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Toxicity and model membrane modifying properties of organolead compounds

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The influence of trialkylleads on haemolysis of red blood cells (RBCs), growth of *Spirodela oligorrhiza* and stability of planar lipid membranes (PLMs) at different pH of solution has been studied. The results obtained show that the efficiency of trialkylleads (methyl-, ethyl-, propyl- and butyl-lead chlorides) in modifying the physiological and mechanical properties of the objects studied depended both on pH of solution and hydrophobicity of the compounds. Namely, it was found that this efficiency increased with pH of solution. The most significant increase was observed in PLM experiments. Also, the hydrophobicity of trialkylleads influenced the properties mentioned. The more hydrophobic a compound the greater was its haemolytic toxicity. The same applies to the physiological toxicity of the compounds, whose measure was 50% inhibition of plant growth. Generally, the sequence of modifying possibilities of the compounds studied at any pH of the solution was the following:

tributyllead > tripropyllead > triethyllead > trimethyllead

A possible mechanism of the interaction of organolead species with model and biological membranes is discussed. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: triorganolead chlorides; erythrocyte haemolysis; physiological toxicity; planar lipid membranes destabilization; interaction mechanism.

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INTRODUCTION

Organic derivatives of lead are very toxic and are often used and so occur in the environment. Mostly, they originate from lead antiknock additives to gasoline, but there are also organolead compounds that are purposefully introduced into environment as biocides.[1–3] Once they invade a living organism they may cause multiple and diverse pathological changes.[4] However, in order to do that they must interact with the biological membrane or cell wall as a place of first contact, and many toxic effects are the consequence of this contact.[2,5–10] The toxicity of organolead compounds depends on various factors. The most important seem to be lipophilicity[1,2,11] and speciation due to the environment in which a particular species is deposited. The latter determines the persistence of organoleads,[11] and one of the environmental properties is its pH.

This work contains the results of studies on the interaction of trialkyllead chlorides with erythrocytes (red blood cells, RBCs), planar lipid membranes (PLMs) and the aqueous plant *Spirodela oligorrhiza*, and its main aim was to determine how this interaction depends on the pH of the solution in which the above species were studied. The measures of the interaction were the concentrations of triorganolead compounds that caused destruction of PLMs in a predetermined time (3 min), 50% and 100% haemolysis of erythrocytes and 50% inhibition of plant growth.

The first two measurement types proved useful in studies on the interactions of different biologically active substances with biological and lipid model membranes,[12,13] and were thought to explain organolead compound interactions with the lipid phase of biological membranes; while physiological tests, connected with perturbation of metabolic processes, were expected to elucidate their potential toxicity at the molecular level.

MATERIALS AND METHODS

Materials

All the triorganolead compounds studied [chlorides of trimethyllead (TML), triethyllead (TEL), tripropyllead (TPL) and tributyllead (TBL)] were purchased from Alfa Products (Germany). PLMs were formed from azolectin dissolved in a mixture of *n*-decane and *n*-butanol. Azolectin was purchased from Avanti (USA) and *n*-decane and *n*-butanol from Sigma (USA). All the chemicals used were of analytical grade.

PLM experiments

PLMs were formed from a 3% (w/v) solution of azolectin in *n*-decane:*n*-butanol (1:1 v/v) on a hole of 1.5 mm diameter in a Teflon two-chamber measuring cell. The compounds studied were dissolved in ethanol–water (ethanol concentration did not exceed 5%) solutions to give 0.01 M solutions. These were pipetted by means of calibrated micropipettes directly into the bath solution (volume of bath solutions was 12 ml) until their concentration reached a value at which membrane life-time was no longer than 3 min. This concentration is further referred to as the critical concentration (CC). Phosphate buffer solutions of pH 4.0 and 8.0 were used as the bath solutions. The time necessary for lipid membranes to achieve bimolecular arrangement was about 15 min at room temperature (~22 °C). This means that under CC conditions no new membrane could be formed. The process of membrane formation was monitored optically by means of a microscope. PLMs were also controlled continuously by observing the current with a measurement system consisting of a Keithley 617 Programmable Electrometer and a standard voltmeter controlling the DC voltage (20 mV) applied to the membrane by means of calomel electrodes immersed directly in the bath solution. This enabled us to determine the moment a PLM broke. Each experiment was repeated at least three times.

RBC experiments

Measurements were performed with fresh heparinized pig blood. Phosphate buffers at pH 8.0, 7.4, 6.0 and 5.0 were used as bulk solutions. Erythrocytes were washed four times in the bulk solution and incubated in the same solution containing a chosen concentration of organolead species. Modification was conducted at 37 °C for 4 h. Haematocrit was 2%. The percentage of haemolysis was measured for 1 ml samples, taken after 0.5, 1, 1.5, 2, 3 and 4 h. The samples were centrifuged and the haemoglobin content in the supernatant measured with a spectrophotometer (Specol 11, Carl Zeiss, Jena) at 540 nm wavelength. Haemoglobin concentration was expressed as percentage of haemolysed cells, calculated relative to a sample containing totally haemolysed erythrocytes. All triorganolead compounds were dissolved in ethanol, the concentration of which in the samples did not exceed 1% (v/v). Each experiment was repeated at least three times.

Plant experiments

Studies on physiological activity of triorganolead compounds were carriedout on *S. oligorrhiza*. Two equal fronds were placed in Erlenmayer flasks containing modified Hoagland's solution.[14] Two solutions of pH 5.2 and 6.2 were used. The plants were cultivated under a constant illumination of 120 $\mu Em^{-2} s^{-1}$ at 25 °C. After 8 days the dry mass of the plants was determined. Biomass data were expressed as percentage control response. Calculation of the effective concentration of triorganolead compounds resulting in 50% growth inhibition (EC_{50}) compared with controls was carriedout by using non-linear regression for the logistic model[15] (dose response). Each experiment was repeated at least three times.

RESULTS AND DISCUSSION

The results of experiments with PLMs are presented in Figure 1. It can be seen that the values of the CCs of triorganolead compounds depend distinctly on pH. For both pH values (4.0 and 8.0), the weakest PLM-destabilizing organolead compound is TPL. The efficiency of other compounds in the less acidic solution is about two to three times higher than in the more acidic one. Only slight differences are found in the efficiencies of individual compounds to break model membranes under the same pH conditions, with the exception of TPL.

The results of haemolytic experiments are summarized in Table 1, which contains values of 50% (C_{50}) and 100% (C_{100}) haemolysis of erythrocytes caused by triorganoleads in buffer solutions at different pH. The values of C_{100} were

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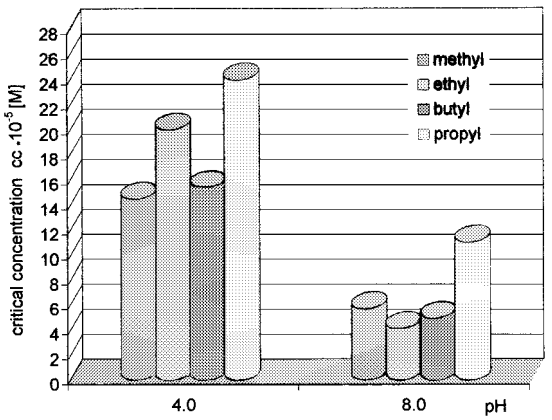

Figure 1 CC of triorganolead compounds for various phosphate buffer solutions in PLM experiments. The standard deviation did not exceed 10%.

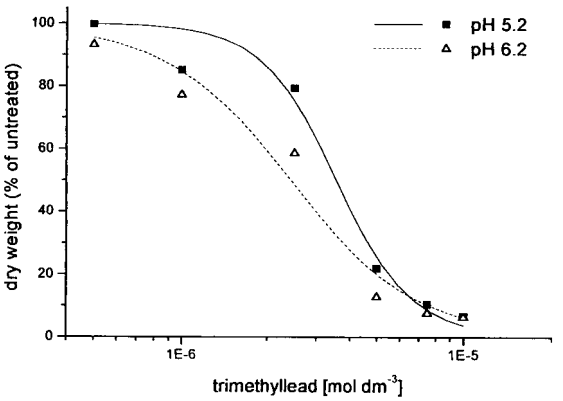

Figure 2 Dependence of growth inhibition of *S. oligorrhiza* on the concentration of trimethyllead chloride (TML) at different pH. The standard deviation was 0.3.

obtained by extrapolation of haemolytic curves and the aim was to obtain a parameter qualitatively equivalent to the one used in PLM experiments (CC), i.e. a parameter describing total disruption of the erythrocyte membrane.

The results of studies on inhibition of growth of *S. oligorrhiza* are presented in Figures 2–5 and show the dependence of dry mass change on concentrations of organoleads. Values of EC_{50} calculated from these plots are collected in Table 2.

All the results obtained show that trialkylleads were effectively interacting with the plant and model membranes studied. As expected, on the basis of previous model experiments and studies on the toxicity to some bacteria and algae, the interaction increased with the increase in lipophilicity of trialkylleads.[16–18] This was clearly seen in haemolytic experiments, where TEL influenced erythrocytes about 10–20 times less efficiently than TPL and TBL, and in plant experiments where the efficiencies of TPL and TBL were about one order of magnitude higher than those of TML and TEL. No similar big differences in destabilization of PLMs by particular triorganoleads were observed.

A gradual increase in pH of the buffer solution from 5.0 to 8.0 caused an increase in haemolytical properties of triorganoleads. This increase was biggest for lower values of pH.

Generally, the efficiencies of the triorganoleads in influencing the model and biological membranes studied, as well as their physiological toxicities, were found to be dependent on their lipophilicities and, to a greater extent, on the pH of the solution in which the experiments were performed. It seems that such results may be explained by the fact that trialkylleads exist as various species in the vicinity of neutral pH. These are hydrated cations, hydroxide cations or hydrated neutral forms existing in equilibrium in various ratios depending on the organolead concentration.[19] There are probably no

Table 1 The values of concentrations of triorganolead compounds causing 50% (C_{50}) and 100% (C_{100}) haemolysis of erythrocytes in phosphate buffer solutions of different pH

	C_{50} (mM)			C_{100} (mM)		
Compound	TEL	TPL	TBL	TEL	TPL	TBL
pH 5.0	1.500	0.150	0.075	2.000	0.300	0.110
pH 6.0	1.000	0.095	0.048	1.200	0.250	0.087
pH 7.4	0.850	0.075	0.040	1.000	0.200	0.070
pH 8.0	0.700	0.060	0.031	0.850	0.150	0.055

Standard deviation was 0.04.

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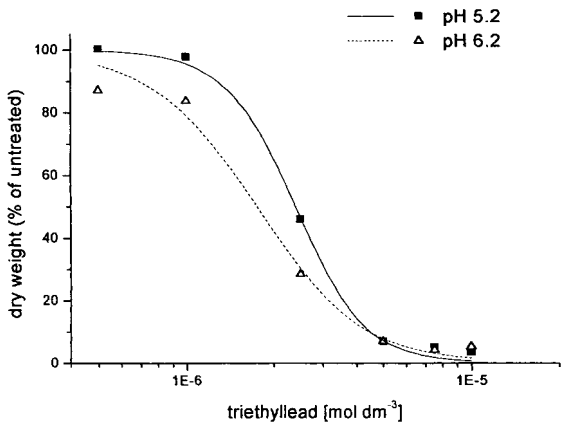

Figure 3 Dependence of growth inhibition of *S. oligorrhiza* on the concentration of triethyllead chloride (TEL) at different pH. The standard deviation was 0.3.

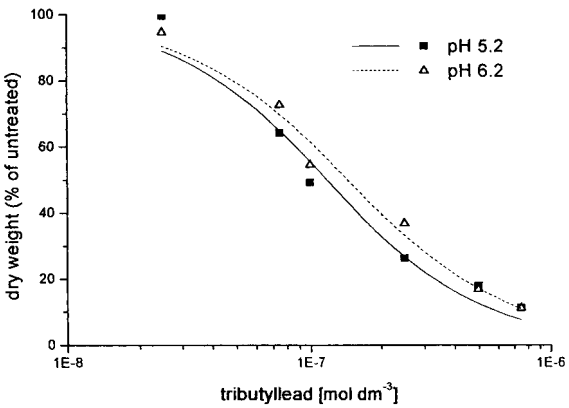

Figure 5 Dependence of growth inhibition of *S. oligorrhiza* on the concentration of tributyllead chloride (TBL) at different pH. The standard deviation was 0.014.

other forms, as measurements were carried out under triorganoleads concentrations not promoting the appearance of those forms. Overall, the interaction of these species with the objects studied is then a combination of hydrophobic and polar interactions. The polarity should be greater in the case of hydrated cations whose available positive charge is not so much as that of other species. A decrease of pH below 7.00 means that hydroxide cations and neutral forms disappear. That leaves the hydrated cation the only species in that pH area. Its polar interaction with the surface charge of the polar head of lipid molecules of model and erythrocyte membranes, especially with choline groups, should be quite intensive and should not permit deep intercalation of triorganoleads into these membranes. Additionally, it must be taken into account that a change in mechanical properties of the erythrocyte membranes caused by changed membrane organization in comparison with that at physiological pH may occur.[20] This may explain the differences in efficiency of triorganoleads in that pH region. Such an approach may explain the results obtained showing a weaker influence of triorganoleads on the objects studied at lower pH values of the solution.

In contrast, the less polar or neutral species appearing in the neighbourhood of pH 8.00 permit a more intensive interaction of triorganoleads with model membranes. Studies on physiological toxicity of triorganoleads do not invalidate the above formulated conclusion. However, it must be underlined that the concentrations of organoleads in which toxicological effects were observed are about three orders of magnitude lower.

The qualitative similarity of these studies

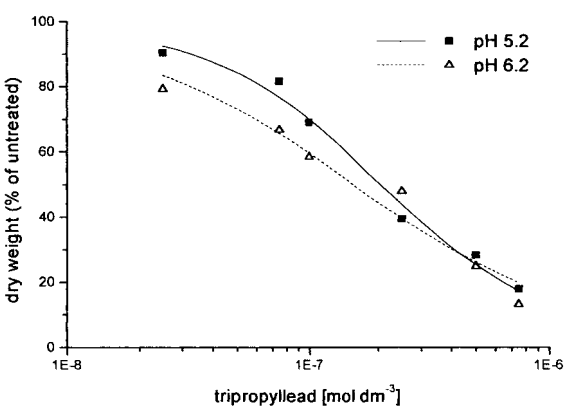

Figure 4 Dependence of growth inhibition of *S. oligorrhiza* on the concentration of tripropyllead chloride (TPL) at different pH. The standard deviation was 0.014.

Table 2 The values of concentrations of triorganolead compounds causing 50% (EC_{50}) inhibition of *S. oligorrhiza* growth in nutrient solutions with different pH

	C_{50} (μM)			
Compound	TML	TEL	TPL	TBL
pH 5.2	3.57	2.41	2.03	1.18
pH 6.2	2.43	1.76	1.55	1.44

Standard deviations were 0.3 for TML and TEL, 0.014 for TPL and TBL.

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suggests that the lipid phase of the plant cell membrane may be involved. However, a direct interaction of organoleads with membrane proteins and the resulting toxicity cannot be excluded.

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