Role of Hydrophobic and Hydrophilic Interactions of Organotin and Organolead Compounds with Model Lipid Membranes

Janina Gabrielska, Janusz Sarapuk and Stanisław Przestalski

Department of Physics and Biophysics, Agricultural University, Norwida 25, 50–375 Wrocław Poland

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Organotin and Organolead Compounds, Model Lipid Membranes, Interaction Mechanism

The present study was conducted to clarify the mechanism of toxicity of organic compounds using lipid model membranes (liposomes and planar lipid membranes).

The compounds studied were trialkyltin and trialkyllead chlorides, dialkyltin dichlorides and some inorganic forms of those metals. Two different (anionic and cationic) detergents were also used in the experiments to change the surface properties of liposomes. As a measure of interaction between the compounds studied and model membranes were the release of liposome bound praseodymium and the change in stability of planar membranes under the influence of those compounds.

On the basis of the results obtained it was postulated that the mechanism of interaction between tin- and leadorganics and model lipid membranes is a combination of different factors featuring interacting sides. The most important properties determining the behaviour of organic compounds in the interaction were lipophilicity and polarity of different parts of the organics and the steric arrangement they can take in the medium. On the other hand, the surface potential of the lipid bilayer and the environment of the lipid molecules, that play a significant role in the availability of the lipid bilayer to the organics, were important factors in the interaction.

Introduction

Organotin and organolead compounds studied in this work are anthrogenic toxic agents occuring in the environment which are responsible for quite a number of pathological effects occurring in living organisms. Toxicity of organotin compounds is extensively used and, depending on individual estimates by different authors, about ten to thirty percent of organotin production is introduced directly to the environment as biocides (Crowe, 1987 ; Attar, 1996; Fent, 1996). The appearance of organolead in the environment is mostly the effect of using tetraethyllead, the well known and controversial antiknock agent in gasoline. At least 60% of organolead content in natural habitats comes from this compound and its derivatives which also exhibit biocidal properties. It is postulated that the toxicological action of organometals can be referred to two different levels. One is the molecular level where toxic effects occur at small concentrations of the compounds {10^{-7} – 10^{-6} M} (Thayer, 1974) and concern perturbation or disruption of metabolic processes in living objects. The second level is the cellular one where the concentration of organometals is usually, but not nesessarily, much higher (about two orders of magnitude) and the effects observed relate to physicochemical and/or mechanical properties of biological walls and membranes (Heumann, 1987; Gray *et al.*, 1987; Hamasaki *et al.*, 1995). However, it must be underlined that specified concentration ranges have a general sense only, as toxic effects at both molecular and cellular levels can sometimes be observed for similar values of organometallic compounds concentrations. It seems that the capability of cell membranes to undergo structural perturbation by organometallic compounds strongly depends, among others, on their lipophilicity, as was postulated or shown by different authors (Gray *et al.*, 1987; Heumann, 1987; Musmeci *et al.*, 1992; Radecki 1993; Ambrosini *et al.*, 1996).

This work contains the results of studies on the interaction of some organotin and organolead compounds, differentiated by their polar and/or hydrophobic parts, with lipid model membranes

Reprint requests to Janina Gabrielska.
Fax: (+48)-71-205172.

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and its main objective was to determine the role played by those parts in this interaction. So far, toxicological activities of both organolead and organotin compounds have already been found to be dependent at least on the kind and number of organic attached to the metal atom (Thayer, 1974; Eng et.al., 1991; Nagase *et al.*, 1991).

This paper contains also the results on the interaction of lipid membranes used (planar lipid membranes – BLM, unilamellar liposomes) with some inorganic compounds of lead that are known to be far less toxic than their organic forms (Röderer, 1983,1986; Heumann, 1987) and whose toxicities are dependent on their solubility in water and dissociation constants (Sharma and Chopra, 1987). Also some inorganic compounds of tin, which is generally accepted as a vital trace element were studied.

Some other experiments performed with liposomes should facilitate the interpretation of data obtained on the interaction between organic compounds and model membranes. These included modification of the liposomal membrane by some cationic and anionic detergents. They were thought to premodify the electrical properties of the model membrane surface and make it more or less accessible for organometallic compounds.

The marker of the interaction between the compounds studied and model membranes was praseodymium ion desorption from liposomes and change of stabilities of BLMs in the presence of the compounds mentioned. Both types of measurements proved useful in studies on interactions of different biologically active substances with biological and lipid model membranes (Kleszczyńska *et al.* 1990; Sarapuk *et al.*, 1992; Gabrielska *et al.*, 1994).

Materials and Methods

Materials

All studied organolead and organotin compounds (chlorides of trimethyltin-TriMT, triethyltin-TriET tripropyltin-TriPT, tributyltin-TriBT, and trimethyllead-TriML, tripropyllead-TriPL, tributyllead-TriBL, dichlorides of dimethyltin-DiMT, dipropyltin-DiPT, and dibutyltin-DiBT,) were purchased from Alfa Products (Karlsruhe, Germany). Both, cationic (trimethyldodecylammonium bromide, TMDA) and anionic (dodecanesulfonic acid sodium salt, AS) detergents were from Fluka (Buchs, Switzerland). The following inorganic lead and tin compounds were studied: $SnCl_2$, $SnCl_4 \cdot 5H_2O$, $Pb(NO_3)_2$ and $Pb(CH_3COO)_2 \cdot 3H_2O$ (Ciech, Gliwice, Poland). Heavy water and praseodymium chloride ($PrCl_3 \cdot 6H_2O$) used in liposome experiments were obtained from Polatom (Świerk, Poland) and Aldrich (Steinheim, Germany), respectively. Liposomes were formed from egg lecithin prepared according to the method described by Singleton *et al.* (1964).

Planar lipid membranes were formed from azolectin dissolved in a mixture of n-decane and n-butanol, all purchased from Sigma (St. Louis, USA). All used chemicals were of the analytical grade.

^{1}H-NMR experiments

The stock solution of lecithin in chloroform was stored at -20 °C. The lecithin was dried in nitrogen atmosphere and dispersed in D_2O. The final concentration of lecithin was 25 mg per ml. The suspension was sonicated for 30 min with 20 kHz sonicator with a titanium probe. During sonication the temperature was kept at 0–2 °C. Obtained dispersion was divided into 0.5 ml samples to which praseodymium ions and then studied compounds were added to achieve wanted concentrations. Liposomes were first incubated for one hour with proper solutions of detergents in the case of experiments performed with premodified liposomes. ^{1}H-NMR spectra were recorded on Bruker Avance DRX 300 Spectrometer at 30 °C.

The 300 MHz spectrometer parameters were: spectral window 6173 Hz; digital resolution 145.2145 Hz/cm or 0.48348 ppm/cm; pulse width 10.7 μs; acquisition and delay times were 2.65 and 1 s, respectively.

BLM experiments

Planar lipid membranes were formed from 1.5% solution (w/v) 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. Studied compounds were pipetted directly into the bath solution (0.9% NaCl) until their concentration reached a value (CC) at which membrane life-time was not longer than 5 min. A time necessary for lipid membranes to achieve bimolecular arrangement was about

15 min at room temperature (~ 22 °C). It means that under CC conditions no new membrane could be formed. The process of membrane formation was monitored optically and electrically. Each experiment was repeated at least three times.

Results

Fig. 1A shows the complete 300 MHz ^{1}H-NMR spectrum for unmodified egg lecithin vesicles in heavy water. The major -$(CH_2)_n$, -CH_3 and choline headgroup -$N^+(CH_3)_3$ resonances of phosphatidylcholines are clearly recognizable (Jendrasiak *et al.*, 1994). The single peak representing the choline headgroup is split by addition to the liposome dispersion of Pr^{3+} as can be seen in 1B. The distance $\Delta\nu$ [Hz] between extravesicular and intravesicular choline headgroup signals is proportional to the concentration of the praseodymium added to the extravesicular medium (Kaszuba and Hunt, 1990). Any change in the concentration of praseodymium bound to the external lipid monolayer of liposome changes $\Delta\nu$ and is a measure of praseodymium desorption from (or Pr^{3+} adsorption to) liposome as seen in the example spectrum presented in Fig. 1C. In this case the desorption of praseodymium was caused by tripropyltin. Figs 1A*, 1B* and 1C* are magnified fragments of spectra 1A, 1B and 1C showing the peak of choline headgroup, its splitting and the change of distance between signals coming from the outer and inner layer choline headgroup under the influence of TriPT, respectively. The percent of praseodymium desorption (release) is calculated as the ratio of the change in the choline headgroup-signal splitting before and after addition of studied compound. The results obtained are presented also in Figs. 2 (trialkylchlorides of tin, TriAT), 3A (trialkylchlorides of lead, TriAL,) and 3B (inorganic tin and lead). These results show that the strongest desorption effect for the organic tin series was caused by the compound of longest alkyl chain. Lead compounds caused slight release of praseodymium and in the case of leadmethyl chloride the opposite effect, i.e., sorption of praseodymium, was observed. Quantitatively, comparison of corresponding TriAT and TriAL compounds points at these first as significantly stronger interacting with liposomal membranes. Somewhat surprising fact is that also inorganic tin compounds were causing greater desorption of praseodymium from liposomes than alkyl lead chlorides. Inorganic lead {$Pb(NO_3)_2$}, like trimethyllead, caused slight sorption of praseodymium ions (see Fig. 3).

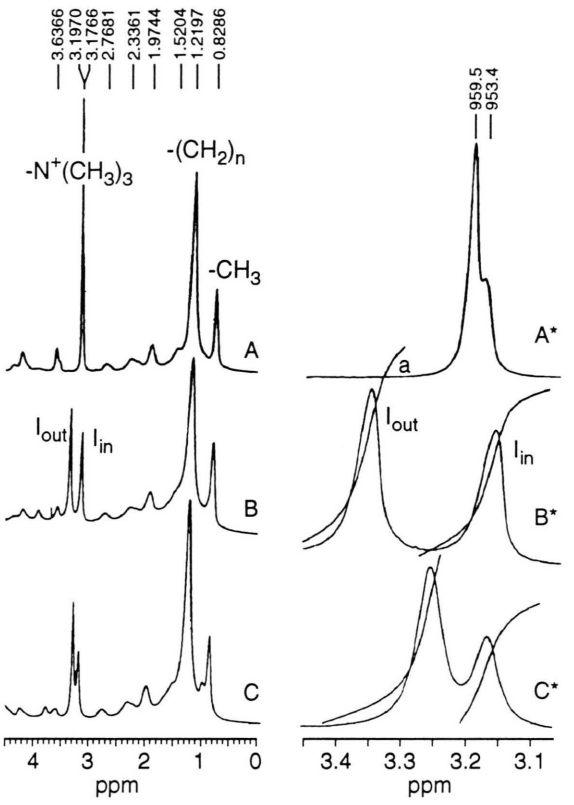

Fig. 1. An example of a typical 300 MHz ^{1}H-NMR spectrum of a sample of egg yolk lecithin liposome suspension (25 mg lipid/ml) in D_2O at 30 °C. The peak water (HOD) at 4.700 ppm was used as the reference signal (not seen in Fig. 1)
A – obtained for vesicle suspension with the main signals coming from the choline headgroups -$N^+(CH_3)_3$, acyl chain methylenes -$(CH_2)_n$, and the terminal methyl groups -CH_3.
B – for liposomes pretreated with Pr^{3+}. Extravesicular relative concentration of Pr^{3+} to lecithin was 1:6.76. Upfield (I_{in}) and downfield (I_{out}) signals come from intra- and extravesicular choline headgroup.
C – for praseodymium pretreated liposome under the influence of tripropyltin chloride (TriPT) at 3.73 mM concentration.
A*, B*and C*- magnified part of the same spectrum showing signals coming from choline headgroup. Additional lines a and b in Fig.B* are integral lines helping to calculate I_{out} and I_{in}. The ratio I_{out}/I_{in} = 1.7.
Signals positions in Fig. A* are given in Hz.

The results of experiments performed with (previously) modified liposomes are shown in

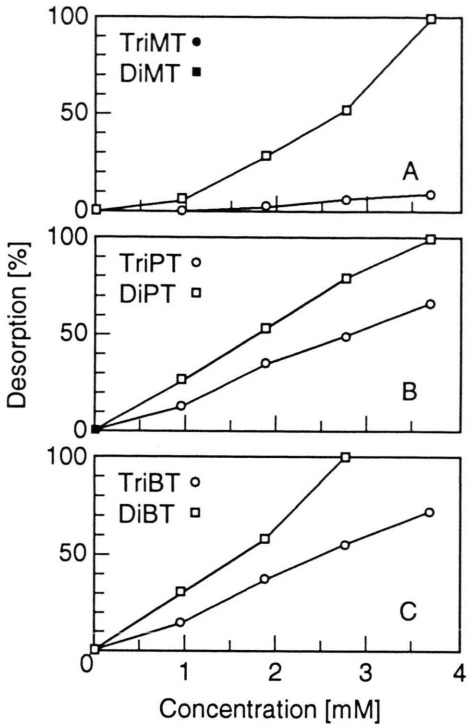

Fig. 2. Percent of Pr^{3+}ion desorption versus concentration of organometallic tin compounds added to liposome suspension (25 mg lecithin/ml). Extravesicular relative concentration of Pr^{3+} ions to lecithin was 1:6.76.
A) o- for trimethyltin chloride (TriMT), □- for dimethyltin dichloride (DiMT),
B) o- for tripropyltin chloride (TriPT), □ – for dipropyltin dichloride (DiPT),
C) o – for tributyltin chloride (TriBT), □ – for dibutyltin dichloride (DiBT).

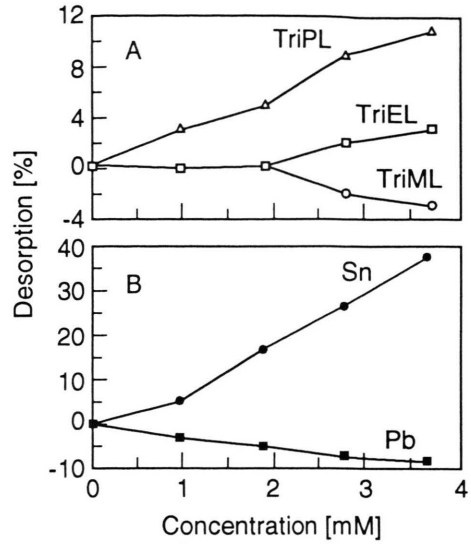

Fig. 3. Percent of Pr^{3+}ion desorption versus concentration of organometallic lead compounds (A) or inorganometallic compounds (B) added to liposome suspension (25 mg lecithin/ml). Extravesicular relative concentration of Pr^{3+}ions to lecithin was 1:6.76. (A) o – for trimethyllead chloride (TriML); □ – for triethyllead chloride (TriEL); Δ – for tripropyllead chloride (TriPL); (B) • – for inorganic tin chloride ($SnCl_2$); ■ – for inorganic lead [$Pb(NO_3)_2$].

Figs 4A and 4B. They reveal that the compounds studied when present in the dispersions of liposomes modified by detergents cause opposite effects depending on the detergent used. Namely, when liposomes were pretreated with trimethyldodecylammonium bromide (TMDA), the tinorganics caused decreased praseodymium release from liposomes compared with Pr^{3+} release by the same compounds from unmodified liposomes (Fig. 4A). When liposomes were pretreated with dodecane-sulfonic acid sodium salt (AS) the increase of praseodymium ion release was observed relative to the release of the ions from unmodified liposomes (Fig. 4B).

The results obtained for praseodymium release from unmodified and premodified liposomes under the influence of the DiAT dichlorides studied are summarized in Tab. I and the former are compared with TriAT in Fig. 2. Qualitatively, these results are similar to those obtained for tin chlorides, i.e., studied salts release Pr^{3+} from detergent unmodified liposomes depending on the length of their hydrocarbon chains; there is small difference in the effectiveness of DiBT and DiPT dichlorides. The comparison of the release of praseodymium ions from liposomes under the influence of TriAT chlorides and DiAT dichlorides shown in Fig. 2 reveals that the degree of the release is somewhat greater for DiAT dichlorides. The same can be said about the results of experiments performed with modified liposomes (see Table I).

The results of experiments with planar lipid membranes showed that practically all the tinorganics were not able to destroy the membranes at concentrations lower than the saturation concentrations; the values of those being of the order ~ 1×10^{-4} M. The only exception is dibutyltin dichloride. No dilution of tinorganic solution in methanol was applied to ensure "purity" of ex-

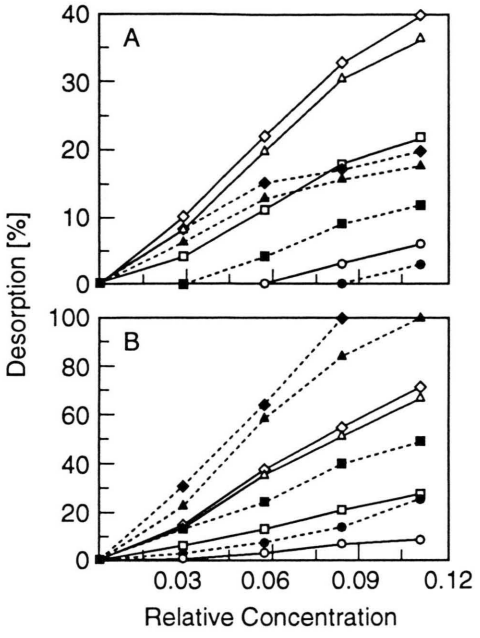

Fig. 4. Percent of Pr^{3+} ion desorption versus relative concentration of organotin compounds added to liposome modified by:
A – TMDA (trimethyldodecylammonium bromide) – dotted line; compared to Pr^{3+}release from unmodified liposome membrane – continous line. Relative concentration of Pr^{3+} ions to lecithin was 1:3.38;
B – AS (dodecane-sulfonic acid sodium salt) – dotted line; compared to Pr^{3+} desorption from unmodified liposome membrane – continous line. Extravesicular relative concentration of Pr^{3+} ions to lecithin was 1:6.76.◆,◊ – for tributyltin chloride (TriBT); Δ,Δ – for tripropyltin chloride (TriPT); ■,□ – for triethyltin chloride (TriET); •,o – for trimethyltin chloride (TriMT).

Table I. Percent of Pr^{3+} ion release from lecithin liposome membrane modified with cationic, TMDA, and anionic, AS, surfactants of 1.89 mM concentration, induced by dialkyltin-DiAT, compounds. DiMT-dimethyltin dichloride, DiPT-dipropyltin dichloride and DiBT-dibutyltin dichloride.

Studied compounds	Pr^{3+}desorption [%] with AS	Pr^{3+}desorption [%] with TMDA	Control
DiMT	41	20	27
DiPT	75	33	54
DiBT	80	34	56

periments. No such demand was necessary in the case of the experiments with TriAL. They were destroying lipid membranes at concentrations lower than saturation concentrations and the effectiveness of doing that can be presented as follows: TriBL > TriPL > TriEL > TriML (Fig. 5).

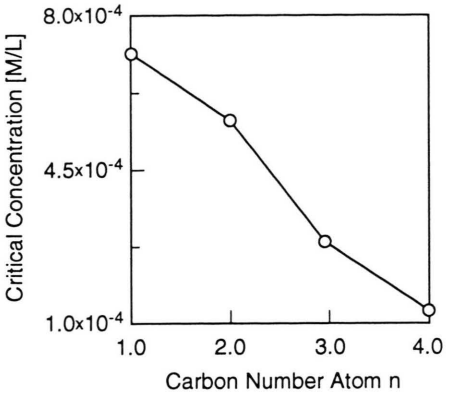

Fig. 5. Dependence of the critical concentrations of trialkyllead compounds on the number of carbon atoms in the alkyl chains.

Inorganic lead compounds critical concentrations were roughly twice of those obtained for leadorganics. The interactions of both lead acetate and lead nitrate with planar membranes were similar, although lead diacetate destroyed membranes when present in the bath solution at slightly lower concentration than the CC of lead nitrate (CC was 2.1×10^{-3} M and 3.4×10^{-3} M for lead acetate and nitrate, respectively). Inorganic tin compound $SnCl_4$ $5H_2O$ did not destroyed BLM at concentrations lower than 5×10^{-3} M.

Inorganic tin compound showed poor efficiency in breaking BLMs.

Discussion

The praseodymium binding takes place at negatively charged phosphate groups of the lecithin polar head, thus making this zwitterionic lipid positively charged. The interaction of any compound with such charged barrier must depend on various properties of the compound. The most important factors governing transport through or incorporation of a molecule to studied liposomal membranes seem to be the molecules stereometry, netto charge or charge density with resulting polarity or hydrophilicity, partition coefficient and the preferable structure the molecule takes in its environment. The role of particular factors can be discussed when the comparison is made for interaction of different compounds with liposomes.

However, some general conclusions can be drawn on the basis of the results obtained. Namely, all the tinorganics studied were releasing praseodymium ions from liposomes and the longer the alkyl chains of the compounds the greater the number of released Pr^{3+}. The obvious conclusion is that the desorption of praseodymium is connected with lipophilicity of the compounds, i.e., with growing possibility of entering of tinorganic molecules of into the lipid bilayer. It was suggested that TriAT compounds are present at physiological pH in aqueous phase as hydrolysis product Alk_3SnOH (Musmeci *et al.*, 1992) or as cationic diaquo forms at pH<5 $\{Alk_3Sn(H_2O)_2\}^+$ (Davies and Smith, 1982). They can interact with phosphate group of lecithin by weak Coulombic interactions (Heywood *et al.*, 1989; Musmeci *et al.*, 1992) and cause release of praseodymium ions from liposomes. Still, the act of organometalls ion enter into the lipid bilayer is dependent on the hydrophobic behavior of the compound.

The above mentioned sequence of effectiveness of the interaction between tinorganics and liposomal membranes, according to their lipophilicity, was observed also in our experiments with TriAL compounds and is commonly found for the interaction of different detergents with model lipid and biological membranes (Kleszczyńska *et al.*, 1990; Sarapuk *et al.*, 1992; Przestalski *et al.*,1996) as well as for the toxic action of organometallics on many biological objects (Thayer, 1974; Vighi and Calamari, 1985; Gray *et al.*, 1987; Eng *et al.*, 1991; Nagase *et al.*, 1991; Hamasaki *et al.*, 1995) and seem to point at the lipophilic properties of studied compounds as responsible for obtained effects. However, because of usually different levels of organometallics concentrations at which toxicity and membrane disturbances are observed no direct conclusions on toxicity can be made on the base of obtained results. Rather, the modifications of the lipid phase of biological membranes by organometallics induce further biological effects via the lipid-protein interactions. A good example illustrating the problem is TriEL which when present in plant membranes at nanomolar concentrations act as potent transmembrane anion antiporter (Cl^-/OH^-) (Hager *et al.*, 1987). No damage of the membrane is expected to occur at so small concentrations of this compound.

The quantitative differences between interactions of TriAT and DiAT, the latter showing the same sequence of effectiveness as trialkyltins, with liposomes may be the consequence of different structures they prefer when in aqueous media and also the preference of particular series to localize at different regions of the lipid bilayer (Ambrosini *et al.*, 1996).

Premodification of liposomes with detergents results in changes in polarities of the polar part of the lipid bilayer or in a change of surface charge of the bilayer. It must be noted that the electrical properties of the lipid bilayer surface are playing essential role in those experiments. Such conclusion is confirmed by experiments with inorganic lead and tin compounds. They have no lipophilic parts and any observed changes in the praseodymium release under their influence can be a consequence of electrical interactions. ^{1}H-NMR spectra revealed that tin compound behave as positive ions competing with bound praseodymium ions while lead acetate causes additional sorption of Pr^{3+} to liposomes. A similar sorption was also caused by TriML (see Fig. 3). Both lead compounds promote in a way a small additional (not exceeding 8%) sorption of praseodymium ions . Explanation of this effect is unclear and needs further investigation. Perhaps the reason is the strong hydration of these compounds and resultant decrease in charge density of lead atom, and, hence, inability to release Pr^{3+}. However weak, interaction of such hydrated compound with the polar part of the lipid molecule can change its polar head conformation, which lead to an additional sorption of praseodymium.

In view of those results, purely lipophilic or hydrophobic approach with simultaneous neglect of electronic effects, as was suggested by some studies (Laughlin *et al.*, 1985) seems to be an oversimplification of the problem of the organometallics interaction with biological objects, even if the electrical part of the interaction plays a role which is not so significant as lipophilicity or stereometry of organometallics.

The results of BLM experiments concerning TriAL compounds indicate at lipophilicity as the factor deciding about the interaction of organoleads with those membrane. The most actively interacting compound was TriBL and the shortest alkyl chain compound (TriML) was breaking lipid

membranes at critical concentration (CC) about five times lower than that found for TriBL compound.

Those results confirm that the interaction of organoleads with the lipid phase of biological membranes takes place in a way suggested elsewhere (Krug and Culig, 1991; Donaldson and Knowles, 1993). It seems that the mechanism of the organolead compounds interaction with lipid membranes is similar to that described earlier for detergents (Sarapuk *et al.*, 1992; Gabrielska *et al.*,1994). There it was postulated that the overall interaction of detergents with model lipid membranes is a combination of hydrophobic and polar interactions between lipid molecules and molecules of the substance incorporated into model membranes. Hydrophobic interactions were found to be predominant for detergents possessing long enough alkyl chains while electronic properties of their polar parts were playing essential role in those cases where hydrocarbon chains were short.

As it was already said, tinorganics were not, with exclusion of dibutyltin dichloride, breaking planar membranes at concentrations lower than their saturation concentrations. It could be connected with the fact that tinorganics poor lipophilicities limited their possibilities of entering into solvent matrix of lipid molecules. DiBT has longest lipophilic chains but its solubility in water (1.5 M) is also the best among tinorganics. Therefore, it seems that combined hydro- and liphophilic properties of this compounds enabled it to incorporate into the polar head region of BLM in amount causing the break of membrane. However, the CC value obtained for this compound evidences ($CC=7\times10^{-4}$ M) a weak interaction with BLM. By contrast, the CC values obtained for organoleads are significantly smaller and do not differ from those obtained for biologically active substances of greatest efficiency (Kleszczyńska *et al.*, 1990; Sarapuk *et al.*, 1992; Gabrielska *et al.*,1994). The general conclusion formulated on the basis of BLM experiments is such that leadorganics are more efficient in destroying model membranes than tinorganics and both are highly more effective than inorganic compounds.

To summarize, the lipophilicity, steric arrangement and polarity were important factors determining the behaviour of the compounds studied. The surface potential of the lipid membrane and the environment of lipid molecules were postulated to play a significant role in incorporation of tin and lead into lipid bilayers.

Acknowledgements

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