

Erythrocyte Hemolysis by Organic Tin and Lead Compounds

H. Kleszczyńska, J. Hładyszowski, H. Pruchnik and S. Przestalski

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

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The effect of trialkyllead and trialkyltin on pig erythrocyte hemolysis has been studied and compared. The results of experiments showed that the hemolytic activity of organoleads increases with their hydrophobicity and follows the sequence: triethyllead chloride < tri-n-propyllead chloride < tributyllead chloride. And similarly in the case of organotins: triethyltin chloride < tri-n-propyltin chloride < tributyltin chloride. Comparison of the hemolytic activity of organoleads and organotins indicates that the lead compounds exhibit higher hemolytic activity. The methods of quantum chemistry allowed to determine the maximum electric potential of the ions R_3Pb^+ and R_3Sn^+ , and suggest a relationship between the potential and toxicity.

Introductions

The practical importance of studies on the interaction between organic compounds of tin and lead and living organisms follows from the fact that the compounds accumulate in our environment and the biosphere and exert a marked effect on living cells and higher organisms (e.g. Krug, 1992; Kumar *et al.*, 1993; Falcioni *et al.*, 1996). Contaminations of living organisms with organic compounds of tin and lead depend on the local concentration of the compounds and the kind of living organism. For instance, in large city areas atmospheric concentration of organolead compounds can reach level $> 2\mu g/m^3$. In the humans trialkyllead is sometimes found at toxic levels in the brain, kidney and blood. Both trialkyllead and trialkyltin inhibit oxidative phosphorylation (Davies and Smith, 1982). The compounds come from various sources, because they have many users in industry, agriculture and in laboratory. In particular, organotins are used by the paint industry, mostly as antifouling paints for ships, boats or fishing nets because they contain tri-n-butyltin which is toxic to most cells. Organotin compounds have wide application as biocides, heat stabilizers of polyvinyl chloride, catalysts for production of urethanes or for esterification, as treatment for some infections

etc. (Crowe, 1987). Organolead compounds, mainly tetraethyllead compounds have been used in large quantities as antiknock petrol additives, triphenyllead salts have been introduced, among others, as pesticides having a similar biocidal action as tin compound and others (Zimmermann *et al.*, 1988). Organotin and organolead compound are toxic to humans, animals, plants and cells (Röderer, 1986; Radecki *et al.*, 1989; Eng *et al.*, 1991; Aldridge and Cremer, 1995). Studies on the cellular level indicate that the compounds are membrane toxicant and produce various membrane effects (Gray *et al.*, 1987a; Gray *et al.*, 1987b). Particularly, in the case of erythrocytes the organotin compounds produce hemolysis (Porvaznik *et al.*, 1986; Gray *et al.*, 1987a; Musmeci *et al.*, 1992; Hamasaki *et al.*, 1992).

Hemolytic effects can be treated as resulting from toxic effects of external factors – organometallic compounds in this case. As a measure of toxicity is often assumed EC_{50} , i.e. concentration of toxic substance that causes 50% hemolysis and apply a structure-activity relationship (QSAR) method in order to predict EC_{50} values of tested organotin compounds, using various descriptors which represent their physicochemical properties or molecular structure (Porvaznik, 1986; Eng *et al.*, 1991; Combes, 1995; Hamasaki *et al.*, 1995).

The studies mentioned above refer to the effect of organotins on the extent of hemolysis. It is of interest to see what is the analogous effect of organoleads on the process. In general, the opinion on

Reprint requests to Dr. Kleszczyńska.
Fax: 48-71-2051 72.

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the properties of organotins and organoleads vary. Harrison (1982) suggest that toxicity of organotins is similar to that of the corresponding alkyllead compounds, whereas Thayer (1974) says that in general the alkyllead compounds are more effective than alkyltin compounds.

In our investigations we have utilized red blood cells for two reasons. In the first place, they become damaged as a result of contamination with organoleads and organotins. In the second place, blood cells are very often used as a good membrane model to study membrane toxicity.

However, the overriding purpose of the present work has been to investigate the relation between toxicity (its measure being hemolysis) of alkyltin and alkyllead compounds and their electrical properties.

Materials and Methods

The following organotin and organolead compounds were used in the investigation: TET – triethyltin chloride ($(C_2H_5)_3SnCl$), TPT – tri-*n*-propyltin chloride ($(C_3H_7)_3SnCl$), TBT – tributyltin chloride ($(C_4H_9)_3SnCl$) and TEL – triethyllead chloride ($(C_2H_5)_3PbCl$), TPL – tripropyllead chloride ($(C_3H_7)_3PbCl$), TBL – tributyllead chloride ($(C_4H_9)_3PbCl$) (Alfa, Johnson Matthey, Karlsruhe; Aldrich – Chemie, Steinheim and Merck – Schuchardt, Hohenbrunn).

The investigation was conducted with fresh heparinized pig blood. For washing erythrocytes and experimentation was used an isotonic phosphate buffer of pH 7.4 (1.31 mM NaCl, 1.79 mM KCl, 0.86 mM $MgCl_2$, 11.79 mM $Na_2HPO_4 \cdot 2H_2O$, 1.80 mM $NaH_2PO_4 \cdot H_2O$). Erythrocytes collected from plasma were washed four times in the phosphate buffer, and then incubated in the same solution but containing proper amounts of the studied organometallic compounds of tin and lead. Modification was conducted at 37 °C for 4 hr, the samples of 10 ml volume contained erythrocytes at 2% concentration, the suspension was stirred continuously. During modification the percent of hemolyzed cells was measured, taking 1 ml samples at 0.5, 1, 1.5, 2, 3 and 4 hr of incubation. The samples taken were centrifuged and the hemoglobin content was measured in the supernatant using a spectrophotometer (Specol 11, Carl Zeiss, Jena) at 540 nm wavelength (Hamasaki *et al.*, 1995; Boyer

et al., 1993). Hemoglobin concentration in the supernatant expressed in percentage was taken as percent of hemolyzed cells, calculated relative to a sample containing totally hemolyzed erythrocytes. All the compounds studied were dissolved in ethanol in such amounts that after addition to erythrocyte suspension the concentration of ethanol in the samples did not exceed 1%.

In water solution the compounds studied can form various structures. However, according to Thayer (1974), both tetraalkyllead and tetraalkyltin compounds owe their toxicity to the cleavage *in vivo* of one metal – carbon bond to form R_3M^+ (where R – alkyl radical, M – metal). The occurrence of these ions is taken into account in many publications (Zimmermann *et al.*, 1988; Nygren, 1994; Hamasaki *et al.*, 1995 and Attar, 1996). In order to get an idea about the probable electrical properties of the ions, we have used the method of molecular modeling and methods of quantum chemistry. All the structures studied were created from scratch with molecular modeling software package Sybyl v 6.2 (Tripos, Inc.) on a Silicon Graphics workstation. At first, Gasteiger-Hückel charges were assigned to all atoms. The initial raw form of each structure was optimized with molecular mechanics methods (using Tripos Force Field) to remove “mechanical stress”. The structure were then taken to Sybyl/Mopac (Stewart, 1990) module for further elaboration. The quantum chemistry PM3 method (Stewart, 1989) was applied to all structures to optimize their geometries and to determine their net atomic charges at the highest precision. The final forms of the structures were further analyzed with Sybyl/MolCad module to study electric potentials of the compounds mapped onto Connolly surfaces (surfaces which are unpenetrable by solvent molecules).

Results

Figures 1–3 show kinetic profiles of the process of hemolysis induced by the studied compounds of tin and lead. Concentrations of the compounds were in the range between 50 and 3000 μM , and 4 h was the time span that hemolysis was studied. In Figs 1A and 1B are shown the relationships between percent of hemolysis and action time for various concentrations of the compounds TET and TEL. The results obtained indicate that TEL com-

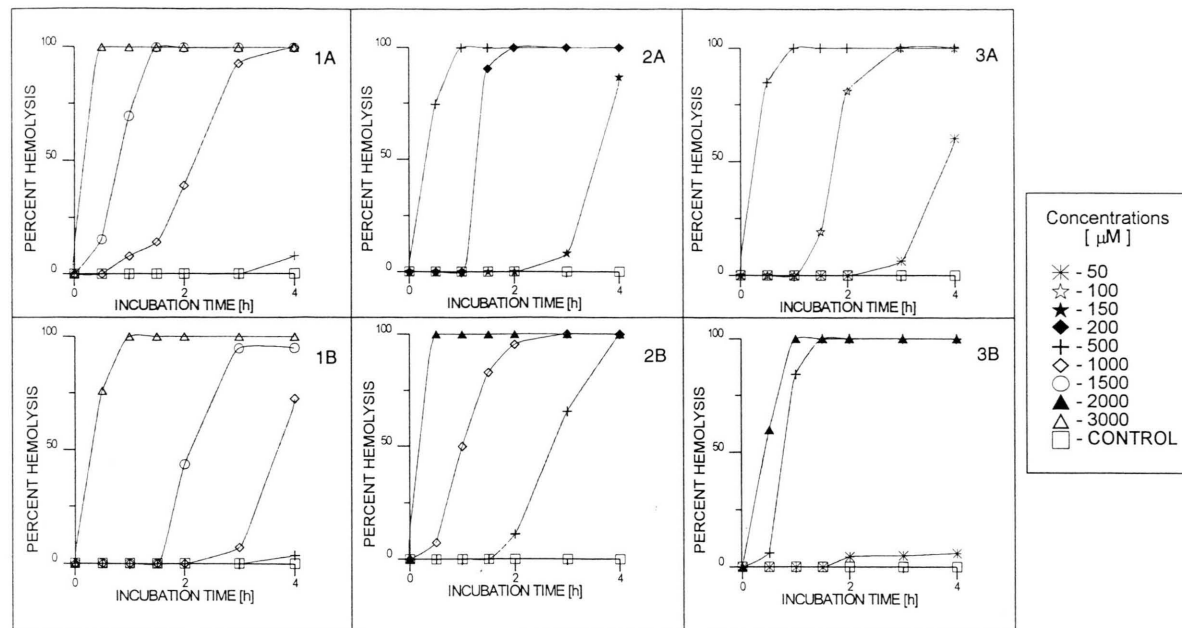


Fig. 1. Percent hemolysis as a functions of time for various concentrations of $(C_2H_5)_3PbCl$ (1A) and $(C_2H_5)_3SnCl$ (1B).

Fig. 2. Percent hemolysis as a functions of time for various concentrations of $(C_3H_7)_3PbCl$ (2A) and $(C_3H_7)_3SnCl$ (2B).

Fig. 3. Percent hemolysis as a functions of time for various concentrations of $(C_4H_9)_3PbCl$ (3A) and $(C_4H_9)_3SnCl$ (3B).

pound is more active than TET, because for equal concentrations of the two the percent of hemolysis for any time chosen is greater for TEL than TET. Yet bigger differences appear with the TPL and TPT compounds (Figs 2A and 2B), indicating also at bigger hemolytic activity of compounds TPL with respect to TPT. Similar relations can be observed for TBL and TBT (Figs 3A and 3B). Thus in every case the respective trialkyllead compounds are more active than trialkyltin compounds. On the basis of the results obtained one can also find that in a homologic series of one kind the hemolytic activity increases with length of the hydrophobic part as follows: $TEL < TPL < TBL$ and $TET < TPT < TBT$. For all the calculations the standard deviation is 8%.

Table I shows the results of calculations of maximal electric potential (on Connolly surface) by using the PM3 quantum chemical method.

As seen from comparing the analogous organolead and organotin compounds, the maximal electric potential for R_3Pb^+ is greater than that for R_3Sn^+ , whereas in the homologous series of lead

Table I. Comparison of electrical data obtained by molecular modelling of the ions R_3M^+ studied. EP denotes electrostatic potential maximal value (expressed in $e/\text{\AA}$, where e is a atomic charge unit, \AA – angstrom) on the Connolly surface.

Ions	EP [$e/\text{\AA}$]	Ions	EP [$e/\text{\AA}$]
$(C_2H_5)_3Pb^+$	149.86	$(C_2H_5)_3Sn^+$	145.50
$(C_3H_7)_3Pb^+$	143.68	$(C_3H_7)_3Sn^+$	137.36
$(C_4H_9)_3Pb^+$	142.00	$(C_4H_9)_3Sn^+$	135.53

compounds the potential decreases with increasing length of the alkyl chain. The same situation occurs in the homologous series of tin compounds.

Discussion

Our considerations were limited to the interaction between the R_3M^+ ions and red cell membrane. In aqueous solution of erythrocytes and the ions (other ions and molecules are not considered here) will occur their encounters due to thermal motion of the ions. If ions of different compounds differ in their electric properties (other properties

and conditions being similar), their first encounter with the membrane and thus the rate of binding to the membrane may depend on electrostatic interactions. In case of erythrocyte membrane which is known to have negative charge on its outside surface, the attractive electrostatic interaction is stronger the higher positive potential of the ion. Comparison of the results of our experiments show that the interaction of organolead ions with erythrocytes is a bit more active than the interaction of corresponding organotin ions. This can be that the frequency of encounters between R_3Pb^+ cations and erythrocyte membrane is greater than that between R_3Sn^+ cations and membrane.

Once the ions R_3Pb^+ and R_3Sn^+ have come into contact with the membrane, the incorporation of the alkyl chains into membrane interior begins. That process depends on hydrophobic interactions. The more hydrophobic ions incorporate more easily into the hydrophobic layer of the membrane, mostly due to their alkyl chains. The ions with longer alkyl chains and lower electrical potential (in a given homologous series) exhibit greater hydrophobicity (about the connection between some electric properties of amphiphilic compounds and their hydrophobicity see Przestalski *et al.*, (1996). For both the homologous series we studied: $(C_2H_5)Pb^+$, $(C_3H_7)Pb^+$ and $(C_4H_9)Pb^+$

and $(C_2H_5)Sn^+$, $(C_3H_7)Sn^+$ and $(C_4H_9)Sn^+$, one can notice that alkyl chain length increases gradually and the maximum electric potential decreases (Tab. I).

The experimental results obtained can be tentatively explained by two processes occurring in the system. One of them depends on the encounters between ions and erythrocyte membranes, which are affected both by the thermal motion of the ions and their positive electrical potential. The other process consists in incorporating the hydrophobic parts of the ions into erythrocyte membrane interior and disorganizing the membrane structure with resulting hemolysis.

The concluding statement can be made that, the electrical and hydrophobic interactions can not be disregarded, independently of many other factors that can influence the kinetics of hemolysis induced by organolead and organotin compounds which were not considered here.

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