36,400	455	44,120	441
320	NA	NA	25,975	1,039	NA	NA	NA	NA	NA	NA
384	NA	NA	40,075	1,603	NA	NA	NA	NA	NA	NA
448	NA	NA	44,200	1,768	NA	NA	NA	NA	NA	NA
512	NA	NA	47,000	1,880	NA	NA	NA	NA	NA	NA

[a] Wet weight.

[b] The last data point of Phase I is taken as the initial value in Phase II.

--Data shown in phase I, Table 1.

NA No Data. Experiment discontinued at 256 hours.

Experiment at 20 μg/l discontinued at 32 hours.

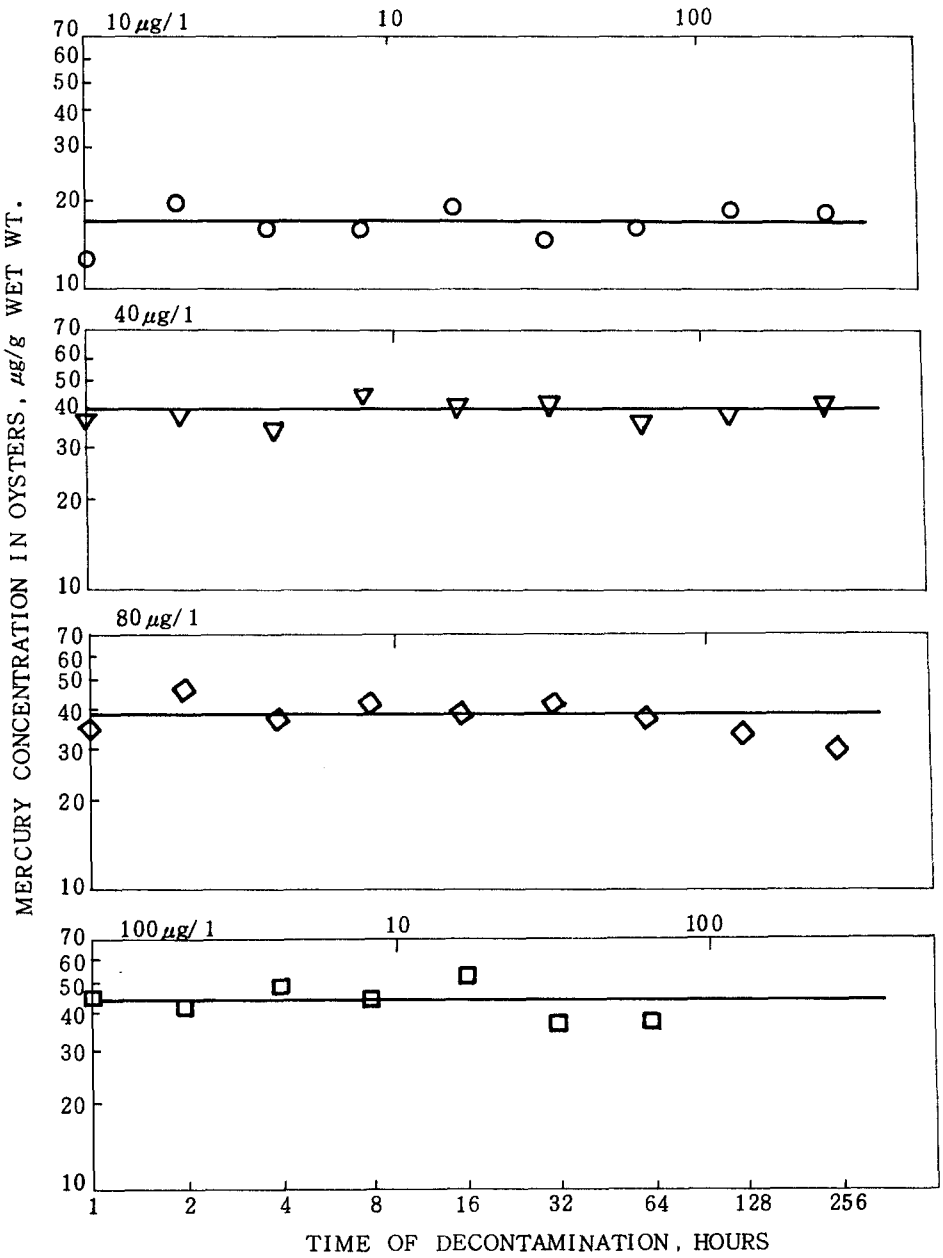

Fig. 6. Mercury concentration in oysters following cessation of exposure.

3. Empirical modeling

The short duration of non-linear uptake and the extended linear period of the second phase suggest that an empirical model may be made for predicting total mercury concentration obtained in oysters exposed to mercuric

chloride. Analysis of the data accumulated in the second phase of uptake shows that while accumulation at each of the concentrations tested is linear with time, the relationship between the rate of accumulation, and concentration of exposure, is non-linear. To simplify calculation, regression equations for uptake were formed on the logs of the data in the five individual experiments and the slopes (b) obtained from the appropriate equation for each concentration of exposure. (see Tables 4 and 5). Subsequent trial showed that these slopes could be fitted as log-log functions of the concentration of exposure. Intercepts from the individual equations were treated similarly but no evidence was found to conclude that

Table 4 : Comparison of experimental slopes (sample data) from uptake experiments at 10, 25, 40, 80 and 100 $\mu g/1$ exposures with the estimated slope (population data) b and test statistics of significance.

Concentration of Exposure, M, $\mu g/1$	Experimental Slopes (log) (b)	Estimated Slope[a] (log) (b)	Calculated Test Statistics[b] (t_b)	Student t at $t_1-\alpha/2, n-2$ ($t_{0.975, 6}$)
10	0.8509	0.8382	1.3678	2.4469
25	0.6715	0.7417	− 9.9139	2.4469
40	0.7664	0.6922	0.9444	2.4469
80	0.6195	0.6192	0.0369	2.4469
100	0.5788	0.5957	− 1.7308	2.4469

Test Statistic :

$(a)_b = \alpha' + \beta' \log t = 1.1087 - 0.2425 \log M$

$(b)_t = \dfrac{(b - b_n) S_x \sqrt{n-1}}{S_{y.x}}$

Analysis of variance for regression of population slope, b_n

Source	Sum of Squares	Degrees of Freedom	Mean Square
Regression	$\dfrac{b_1^2 L_{xx}}{n}$	1	$\dfrac{b_1^2 L_{xx}}{n}$
	0.0383		0.0383
Error	$(n-2)S^2_{y.x}$	3	$S^2_{y.x}$
	0.0109		0.0036
Total	$\dfrac{L_{yy}}{n}$	4	NA
	0.0492		

Test Statistic

$f = \dfrac{b_1^2 L_{xx}}{S^2_{y.x}}$

$= 52.7328$

$F_{0.95, 1, n-2} = 10.13$

Thus we find

$f > F_{0.95, 1, n-2}$

Table 5 : Comparison of experimental concentration ration (sample data elevations) from uptake experiments at 10, 25, 40, 80 and 100 ug/1 exposures with the estimated population intercept.

Concentration of Exposure, M, ug/1	Estimated Values log y-intercept, α[a]	Experimental Values	
		log y-intercept	log 95% C.I. for y-intercept at log M=0
10	1.1356	1.1048	0.9909–1.2187
25	1.1356	1.2139	1.1270–1.3008
40	1.1356	1.0611	0.9647–1.1575
80	1.1356	1.1113	1.0115–1.2111
100	1.1356	1.1869	1.0667–1.3071

a α is accepted as a constant since the null hypothesis cannot be rejected at P= 0.95 for the regression of α against M, the aqueous concentration.

Analysis of variance

Source	Sum of Squares	Degree of Freedom	Mean Square
Regression	$\frac{b_2^2 L_{xx}}{n}$	1	$\frac{b_2^2 L_{xx}}{n}$
	0.0004		0.0004
Error	$(n-2)S^2_{y.x}$	3	$S^2_{y.x}$
	0.0155		0.0052
Total	$\frac{L_{yy}}{n}$	4	NA
	0.0159		

Test Statistic

$$f = \frac{b_2^2 L_{xx}}{S^2_{y.x}} \qquad F\ 0.95,\ 1, n-2 = 10.13$$

$$= 0.3670$$

Thus we find

$$f < F\ 0.95,\ 1, n-2$$

the intercepts (one hour uptake) varied with concentration. Thus, the concentration ratio for mercury may be estimated for any time, t, by:

$$\log C/M = \log \alpha + b \log t \quad \ldots . 2$$

where:

C/M = the concentration ratio

$$= \frac{\text{tissue concentration}}{\text{aqueous concentration}}$$

α = a constant for all exposure concentrations tested

b = the slope of the accumulation curve, a function of the aqueous concentration of mercury

t = duration of exposure, hours

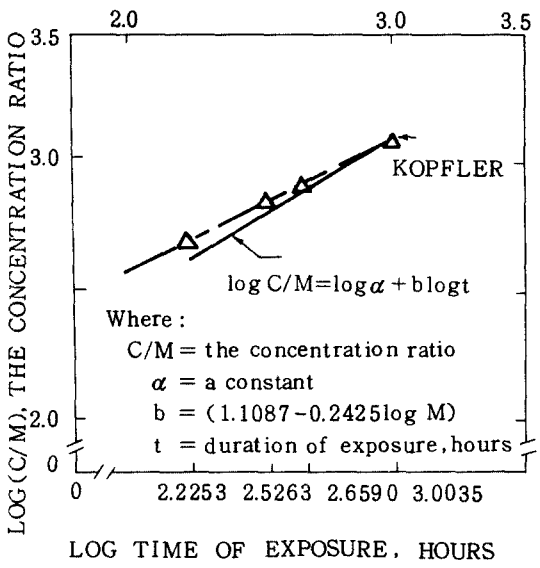

Fig. 7. Concentration ratios versus time. Triangles and dashed line represent data of Kopfler (1973)····· unbroken line represents the author's equation.

Solution of the regression to base 10 for the parameters developed from our data gives the equation and fit illustrated in Figure 7.

Summary and conclusions

Mercury was found to accumulate rapidly in two phases which may be attributed to the first and second compartment of a sequential two compartment system similar to that of Hermione hystrix (Chipman, 1966). Although initial accumulation can be modelled as a reversible system, loss rates measured in the second phase confirm the irreversibility of accumulation after changeover to the linear form (Ruzic, 1972). The parameters defining changeover to linear accumulation can be fitted to functions of the form $(M-a)(t_0-b) = K$ or $M = M_0 e^{-kt}$. Somewhat better fit is found for the latter function which suggests that the changeover may not require a threshold concentration.

Levels of mercury exceeded 0.5 μg/gm in 8.6 hours and 0.5 hours respectively at 10 μg/l and 100 μg/l concentrations.

All uptake studies were performed in the absence of appreciable food and detritus concentrations as evidenced by turbidities of less than 1 Jackson Turbidity Unit (JTU), which lends strong support to the proposal that trace metals may be assimilated directly from water through gill and mantle tissues. Work currently in progress in our laboratory also indicates highest rates of accumulation in the gills and mantle and probably accounts for the closeness of fit of the proposed regression model to the work of Kopfler (1973) which was performed in a flow through system using natural estaurine water.

It is our suggestion that further experiments be performed on the initial uptake of mercury compounds to better define conditions affecting the shift to irreversible accumulation. Special emphasis on the determination of a possible threshold below which the shift does not occur should be made since it is of critical importance in considering the effects of chronic low levels of exposure on accumulation and the usefulness of depuration processes to decontaminate contaminated oysters.

Acknowledgement

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