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Bioaccumulation of alkyllead compounds from water and from contaminated sediments by mussels

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The bioconcentration of alkyllead compounds from water and from contaminated sediments by freshwater mussels (*Elliptio complanata*) has been investigated. Higher levels of trimethyllead than triethyllead species are accumulated for the same exposure period. *In-vivo* transformation of the trialkyllead species by a series of dealkylation reactions giving dialkyllead and inorganic lead(II) species appears to take place. Rates of accumulation are higher for the more contaminated sediments.

Keywords: Bioaccumulation, alkyllead, sediment, mussels

INTRODUCTION

It is well known that chemicals in the environment may be accumulated in living organisms to a very high level. In aquatic systems, sediment is the ultimate storage sink for metallic and organic compounds. Current knowledge of point and diffused sources to rivers and lakes has indicated that persistent organic compounds are present in surficial sediment layers in nearshore and depositional basins. Thus the bioavailability of these adsorbed compounds could pose a severe threat to the ecosystem.

Studies on bioaccumulation are mainly conducted in water systems and seldom with regard to the underlying sediment.[1] Recent studies of bioavailability of pollutants from contaminated sediments and their impact have been the topic of an international conference.[2] Alkyllead compounds, notably tetraalkyllead (R_4Pb; R = Me, Et), as a result of anthropogenic input, have been found in fish, sediment and other biota near the alkyllead production plants.[3,4] These compounds are either physically bound to sediment by adsorption or chemically bound by complexation processes. They can become available to biota through leaching or exchange. The lipophilic properties of tetra-alkyllead compounds further enhance their partition into lipid-containing organisms. The present study was conducted with two objectives: first, to investigate the direct bioconcentration of different alkyllead compounds from water and from contaminated sediment by freshwater mussels; and secondly, to investigate the possibility of *in-vivo* transformation of these compounds through methylation, demethylation, or degradation processes in mussels.

MATERIALS AND METHODS

Indigenous freshwater mussels (*Elliptio complanata*), 6.0–7.0 cm in length, were collected from Balsam Lake, Ontario, for laboratory and field experiments. These mussels were free from contamination by alkyllead.

Glass tank aquaria (40 × 20 × 25 cm, LWH), filled with Lake Ontario water, were used for experiments. Mussels were exposed to alkyllead solutions to study the concentrations of these compounds in different organs of the mussels. In these experiments, well-washed fine white sand was used in each aquarium (*ca* 3 cm deep) to provide a bed for the mussels. The tanks were filled with lake water, spiked with solutions of trimethyllead chloride and triethyllead chloride at 1 mg dm^{-3} (1 ppm) expressed as lead (Pb). The concentrations of the lead compounds in water were checked before and after the experiment by the gas

chromatography–atomic absorption (GC AA) spectrometry method[5] to ensure that the test compound was still present at the experimental concentration, and in the designated form. At 7, 10, 14 and 17th day intervals, three mussels were sacrificed and dissected for analysis of the alkyllead compounds in the following organs: gill, mantle, muscle (adductor muscle and foot), and the visceral mass. After dissection, the organ was blotted, dried with tissue papers, weighed and processed accordingly for analysis as described in the following section.

Contaminated sediments used in the exposure experiments were collected by an Ekman grab from the St Lawrence River off Maitland and from the St Clair River off Corunna near lead-alkyl production plants. The sediments were stored in plastic bags, transported to the laboratory, and stored in a cold room (2–5°C) before use. About 3 kg of the wet sediment was placed in the aquarium and allowed to equilibrate with Lake Ontario water for seven days before the test mussels were put in. The sediment samples were analyzed for alkyllead compounds before and after the experiments. The overlying water was also analyzed for alkyllead species occasionally, in parallel with the mussel analysis.

Both the water and contaminated sediment exposure experiments were conducted under static conditions, without agitation or continuous water circulation, except with air bubbling in at mid-level to supply the system with oxygen, without disturbing the sediment. Water levels in the aquarium were maintained occasionally by replenishing with distilled water when necessary. The mussels were fed with live algae every three days. During the exposure experiment, two mussels were removed from each of the sediment tanks and the control tank at every three to four days interval. The mussels were mechanically opened, blotted, dried with filter papers, weighed and analyzed individually on a whole-clam basis for alkyllead species according to the techniques given by Chau *et al.*[6] In this method, the whole mussel was digested in a 20% tetramethylammonium hydroxide (TMAH) solution. After neutralization, the tetra-alkyllead, ionic trialkyl- and dialykl-lead, and lead [Pb(II)] compounds were extracted into a benzene solution containing a chelating agent, sodium diethyldithiocarbamate (NaDDTC), followed by butylation to convert all the ionic alkyllead and lead(II) species to the tetra-alkyl-substituted forms finally determined by a GC AA technique. The following alkyllead species were determined: R_4Pb, R_3Pb^+, R_2Pb^{2+}, Pb(II) (R = Me, Et).

RESULTS AND DISCUSSION

Exposure to alkyllead solutions

Mussels exposed to solutions of triethyllead and trimethyllead (1 mg dm^{-3}) were observed to concentrate these compounds at steady rates with no noticeable physiological or behavioral changes before the 17th day of exposure to triethyllead. The mussels grew normally without loss in body weight. The burdens of trimethyllead and triethyllead in different mussel organs as a function of time are given in Figs 1 and 2 respectively. The concentrations of alkyllead in the various organs were mean values from three individual mussels, with relative standard deviations ranging from 5 to 18% of the mean values. A much higher concentration of trimethyllead was accumulated than triethyllead for the same exposure period. For

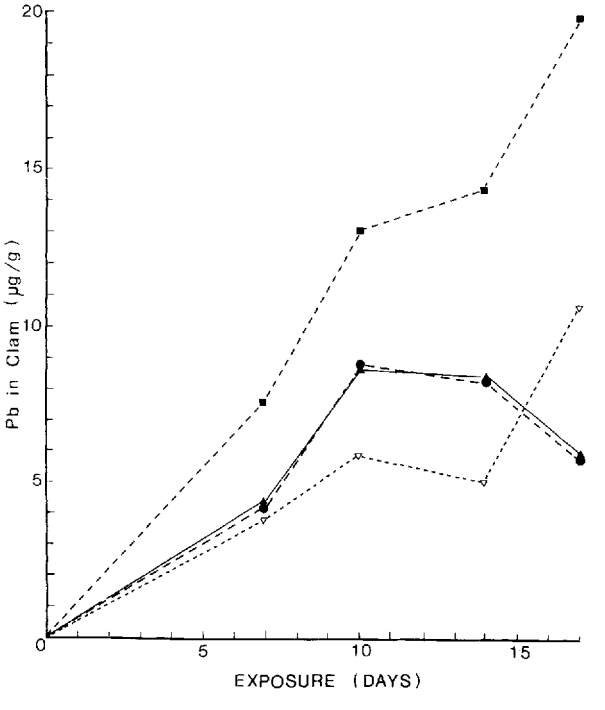

Figure 1 Concentration of trimethyllead in different mussell tissues after exposure to a trimethyllead chloride solution (1 mg dm^{-3} as lead) for 17 days. Data from average of three clams: ■, muscle; Δ, viscera; ▽, gill; ●, mantle.

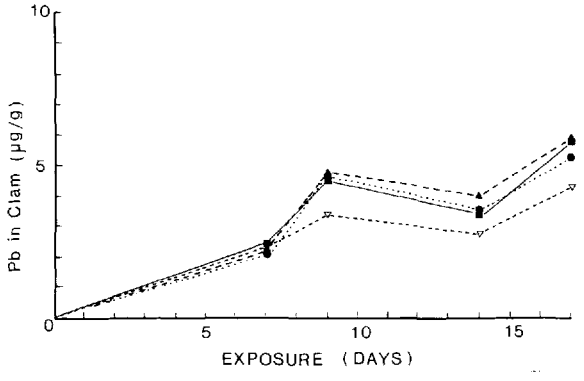

Figure 2 Concentration of triethyllead in different mussel tissues after exposure to a triethyllead chloride solution (1 mg dm^{-3} as lead) for 17 days. Data from average of three clams: ■, muscle; Δ, viscera; ▽, gill; ●, mantle.

trimethyllead, the highest concentration was found in the muscle tissue (19.7 $\mu g\ g^{-1}$) followed by gills (10.5 $\mu g\ g^{-1}$), visceral mass (6 $\mu g\ g^{-1}$) and mantle (5.9 $\mu g\ g^{-1}$). The distribution of triethyllead in the organs was in general similar to that for trimethyllead, except the differences among the organs were not as remarkable. Muscle (5.9 $\mu g\ g^{-1}$), visceral mass (5.9 $\mu g\ g^{-1}$) and mantle (5.7 $\mu g\ g^{-1}$) contained the highest concentrations of triethyllead. The rate of accumulation was in general much higher for trimethyllead than for triethyllead. Whether or not this is an effect of the alkyl group functionality was not investigated. The *in-vivo* stability of the trimethyllead species as discussed in the subsequent section may explain its high accumulation. It has been shown, however, that marine mussel and dab also accumulate higher concentrations of trimethyllead than triethyllead.[7]

As estimated from the accumulation curves, the concentration factors for trimethyllead and triethyllead by mussels were eight fold and three fold respectively over a 14-days exposure period in solutions of 1 ppm of the alkyllead compounds. These concentration factors are relatively low in comparison with those of other aquatic organisms. Bioaccumulation for organometallic compounds, even for those with strongly ionic characteristics, is much higher in the lipid-containing tissue. Mussels and other bivalves usually have a lower lipid content than most other fish. This could be the reason for their low concentration factors for the ionic alkyllead compounds. For comparison Table 1 summarizes some concentration factors for different species of alkyllead compounds by various aquatic organisms.

At the 17th day, the triethyllead test solution began to turn turbid. The colloidal particles, soluble in dilute acid, were analyzed to be lead-containing compounds, probably hydrous lead oxide precipitates, formed due to slow decomposition of triethyllead. Two mussels died as a consequence of fouling of the solution and the experiments were terminated. Such a phenomenon was not observed in the trimethyllead test solution.

Tetra-alkyllead compounds are volatile and form only unstable physical solutions. It is difficult, although not entirely impossible, to maintain test solutions at constant concentration.[8] Their environmental occurrence is scarce owing to lack of stability and therefore they were not included in the experiments.

In the course of the exposure experiment, no mussel died before the 17th day, indicating that the concentration to cause acute toxicity of alkyllead compounds to mussel may have not been reached. No data are available for acute toxicity of these compounds to freshwater mussels.

Transformation of alkyllead compounds

In-vivo transformation of alkyllead compounds was observed in the course of mussel analysis. Using the temporal accumulation of trialkyllead in the visceral mass of the exposed mussels as an example, both diethyllead and inorganic lead [Pb(II)] species were found to increase as the triethyllead accumulation increased with time during the first 10 days of incubation (Fig. 3). Similarly, dimethyllead and lead(II) species were also found in the organs of mussels exposed to trimethyllead solution. During this period of time, concurrent analyses of both test solutions did not show the presence of the diethyllead or dimethyllead species except traces of lead(II), which was always present. It is evident that the dialkyllead and lead(II) species must have been formed *in-vivo* by the mussels by dealkylation of the trialkyllead species during this time period. It is also apparent that more degradation occurred with the triethyllead species than with the trimethyllead, such that the accumulated products of dialkyllead and lead(II) in the mussel organ were much higher in the triethyllead exposure experiments. At the end of the 17-day exposure experiment, more lead(II) was accumulated than the diethyllead species, indicating faster kinetics for the conversion of diethyllead to lead(II) than that for the conversion of triethyllead to diethyllead. The fact that

Table 1 Concentration factors for alkyllead compounds

Compound	Species	Exposure conc.	Exposure time	Concentration factor (fold)[a]	Ref.
Me_4Pb	Shrimp	0.01–0.7 mg dm^{-3}	96 h	20	7
	Mussel	0.05–0.7 mg dm^{-3}	96 h	170	
	Plaice	0.02–0.7 mg dm^{-3}	96 h	60	
	Rainbow trout	3.46 μg dm^{-3}	96 h	500–934	9
	Rainbow trout	3.5 μg dm^{-3}	1 day	100	8
	Rainbow trout	3.5 μg dm^{-3}	7 days	760	8
Et_4Pb	Shrimp	0.01–0.2 mg dm^{-3}	96 h	650	7
	Mussel	0.01–0.2 mg dm^{-3}	96 h	120	
	Plaice	0.02–0.2 mg dm^{-3}	96 h	130	
	Rainbow trout				
	Viscera fats	2.5 μg g^{-1}	21 days	3189	10
	Intestine	2.5 μg dm^{-3}	21 days	1824	
	Skin	2.5 μg dm^{-3}	21 days	1500	
	Kidney	2.5 μg dm^{-3}	21 days	843	
	Brain	2.5 μg dm^{-3}	21 days	92	
Me_3Pb	Shrimp	0.2–3.0 mg dm^{-3}	96 h	1	7
	Mussel	0.1–3.0 mg dm^{-3}	96 h	24	
	Plaice	10–50 mg dm^{-3}	96 h	1	
	Mussel (freshwater)	1 mg dm^{-3}	14 days	8	This work
Et_3Pb	Shrimp	1.0–15.0 mg dm^{-3}	96 h	2	7
	Mussel	0.1–25.0 mg dm^{-3}	96 h	10	
	Plaice	1.0–10.0 mg dm^{-3}	96 h	2	
	Mussel (freshwater)	1 mg dm^{-3}	14 days	3	This work
	White sucker	0.54 μg dm^{-3}	FS[b]	88	3
Et_2Pb	White sucker	0.14 μg dm^{-3}	FS[b]	375	3

[a] Concentration factor = concentration of alkyllead compound in animal tissue (μg kg^{-1})/concentration in water (μg dm^{-3}). [b] FS, field samples.

higher accumulation was observed for trimethyllead (preceding section) could well be due to its *in-vivo* stability in comparison with the triethyllead species. Degradation of tetra-alkyllead by a series of dealkylation reactions to trialkyllead, then to dialkyllead and eventually to lead(II) has been observed in other biota such as fish[11] and also humans.[12]

Exposure to contaminated sediment

Results from experiments with mussels exposed to sediment contaminated with alkylleads agree well with the findings of the spiked lake water experiments, except that the uptake rates of alkyllead concentration are different. The rates of bioaccumulation of alkyllead compounds are obviously related to the concentrations of the alkyllead species in the sediment. For example, St Clair river sediment contained about eight times as much lead(II) and more than twice as much tetraethyllead than the St Lawrence River sediment (Table 2). The rates of bioaccumulation for triethyllead and lead(II) are 5 times and 14 times faster respectively for mussels placed in St Clair sediment than those placed in St Lawrence River sediment. Unlike the bioaccumulation experiments conducted in alkyllead solutions (which are simple media), the accumulation experiments with contaminated sediment may be complicated by many other parameters controlling the

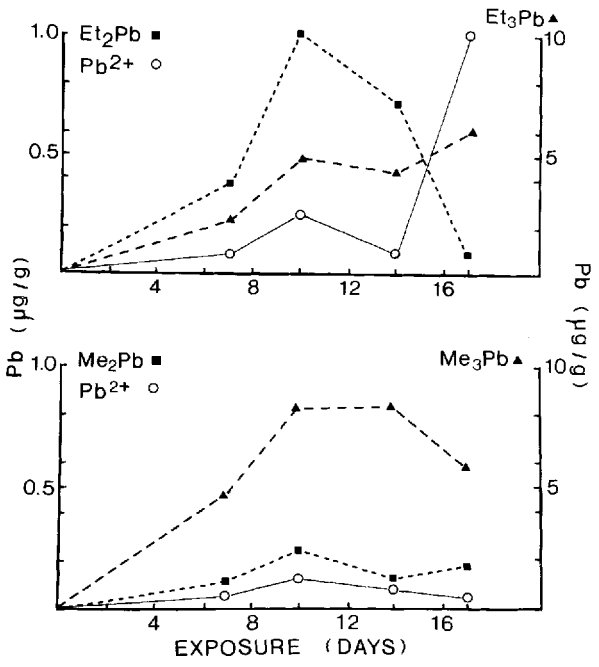

Figure 3 Biotransformation of trialkyllead in viscera mass.

absorption and desorption of alkyllead compounds from the sediment surfaces. Apart from the differences in the alkyllead loadings of the sediment, the differences in sediment composition, such as organic content, will also play an important role in affecting the bioaccumulation of alkyllead.

In the course of the 52-day exposure, the bioaccumulation rates for mussels for triethyllead, diethyllead and lead(II), from the St Clair River sediment, as estimated from the slopes of the regressed accumulation curves, were 1.96, 0.26, and 4.87 ng g^{-1} per day respectively (Fig. 4A,B). For St Lawrence sediment (Fig. 5A,B), the bioaccumulation of alkyllead and lead(II) species was less pronounced. For triethyllead, the rate was 0.42 ng g^{-1} per day which is about one-quarter of the rate for St Clair sediment under identical conditions. For diethyllead, and lead(II), the slopes of the accumulation curves were almost flat but slightly negative. Accumulation was considered zero. This phenomenon might be attributed to the decrease of the concentration of diethyllead in the water of the St Lawrence sediment tank from 510 ng dm^{-3} on day 14 to below detection on day 46, and that of lead(II), from 3530 ng dm^{-3} to less than half of its value, after 46 days. By contrast, the diethyllead concentration in the water of the St Clair

Table 2 Analyses of sediments and aquarium water in mussel exposure studies

	Sediment (ng g^{-1})		Aquarium water (ng dm^{-3})	
Compound	Starting	33-day	14-day	46-day
St Lawrence River sediment				
Et_4Pb	39	98	nd[b]	nd
Et_3Pb	25	35	1100	270
Et_2Pb	21	nd	510	nd
Pb(II)	4050	3131	3530	1610
St Clair River sediment				
Et_4Pb	90	337	nd	nd
Et_3Pb	28	34	1170	810
Et_2Pb	11	nd	460	150
Pb(II)	23926	29024	6850	8200
Control[a]				
Et_4Pb	—[c]	—	nd	nd
Et_3Pb	—	—	nd	nd
Et_2Pb	—	—	nd	nd
Pb(II)	—	—	150	460

St Lawrence River sediment was taken off Maitland; St Clair River sediment was taken off Corunna; 3 kg of sediment and 10 dm^3 of Lake Ontario water were used in each aquarium.
[a] 4 kg of well-washed aquarium white sand and 10 dm^3 of Lake Ontario water were used in the control aquarium. [b]nd, not detected. [c]—, not analyzed.

sediment tank did not decrease as much, and the concentration of lead(II) actually increased by about 20% after 46 days (Table 2). The release of alkyllead compounds from sediment is a complex process, depending on many parameters such as particle size, organic content, chemical and physical characteristics of the sediment, chemistry of the overlying water, and is very much affected by bioturbation caused by movements of any organisms, in this case the mussels. The present investigations, however, only aim at assessing the direct bioavailability of alkyllead compounds in contaminated sediments with a view to providing a laboratory basis for the interpretation for our continuing *in-situ* work using caged mussels in the polluted sites. The investigation of the release mechanism is beyond the scope of this study.

Although tetraethyllead was present in sediment samples collected from both locations (Table 2), it was not found in the exposed mussels. It is possible that tetraethyllead was strongly adsorbed onto the sediment

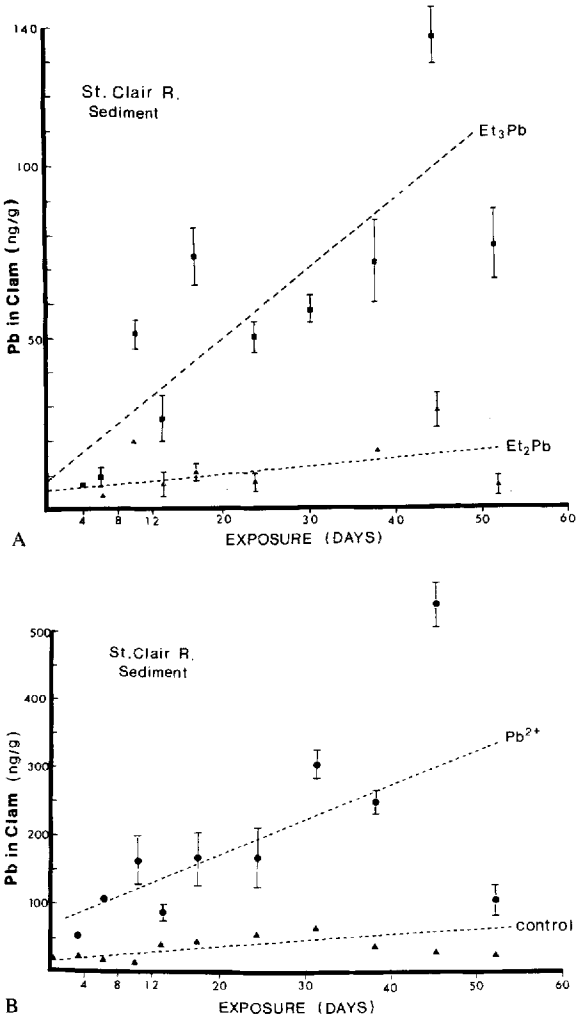

Figure 4A,B Concentrations of triethyllead, diethyllead and lead(II) in mussels after exposure to St Clair River sediment. Data from average of two mussels.

and was not available to the mussels. Alternatively, it may undergo rapid *in-vivo* degradation in the mussel to the triethyllead and diethyllead species after accumulation. Such degradations have been previously reported.[11,12] Our earlier work[8] showed that rainbow trout accumulated tetramethyllead from water and the highest concentration was found in the lipid layer of the fish. Unfortunately, only tetramethyllead was determined because methods were not available for the determination of trimethyllead and dimethyllead species in fish and water at the time of the investigation. We were not able to substantiate the *in-vivo* degradation of tetra-alkyllead compounds in aquatic organisms.

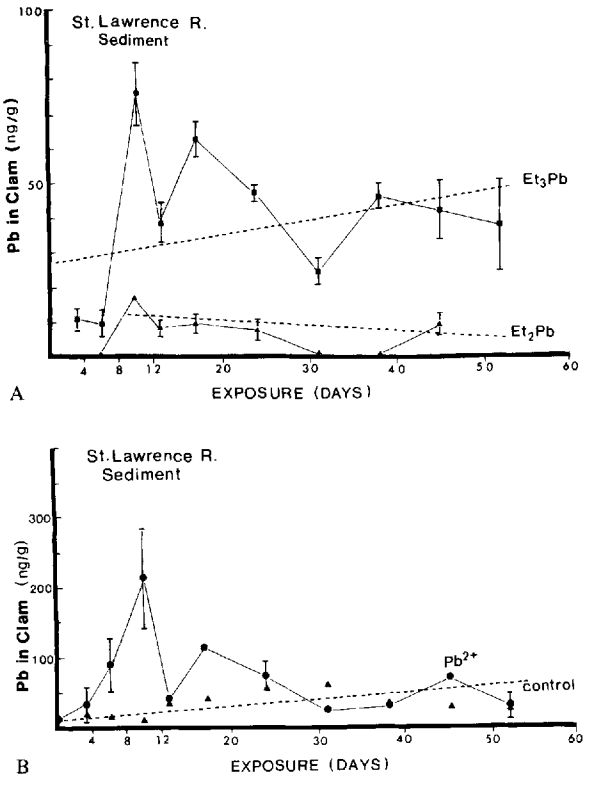

Figure 5A,B Concentrations of triethyllead, diethyllead and lead(II) in mussels after exposure to St Lawrence River sediment. Data from average of two mussels.

CONCLUSIONS

Alkyllead compounds are accumulated by mussels, with much higher concentration of trimethyllead being accumulated than triethyllead for the same exposure period. The highest concentrations of trimethyllead are found in the muscle, gills and visceral tissue. The distribution of triethyllead in organs is slightly different with the highest concentrations in the muscle, visceral mass and mantle.

There is evidence for the *in-vivo* transformation of the trialkyllead species to dialkyllead and lead(II) species in the mussel organs by a series of dealkylation reactions. As dialkyllead species were not detected in both of the control trialkyllead test solutions within the time frame, the transformations must have taken place *in-vivo* after accumulation.

The rates of accumulation are related to the concentrations of alkyllead compounds in water and sediment. Higher accumulation is observed with St Clair River

sediment (Corunna) than with St Lawrence River sediment (Maitland), as reflected by their alkyllead content. As the release of alkyllead compounds from sediment is probably not a linear process, the accumulation by the exposed mussels would accordingly fluctuate. The bioaccumulation study can only indicate the trend and a cumulative estimation of accumulation over a period of time.

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