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The toxicity of alkyllead compounds to *Scrobicularia plana* (Da Costa)

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Conventional (whole animal) toxicity tests were made with *Scrobicularia plana* (Da Costa), an infaunal estuarine bivalve, and compared with the responses recorded from siphonal preparations. Exposure of *Scrobicularia* to low concentrations of triethyllead chloride and trimethyllead chloride, for 35 and 60 days respectively, produced typical response curves. However, mortality had not become asymptotic with time at lower concentrations, suggesting further mortality with increased exposure time. An approximate incipient lethal concentration was predicted.

A siphonal preparation technique with isolated and *in-situ* inhalent siphons of *Scrobicularia* was used to estimate the lowest effect concentrations of alkyllead compounds. Response to alkyllead was indicated by contraction of the siphon, recorded via an isotonic transducer. Trialkyllead compounds were more toxic than the respective dialkylleads and inorganic lead. Toxicity of trialkylleads increased with alkyl chain length. Pure tetraethyllead did not cause any siphonal contraction even when applied directly to the preparation. It was concluded that tetraethyllead has a low toxicity or is non-toxic in pure form.

Keywords: ***Scrobicularia*, alkyllead compounds, toxicity, inhalent siphon, bivalves**

INTRODUCTION

It has been reported that alkyllead poisoning may have been responsible for the deaths of large numbers of birds on the Mersey estuary (UK) in 1979.[1] The birds may have accumulated the lead through their diet of bivalve molluscs and polychaete worms which, in turn, may have accumulated alkyllead from sources within the estuary via antiknock production site effluent discharges or spills during handling and transport. Both of the antiknock agents tetramethyllead (TML) and tetraethyllead (TEL) have high densities (1.995 $g\,cm^{-3}$ and 1.65 $g\,cm^{-3}$ respectively[2]) and would sink rapidly to the sediment or become adsorbed to particulates. These tetraalkylleads (TALs) would rapidly decompose by chemical and photolytic mechanisms to the tri- and dialkyl analogues, and ultimately to inorganic lead. Sedentary animals such as sediment-dwelling bivalve molluscs or polychaete worms in the vicinity of such contamination would then receive the greatest exposure.

In contrast to the environmental toxicity information available for inorganic lead, which is generally accepted as being many times less toxic than alkylleads,[3] there are relatively few data on alkyllead toxicity. The aim of the work reported here was to assess the toxicity of alkyllead to the widespread and abundant infaunal bivalve *Scrobicularia plana*. *Scrobicularia* is a particularly useful mollusc to assess the toxicity of insoluble or particulate associated chemicals such as alkyllead, because of its sediment-dwelling habit and direct ingestion of surface sediment[4] and because it occurs in areas most likely to become contaminated.

The first sign of response by *Scrobicularia* to adverse stress is the rapid retraction of its inhalent and exhalent siphons.[5] The siphons provide the buried animal with contact with the overlying seawater and it is likely, therefore, that any sensory apparatus of the siphons will first detect adverse conditions. Several bivalves including *Scrobicularia* can isolate themselves completely from the environment by closure of the shell,[6] a response triggered by the sensory apparatus of the animal. When deleterious conditions prevail, the valves are closed but periodic environmental sensing allows the mollusc to reopen its valves and resume pumping as soon as favourable conditions return.

Several methods have been developed to determine the concentrations of pollutants which cause avoidance behaviour in bivalves, including *Scrobicularia*.[5–8] We used a previously tested siphonal preparation technique[5] and compared the lowest alkyllead concentration to cause siphonal contraction with results from more conventional toxicity tests.

MATERIALS AND METHODS

Reagents

Alkyllead compounds were kindly donated by the Associated Octel Company, Ellesmere Port, UK. All other reagents were of AnalaR grade from a commercial supplier.

Alkyllead analysis

Tetra-alkyllead compounds were extracted from aqueous samples by shaking with n-hexane and then either analysed by GC MS or using the indirect method of Hancock and Slater. In this method a solution of iodine monochloride converts the TAL species into the dialkyllead ionic forms which are then extracted from solution as dithizonates and analysed by AA.[9]

Alkyllead salts were analysed by the Hancock and Slater method, after hexane extraction to remove TAL. This procedure dealkyllates TAL and/or trialkyllead to dialkyllead which is then quantified by flameless AA spectrometry. Therefore there is no differentiation of tri- and dialkyllead species.

Scrobicularia bioassays

Scrobicularia were collected at mid-tide level from the Dee estuary (Clwyd/Cheshire, UK) and transported to the laboratory where they were maintained in aerated artificial seawater (34‰)[10] at 10°C prior to testing. The animals used in all tests were 38–43 mm measured along the anterior–posterior axis.

(1) Whole-animal procedure

Assessments of acute toxicity were made by recording the time taken for the exposed group of *Scrobicularia* to reach 50% mortality. This method was preferred to the 96 h LC_{50} determinations often reported because of the avoidance behaviour described above. Animals, cleaned of mud, were exposed to a range of alkyllead concentrations, or used as controls, in replicate two-litre polycarbonate tanks maintained at 10 ±1°C. Test solutions were renewed every two to four days. The animals were fed every two or three days with a culture of *Phaeodactylum tricornutum* grown in the laboratory. The small dilution involved in adding approximately 100 cm^3 algal culture was compensated for by adding the required volume of alkyllead stock to maintain the concentration. Each tank was aerated to ensure that the oxygen saturation remained above 95%; however, it was not possible to aerate TAL-dosed tanks for safety reasons owing to the volatility of TAL compounds.

(2) Siphonal preparation procedure

This method is described in detail by Akberali.[5] The siphons of *Scrobicularia* can either be excised from the rest of the body or used *in situ* with the whole of the soft parts intact and only the left-side valve removed. In both methods the inhalent siphon tip is ligatured and connected via a fine thread to an isotonic transducer. In isolated siphonal preparations the base of the inhalent siphon is also ligatured and fixed to a central position in an organ bath. Fixing of *in-situ* preparations is achieved by attaching the right-side valve to a central pedestal with molten wax. Adjustments to the transducer arm are made to gradually elongate the inhalent siphon to about 3 cm. This length constitutes the relaxed and extended state and was used as the baseline for all recordings of contractions.

All preparations were kept under 150 cm^3 artificial seawater at 10°C. Aeration of the seawater caused an effective mixing of alkyllead additions. All concentrations un the organ bath were calculated from the known dilution of analysed stock solutions.

RESULTS AND DISCUSSION

Toxicity tests

Toxicity tests were performed using Et_3PbCl and Me_3PbCl. Tetramethyllead (TML) was not used because of its highly unstable nature in pure form. Attempts were made to assess tetraethyllead (TEL) toxicity; however, the highly volatile nature of this material precluded any aeration of the exposure tanks, resulting in rapid deterioration of water quality and unacceptable control mortality. A closed flow-through exposure system would have overcome this problem but we did not have this facility and therefore decided to obtain an assessment of TEL toxicity by the siphonal preparation method.

All tests were made without any sediment in the exposure tanks. Although this is a less realistic environment, initial studies with natural sediment indicated a rapid loss of alkyllead to sediment (Fig. 1) which would prevent a steady exposure concentration.

The tests with both trialkylleads indicated the same general response (Fig. 2). For the first 5–10 days exposure there were relatively few mortalities but these were followed by a rapid increase in the mortality rate. In some concentrations the rate then became lower, as 100% mortality approached. This response pattern is common in short-term acute toxicity tests.

Concentrations of the alkylleads showed relatively little variation during the exposure period (Table 1). The initial low rate of mortality may have been partly due to the avoidance behaviour observed in *Scrobicularia*. Observations of the animals indicated that a higher percentage of valve adduction occurred in alkyllead-exposed tanks than in controls. However, although the periods of valve closure were not quantified, they lasted for several hours rather than several days. This is in contrast with the much longer periods of valve closure observed by Akberali.[5,11] The avoidance behaviour, therefore, may have delayed toxicity to some extent, but certainly did not prevent at least intermittent exposure during the first seven-day period.

Maddock and Taylor[12] reported that, in general, ethyl derivatives were more toxic to aquatic organisms than methyl derivatives, although mussels (*Mytilus edulis*) were twice as sensitive to Me_3Pb^+ than to Et_3Pb^3 in 96 h LC_{50} tests ($0.5\,mg\,dm^{-3}$ and $1.1\,mg\,dm^{-3}$ respectively). From these tests they predicted the toxicity threshold value for Et_3Pb^+ as $0.4\,mg\,dm^{-3}$.

Incipient lethal concentrations for *Scrobicularia* cannot be accurately calculated because, at all exposure concentrations causing partial mortality, response curves do not become asymptotic with time by the end of the tests. This means that even longer exposure would be expected to cause further mortality. However, by extrapolating the 50% mortality curves (Fig. 3), tentative incipient lethal concentrations of 0.07–0.1 $mg\,dm^{-3}$ are indicated for both compounds. Mortality occurs more rapidly in Et_3Pb^+-exposed animals, suggesting the shorter chain

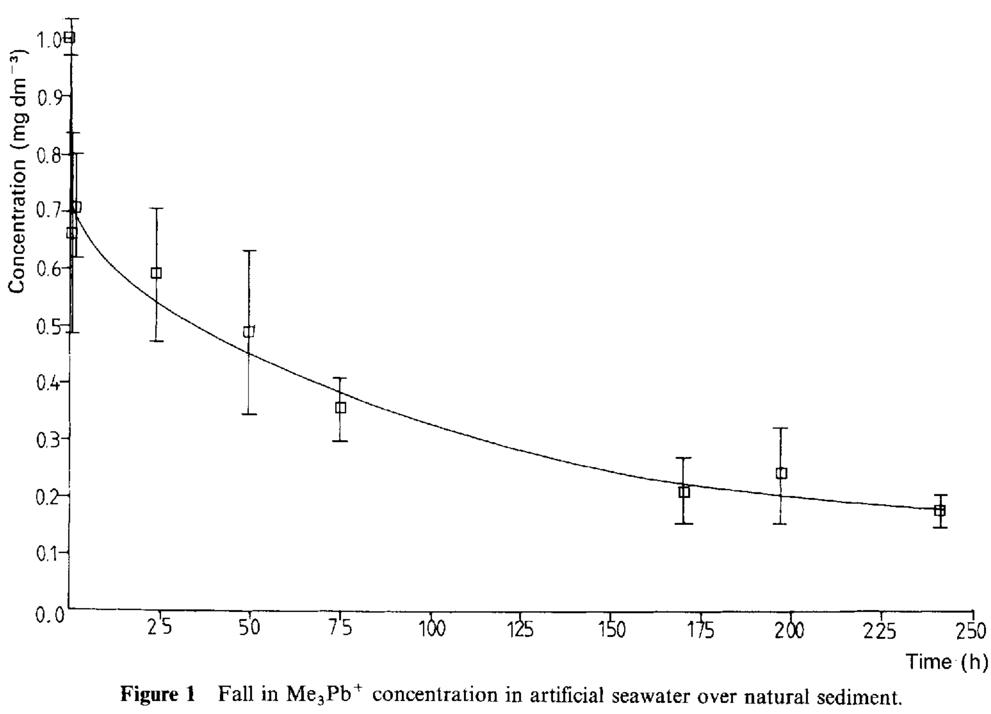

Figure 1 Fall in Me_3Pb^+ concentration in artificial seawater over natural sediment.

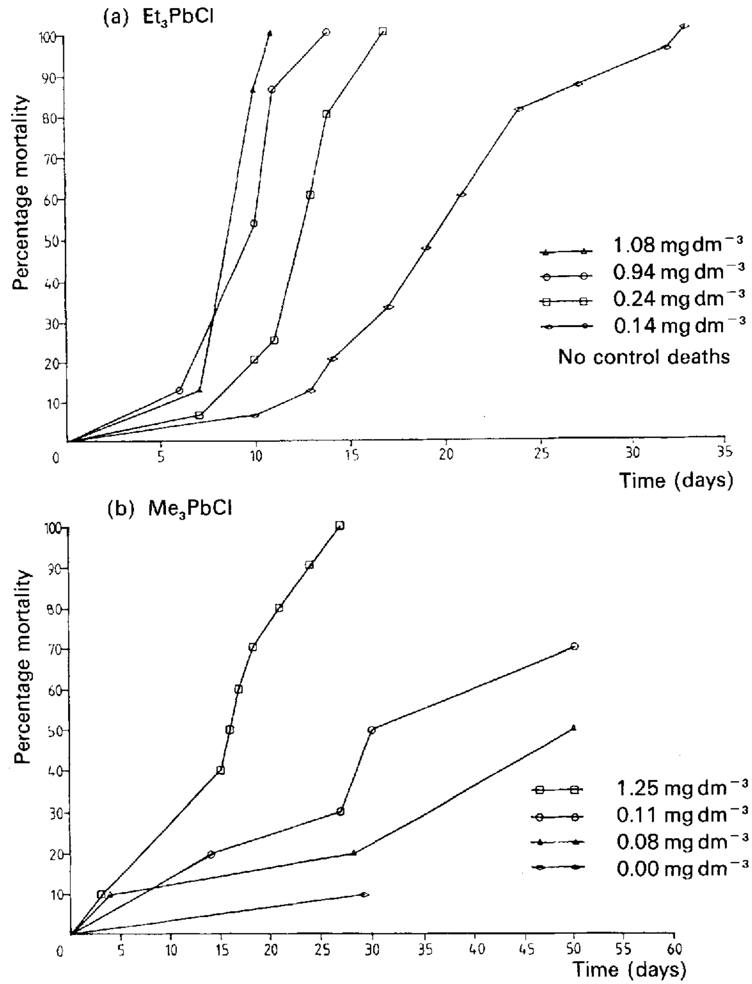

Figure 2 Mortality of *Scrobicularia* exposed to (a) Et_3PbCl and (b) Me_3PbCl.

length compound to be less toxic, although it appears likely that the incipient lethal toxicities are at similar concentrations. Maddock and Taylor suggest a 'safe' exposure level of trialkyllead species to mussels of 0.1 × predicted threshold value, which gives 0.1 mg dm^{-3}. Application of the same safety factor to the incipient lethal concentrations for *Scrobicularia* suggest a 'safe' concentration of approximately 0.01 mg dm^{-3} for Et_3Pb^+ and Me_3Pb^+. The lower value for *Scrobicularia* may be due to interspecific variation in sensitivity to trialkyllead, or to inaccuracies resulting from extrapolation.

Siphonal preparation tests

The initial static exposure test method with *Scrobicularia* was not appropriate for determination of the toxicity of TEL, although useful results were obtained for Et_3Pb^+ and Me_3Pb^+. Without the facility to conduct continuous-flow toxicity tests, another approach was required. Akberali[5] has demonstrated the feasibility of *Scrobicularia* siphonal preparations to test the effects of copper and other environmental stresses. We use the same method to compare the effects of TEL, Et_3Pb^+, Me_3Pb^+, Bu_3Pb^+ (tributyllead), Et_2Pb^{2+}, Me_2Pb^{2+} and Pb^{2+} (inorganic lead). The experiments were conducted during February, July and October and replicate experiments carried out in each month produced similar results, indicating no seasonal variations.

In all preparations the facility of the inhalent siphon to contract was tested before alkyllead addition by tactile stimulation. A viable siphon rapidly contracted and then elongated back to its

Table 1 Nominal and measured concentrations of ionic alkyllead species in seawater in toxicity tests (mg dm^{-3})
(a) Et_3PbCl exposure

Time (days)	0.0	0.15 a	0.15 b	0.30 a	0.30 b	1.00 a	1.00 b
0	<0.003	0.12	0.16	0.25	0.19	0.91	0.93
3		0.15	0.13	0.25	0.31	0.96	1.21
7		0.13	0.04[a]	0.15	0.18	0.96	1.16
14	0.004	0.16	0.15	0.28	0.28	0.92	1.00
21	<0.003		0.14				
28	<0.003		0.14				
Mean	<0.003	0.14	0.14	0.23	0.24	0.94	1.08
S.D.		0.02	0.01	0.06	0.06	0.03	0.13

(b) Me_3PbCl exposure

Time (days)	0.0	0.05 a	0.05 b	0.10 a	0.10 b	1.00 a	1.00 b
7	0.004	0.08	0.10	0.13	0.12	1.50	1.26
10	<0.003	0.07	0.07	0.08	0.11	1.01	1.09
20		0.11	0.09	0.12	0.11	1.16	1.46
31	0.004	0.09	0.07	0.10	0.12		
55	<0.003	0.08		0.11			
Mean	<0.004	0.09	0.08	0.11	0.12	1.22	1.27
S.D.		0.02	0.02	0.02	0.01	0.25	0.19

Note. All concentrations are the sum of trialkylleads and respective dialkylleads.
[a]Not included in calculation of mean.

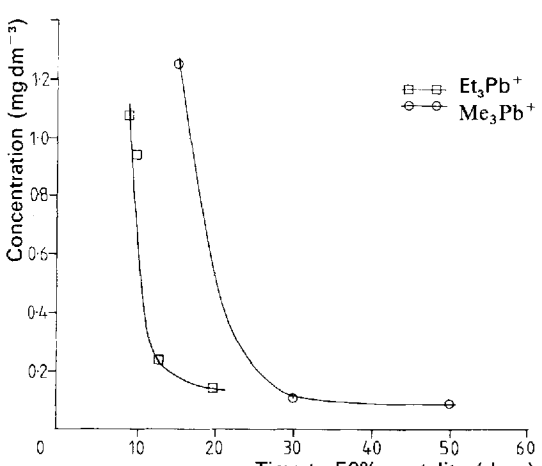

Figure 3 Relationship between concentrations of Et_3Pb^+ and Me_3Pb^+ and time to 50% mortality of *Scrobicularia*.

initial baseline length. Less than 10% of preparations failed to respond in this way and these were discarded. Typical responses are shown in Figs 4–7.

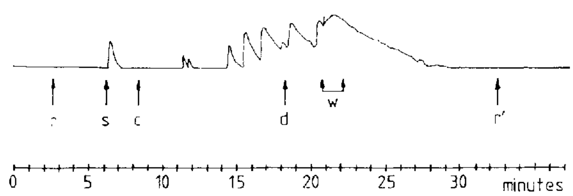

Figure 4 Typical response of an inhalent siphon exposed to alkyllead. The steady baseline reflects the inhalent siphon's relaxed extended state (r). A tactile stimulation, by pinching with forceps, produces a rapid, isotonic contraction (upward deflection), followed by a relaxation (downward deflection) (s). The siphon, in this case *in situ*, is then back to its steady relaxed state. An addition of 7.5 cm^3 of a stock diethyllead chloride solution (100 mg dm^{-3}) to the organ-bath at c, to give a final Et_2Pb^{2+} concentration of 5 mg dm^{-3}, results in a series of contractions, increasing in magnitude, after several minutes of delay. The siphon reaches a permanently semi-contracted state (d). Washing the tissue twice with 250 cm^3 of fresh seawater and refilling the organ-bath, brings about a fairly rapid extension of the siphon to its former extended length (w and r'). This suggests that the contractions are due to alkyllead effects, which can be washed away with fresh seawater.

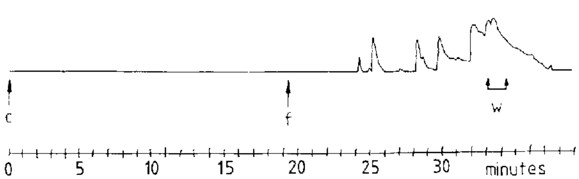

Figure 5 There is no response of the *in-situ* siphon to 1.0 mg dm^{-3} Me_3Pb^+ added at c. A further addition to give a final concentration of 2.0 mg dm^{-3} Me_3Pb^+ (f) causes a contraction after a short delay. A 250 cm^3 wash with fresh seawater returns the siphon to its relaxed state.

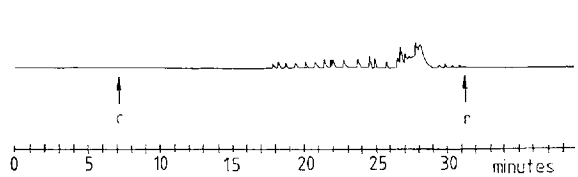

Figure 6 Bu_3Pb^+ (0.1 mg dm^{-3}) added to an *in-situ* preparation (c) causes a response after exposure of about 10 min. Contractions are small and interspaced by short relaxations. An increase in intensity does not last and the siphon returns to its relaxed state (r). No further contractions are observed.

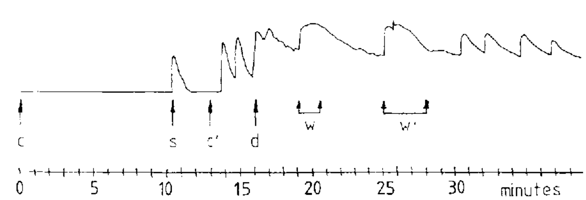

Figure 7 An *in-situ* preparation does not respond to the addition of 0.4 mg dm^{-3} Et_3Pb^+ (c). Mechanical stimulation (s) produces a typical short response. Further addition of Et_3Pb^+ (c′) to give a final concentration of 0.5 mg dm^{-3} results in maximum contraction (d). Successive washes with 250 cm^3 (w) and 500 cm^3 (w′) seawater do not cause the siphon to relax.

Both types of siphonal preparation produced the same range and type of recordings. In some cases unusual responses were observed. For example, a small number of preparations contracted on exposure to alkyllead for only a few minutes and then became relaxed again. Further contractions did not occur for several minutes or not at all (Fig. 6). Another unusual response consisted of the siphon entering into a permanently semi-contracted state but several washing cycles failing to abate the contraction (Fig. 7). The failure to obtain a relaxed preparation by washing suggests that some alkyllead may have remained in contact with the siphon even after several washes.

It is unclear why a small proportion of the preparations responded atypically. However, as the main aim was to estimate the lowest effect concentrations, we did not attempt to identify the cause of atypical responses. Because of the range of possible responses, it was decided that the minimum concentrations should cause at least two consecutive large contractions, or bring about a prolonged series of individual contractions. These criteria are arbitrary, but pragmatic in that only large or repeated contractions are likely to reflect a probable closure of the valves, if the animal had been intact.

The time of response from the addition of the alkyllead until the first contraction varied considerably, typically being between 1 or 2 and 20 min (Fig. 4). For minimum concentrations of alkylleads causing a response (Table 2) an exposure period of about 10 min elapsed before contraction occurred. Higher concentrations brought about contractions after much shorter periods.

Siphons were ten times more sensitive to Et_3Pb^+ than to Me_3Pb^+ and twice as sensitive to Bu_3Pb^+ as to Et_3Pb^+ (Figs 5 to 7 show examples of response to trialkyllead). These results are consistent with the earlier toxicity tests which suggested Et_3Pb^+ to be more toxic than Me_3Pb^+. Both dialkylleads were less toxic than their trialkyllead analogues and inorganic lead, added as lead(II) nitrate ($Pb(NO_3)_2$), did not elicit any response at concentrations up to 100 mg dm^{-3}. Comparing the minimum Et_3Pb^+ and Me_3Pb^+ concentrations for *in-situ* siphonal contraction with the earlier toxicity tests, suggests similar threshold concentrations for Et_3Pb^+ but there is less agreement for Me_3Pb^+. However, the toxicity tests can only be taken as an indication of the threshold of toxicity because of the limited data. It is interesting that the predicted toxicity threshold for mussels of about 1.0 mg dm^{-3} for both compounds[12] is also within the same order of magnitude.

Table 2 Minimum concentrations required for siphonal contraction in *Scrobicularia* (mg dm^{-3})

Compound	Siphonal preparation *In situ*	Siphonal preparation Isolated
$Pb(NO_3)_2$	n.r.[a]	n.r.
Me_2PbCl_2	7.0	n.d.[b]
Et_2PbCl_2	5.0	n.d.
Me_3PbCl	2.0	5.0
Et_3PbCl	0.2	0.5
Bu_3PbOAc	0.1	n.d.
TEL	n.r.	n.d.

[a]n.r., No response. [b]n.d., Not done.

For all compounds tested, *in-situ* siphons responded at lower concentrations than isolated siphons. This suggests that some part of the siphon's sensory apparatus was damaged during excision, reducing its sensitivity. The cruciform muscle at the base of the siphon is thought to take the strain of siphonal contraction and may also be part of the sense organ which is present near the posterior muscle attachment.[13] The intact cruciform muscle in *in-situ* preparations may explain the greater sensitivity exhibited. The contraction of isolated siphons with damaged cruciform muscles suggests that there may also be an intrinsic mechanism of the siphon itself, as suggested by Akberali.[5]

For the alkylleads tested, the minimum response concentration decreased with increasing degree of alkylation and chain length, except for TEL which did not cause a response (Table 2). This trend would be expected as the toxicity of organic compounds is often highly correlated with lipophilicity, even though in this case the most lipophilic compound was TEL.

TEL has a very low solubility in water ($0.3\,mg\,dm^{-3}$)[14] and no siphonal contractions were observed at this nominal concentration. Higher concentrations were tested using a fine $100\,mg\,dm^{-3}$ TEL suspension prepared by sonication. The suspension dispersed rapidly on entry into the organ bath. The TEL was assumed not to be in solution but still to have come into contact with the siphons because of the constant mixing; however, no responses were observed. Higher concentrations, up to $100\,\mu l$ TEL in $1\,cm^3$ ethanol, were added into the organ bath but apart from a small response which was probably due to the ethanol (the same response was obtained by adding $1\,cm^3$ ethanol alone), there were no contractions. The failure to demonstrate any response from TEL is in contrast to the work of Maddock and Taylor,[12] who report TEL to be more toxic than Et_3Pb^+ and Et_2Pb^{2+}.

The issue of TEL toxicity has also been investigated in mammals which have been reported to be more tolerant to TEL than Et_3Pb^+[15,16] and for marine and freshwater algae. Maddock and Taylor[12] showed that TEL was more toxic to the marine diatom *Phaeodactylum tricornutum*. Jarvie and Marshall[17] performed similar experiments and also found significant inhibition of photosynthesis; however, analyses of the exposure medium suggested rapid decomposition of TEL to the lower derivatives. Indeed it was concluded that enough Et_3Pb^+ had been produced to account for the observed inhbition. Further, Roderer[18] and Jarvie and Marshall[17] concluded from experiments with the freshwater Chrysophyte *Poterioochromomonas malhamensis* that TEL was not toxic to the alga under dark (non-degrading) incubation conditions and that toxicity was only observed under high illumination where Et_3Pb^+ and Et_2Pb^{2+} would be present due to the photolytic decomposition of TEL.

It would appear unlikely that highly toxic concentrations of Et_3Pb^+ would occur in exposure vessels in the continuous-flow system used by Maddock and Taylor, even if chemical decomposition of TEL in seawater is significant. However, the siphonal responses observed from all of the alkyllead compounds tested except TEL correlate well with the expected relative toxicities, suggesting that TEL has only a low toxicity in its pure form.

CONCLUSIONS

Triethyllead and trimethyllead species are acutely toxic to *Scrobicularia* at concentrations below $0.14\,mg\,dm^{-3}$ and $0.08\,mg\,dm^{-3}$ respectively. Mortality at these concentrations continues to occur after 35 and 60 days respectively. Approximate incipient lethal concentrations for both compounds fall in the range 0.07–$0.1\,mg\,dm^{-3}$.

In-situ siphonal preparations are more sensitive to toxicants than excised isolated siphons. Trialkylleads cause siphonal contraction at lower concentrations than the respective dialkylleads. Tributyllead is more toxic than triethyllead, which in turn is more toxic than trimethyllead. Tetraethyllead and inorganic lead do not cause siphonal contraction.

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