APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **14**, 40–47 (2000)

Stability of Model Membranes in the Presence of Organotin Compounds

Janusz Sarapuk,* Halina Kleszczyńska and Stanisław Przestalski
Department of Physics and Biophysics, Agricultural University, Norwida 25, 50-375 Wrocław, Poland

The influence of tri- and di-alkyltins (TATs and DATs) as well as di- and triphenyltin compounds (DPhTs and TPhTs) on haemolysis of red blood cells (RBCs) and stability of planar lipid membranes (PLMs) has been studied. The results obtained show that the efficiency of TATs (trimethyl-, triethyl-, tri-n-propyl- and tributyltin chlorides) in destroying PLMs did not differ greatly when the compounds were studied in solutions of physiological pH (phosphate buffer, pH 7.4). A decrease in pH to 5.0 caused small changes in the efficiency of the three largest TAT molecules and a significant decrease in the efficiency of trimethyltin chloride. Both haemolytic and PLM experiments showed that the most active TAT was tri-n-propyltin chloride. The destructive action of DAT (dimethyl- and dibutyltin) and DPhT dichlorides was somewhat more differentiated. Dimethyltin dichloride (DMT) interaction with model membranes was a little weaker than that of DPhT and dibutyltin dichlorides and all these compounds influenced the model membranes to a lesser extent than TATs or TPhT. To bring about comparable haemolysis effects the dichlorides had to be used at much greater concentrations than the chlorides. The haemolytic properties of the dichlorides, especially of that of DMT, significantly increased in solution at pH 5.0. TPhT chloride interacted with model membranes similarly to TAT chlorides. Also, no great difference in efficiency of this compound was found for the two buffer solutions used.

Keywords: organotin compounds; erythrocytes; haemolysis; planar lipid membranes; interaction mechanism

* Correspondence to: J. Sarapuk, Department of Physics and Biophysics, Agricultural University, Norwida 25, 50-375 Wrocław, Poland.
E-mail: js@ozi.ar.wroc.pl
Contract/grant sponsor: Polish Research Committee (KBN); Contract/grant number: 4 S401 024 07.

Received 2 April 1999; accepted 20 August 1999

INTRODUCTION

Industrial and agricultural development may bring about growing environmental pollution, with many substances introduced into the environment purposefully or as waste products. Organometallic compounds are in such categories and they are usually more toxic than inorganic compounds of the same metals.[1–3] The organotin compounds studied here are in this class and are responsible for a number of pathological events occurring in living organisms.[4–6] Although toxic organotin compounds are used extensively as biocides[7–9] and *ca* 10–30% of organotin production is introduced directly to the environment,[5,10] they constitute a lesser threat to the environment than other organometals because they are not as toxic as organic derivatives of some other metals and because they degrade relatively easily to less toxic or inorganic forms that are regarded as nontoxic.[11,12]

It is postulated that the toxicological action of biologically active compounds, including organotins, operates at two different levels. One is the molecular level, where toxic effects occur at relatively low concentrations of the compounds (10^{-6}–10^{-7} mol dm^{-3})[11,13] and concern perturbation or disruption of metabolic processes in living organisms. The second level is the cellular one, where the concentration of biocide is usually, but not necessarily, much higher (about two orders of magnitude) and the effects observed concern changes in physicochemical and/or mechanical properties of biological walls and membranes.[11,14–17] In each case, the primary contact of organotins with living organisms takes place at the cell wall and/or cell membrane. The capability of cell membranes to undergo structural perturbation by organometallic compounds depends strongly on, among other things, the nature of the compound and the number of attached organic groups that determine compound

 CCC 0268–2605/2000/010040–08 $17.50

lipophilicity, the counterion attached to the metal atom, the speciation of organotins under environmental conditions and the structures they may form in the environment.[5,10,11,15–17,18–25] Physicochemical and topological parameters describing organometals can be used to predict their toxicities by the quantitative structure–activity relationship method (QSAR).[16,26–28] However, the validity of the results obtained by this method (the attempt being justified in view of the growing number of compounds being produced for application) depends on a proper choice of available parameters describing these compounds.

This work includes the results of studies on the interaction of several organotin compounds (differentiated in their polar and/or hydrophobic nature) with erythrocytes (RBCs) and planar lipid membranes (PLMs); its main aim was to determine the role played in this interaction by the particular parts of compounds. The interactions were measured in terms of the concentrations of the tin organic compounds that cause destruction of PLMs or 100% haemolysis of erythrocytes.

Both types of measurement proved useful in studies on the interactions of different biologically active substances with biological and lipid model membranes.[29,30]

MATERIALS AND METHODS

Materials

All the organotin compounds studied [chlorides of trimethyltin (TMT), triethyltin (TET), tri-n-propyltin (TPT), tributyltin (TBT); dichlorides of dimethyltin (DMT), dibutyltin (DBT); and chlorides of triphenyltin (TPhT) and diphenyltin (DPhT)] were purchased from Alfa Products (Germany). Planar lipid membranes were formed from azolectin dissolved in a mixture of n-decane and n-butanol, all purchased from Sigma (USA). All the chemicals used were of analytical grade.

PLM experiments

Planar lipid membranes were formed from a 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. The compound studied were dissolved in ethanol–water solutions to give 0.01 M solutions. These were pipetted by means of calibrated micropipettes directly into the bath solution (12 ml) until their concentration reached a critical value (CC) at which the membrane lifetime was not longer than 3 min. Phosphate buffer solutions of pH 5.0 and 7.4 were used as the bath solutions. The time necessary for lipid membranes to achieve a bimolecular arrangement was about 15 min at room temperature (*ca* 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. Each experiment was repeated at least three times.

RBC experiments

Measurements were performed with fresh heparinized pig blood. Two phosphate buffers of pH 7.4 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 the tin organic. The percentage of haemolysis was measured for 1-ml samples, taken at 0.5, 1, 1.5, 2, 3 and 4 h of incubation at 37°C. 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 the percentage of haemolysed cells, calculated relative to a sample containing totally haemolysed erythrocytes. All tin organics were dissolved in ethanol, the concentration of which in the samples did not exceed 1% (v/v).

RESULTS AND DISCUSSION

The results of experiments with planar lipid membranes (PLMs) are summarized in Table 1 which reports the critical concentrations (CCs) of the tin organics, i.e. the concentrations causing destruction the of PLMs in a time not longer than 3 min. The time courses of haemolysis of erythrocytes under the influence of chosen concentrations of organotins at various pH values are shown in Figs 1–6. The general conclusion from the results obtained by both types of experiments is that compounds having three organic groups attached to the metal atom influenced the model membranes more effectively than did dichlorides. This conclu-

Table 1 Critical concentrations (CCs) of tin organics for various phosphate buffer solutions in PLM experiments[a]

	CC (m M)							
pH	TMT	TET	TPT	TBT	DMT	DBT	TPhT	DPhT
7.4	0.40	0.32	0.32	0.48	1.10	0.24	0.41	0.83
5.0	1.10	0.64	0.40	0.40	3.20	0.16	0.48	1.30

[a] Standard deviation did not exceed 10%.

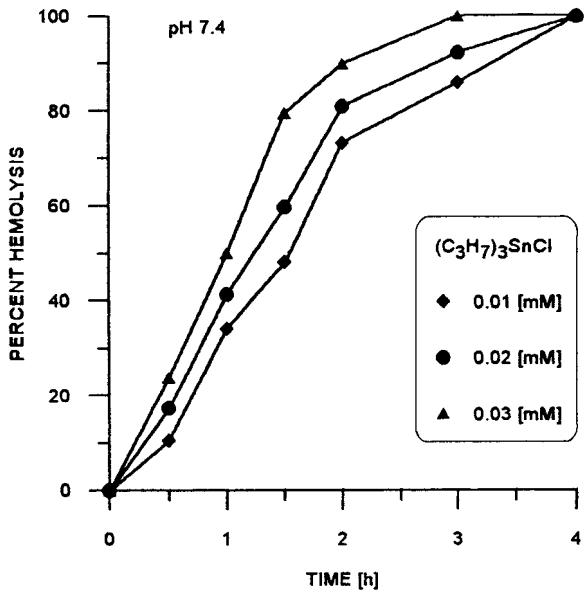
sion agrees with pronons results on the toxicity of organotins. [5,15–17,22,24,28] The most haemolytic compound of those containing three organic groups attached to tin was tri-n-propyltin chloride (Fig. 1). This compound was found to be the most destructive in PLM experiments, although the CCs of triorganotins did not differ very much (Table 1).

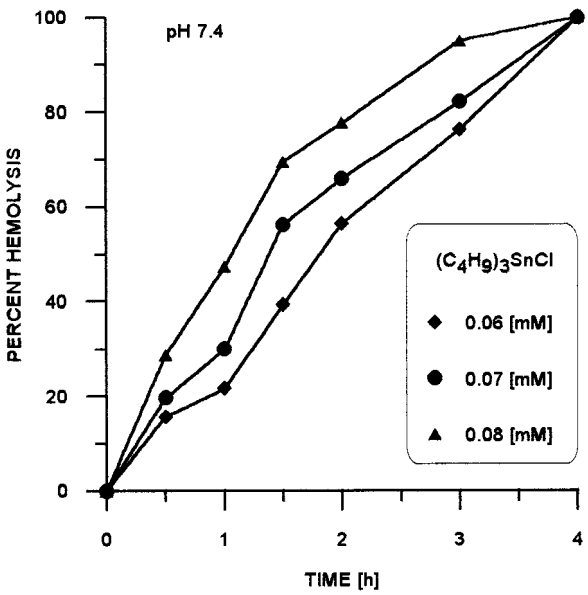
A decrease in the pH of the buffer solution from the physiological value to 5.0 accelerated the haemolysis process slightly in the case of triorganotins, but had no great influence on the compound concentrations causing 100% haemolysis (Figs 1, 2, 5). Also, the CC values of these compounds in general differed (Table 1). The only exception was TMT chloride, for which the critical concentration was found to increase from 0.4 mM at pH 7.4 to 1.1 mM at pH 5.0 (Table 1). It must be noted that some errors could occur in the determination of the CC values for TPT and TBT chlorides due to the poorer solubility of these two compounds under experimental conditions. No similar problem was met when studying the other compounds.

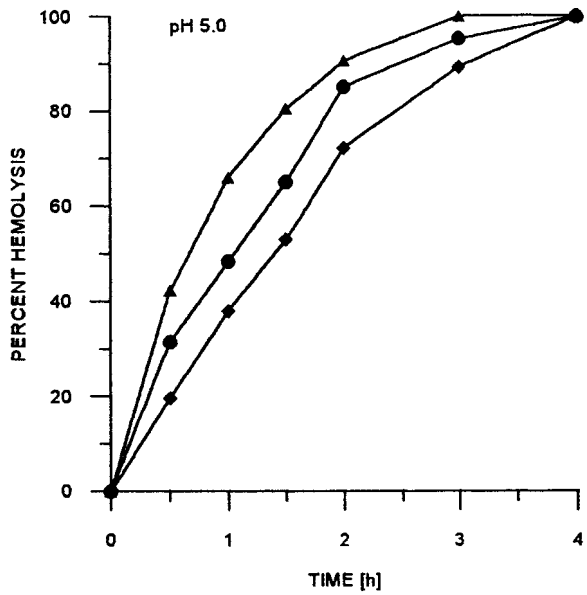
All the diorganotin chlorides studied were markedly weaker in influencing model membranes than the triorganotins with the physiological pH phosphate buffer. However, their haemolytic toxicity (Fig 3, Fig 4, Fig 6) increased significantly in pH 5.0 phosphate buffer (about two- to three-fold for DBT and DPhT, and about seven-fold for DMT). No such improvement in the destructive efficiency of these compounds was found in PLM experiments (Table 1).

The difference in the interaction with model membranes between di- and tri-organotins must depend on various properties of the compounds. The most important seem to be the molecular stereochemistry, the net charge or charge density (with the resulting polarity or hydrophilicity), the partition coefficient; and the preferred structure a molecule adopts in its environment. As already mentioned, some environmental features, as well as those concerning the membranes with which the tin organics interact, must be taken into account.[31,32] Some of these factors have been discussed; for example it is generally accepted that triorganotins form dihydrated trigonal bipyramids in which the organic groups are distributed equatorially around the tin atoms, while diorganotins take a tetrahydrated octahedral geometry.[20,21,26,32] It is clear that lipophilicity is responsible for the stronger interactions observed, in this work and elsewhere, of triorganotins with membranes in comparison with diorganotins and also the stronger interactions of compounds having larger and more lipophilic groups within the same homologous series.[22,33]

However, this conclusion cannot be generalized (as shown in some fluorescence measurements). It was found that the partition coefficient between egg yolk lecithin liposomes and the bath solution for tributyltin chloride seemed to be independent of its lipophilicity.[34] In a sense such conclusions agree with our earlier findings[35] and the results obtained in this work indicated that this compound was somewhat weaker in its interaction with membranes than TPT chloride, especially in pH 7.4 solution. Also, TPhT chloride, a highly hydrophobic compound, was found to be slightly less effective than TPT in haemolysing erythrocytes. These observations underline the fact that lipophilic or hydrophobic properties of organotins can only partially explain the mechanism of their interaction with the model membranes used. The electronic effects at the polar region of the membranes, must also be considered even if the electrical part of this interaction plays a less significant role compared to the lipophilicity or stereochemistry of organotins. The validity of such an approach has been shown in the case of the interaction of various biologically active compounds with the same model membranes as those used in the present work.[29,36] It was postulated that the overall interaction of biocides with model membranes is a combination of hydrophobic and polar interactions between lipid molecules and molecules of the substance incorporated into the model membranes. Hydrophobic interactions were found to be predominant for compounds possessing sufficiently long alkyl chains, while the electronic properties of their polar regions played an essential role in those cases where hydrocarbon chains were short. Qualitatively, tin organic compounds have to interact with model membranes in the same way. The lipid phase of biological membranes has been proposed as one of the possible sites where tin organics can interact with membranes. [5,14,15,20,25,31–35,37–39] Thus, elec-







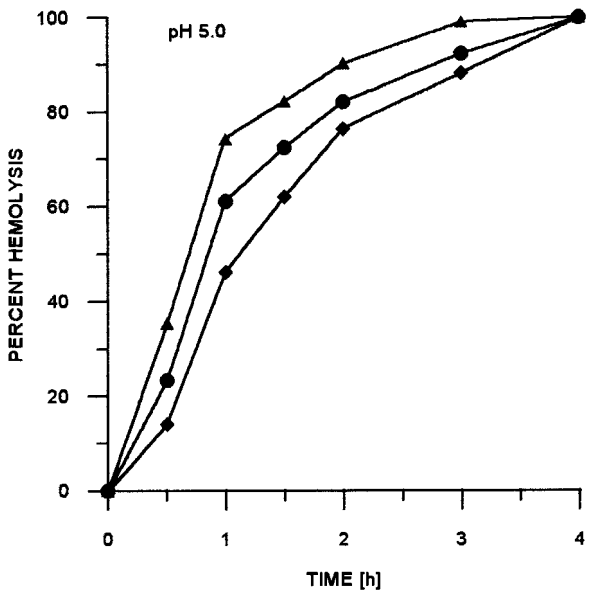


Figure 1 Haemolysis of TPT chloride at pH 7.4 and 5.0. The standard deviations did not exceed 6%.

Figure 2 Haemolysis of TBT chloride at pH 7.4 and 5.0. The standard deviations did not exceed 6%.

trical interactions of tin organics with the lipid phase should take place at the polar part of the lipid bilayer, with the phosphocholine groups of lipid molecules. This interaction should, in turn, depend on the structures that tin organics form in aqueous environments and the degree of their hydration. It is assumed that triorganotin chlorides are present at physiological pH in the aqueous phase as the hydrolysis product R_3SnOH (R = organic group). (It is more correct to say that the hydroxo complex dominates at neutral pH over the ionic form R_3Sn^+.[40])

A decrease in pH below 5–6 (depending on the tin compound) is followed by the appearance of the cationic diaquo form $[R_3Sn(H_2O)_2]^+$.[5,41] The exception is TMT chloride, for which the cationic

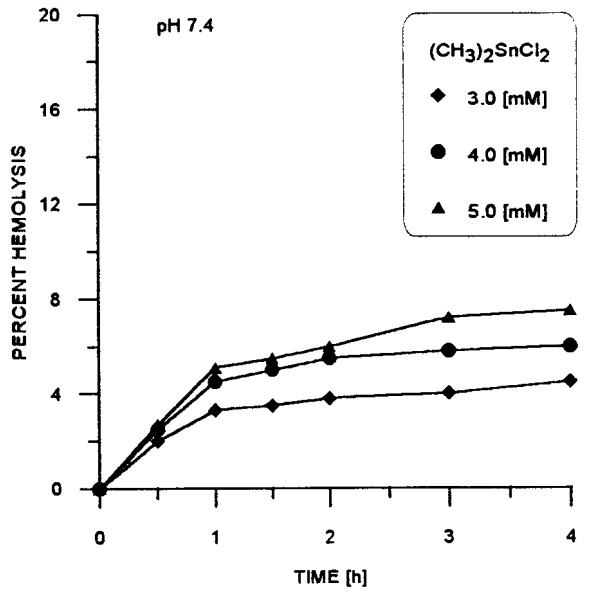


Figure 3 Haemolysis of DMT dichloride at pH 7.4 and 5.0. The standard deviations did not exceed 6%.

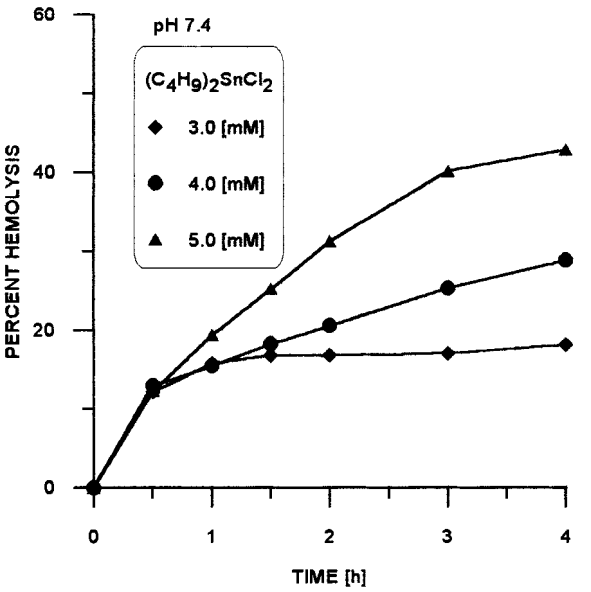
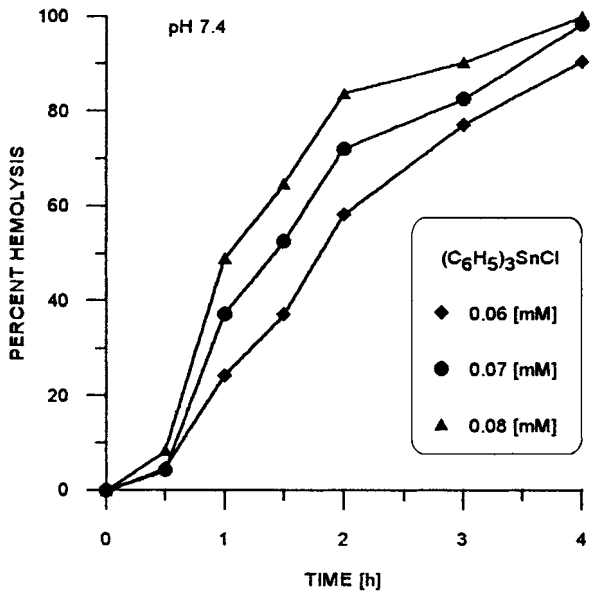
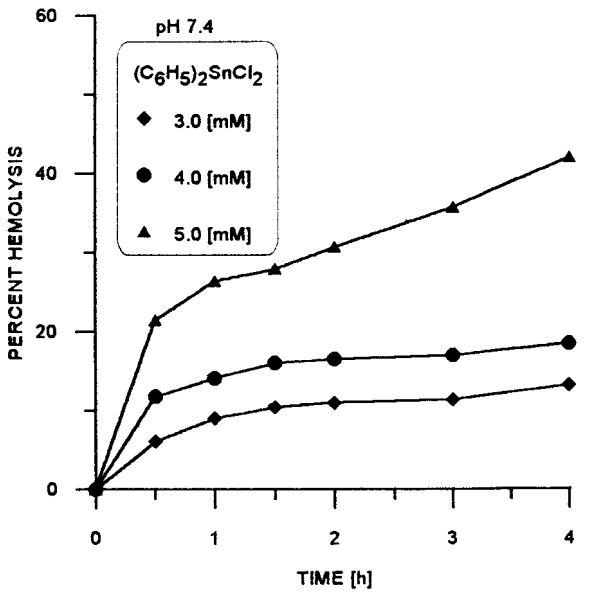
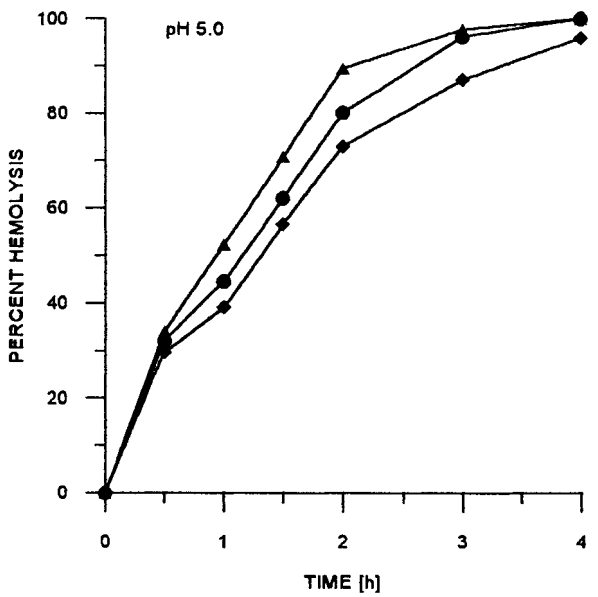


Figure 4 Haemolysis of DBT dichloride at pH 7.4 and 5.0. The standard deviations did not exceed 6%.

form begins to dominate at pH values higher than 5.0. Generally, in the pH range studied we would expect to deal with neutral forms of triorganotins which can interact with the phosphate group of lecithin by weak Coulombic interactions.[20,42] It has been shown that it is also possible that some triorganotins can localize in the hydrophobic part of lipid membranes.[31] The more intensive interaction of triorganotins observed with model membranes, in comparison with diorganotins, is linked with their localization near or at the hydrophobic part of the lipid bilayer, and that localization depends on the lipid species. As follows from PLM experiments, the destructive efficiency of TMT chloride decreased very sharply in bulk solution at pH 5.0 due to the appearance of the cationic diaquo form.







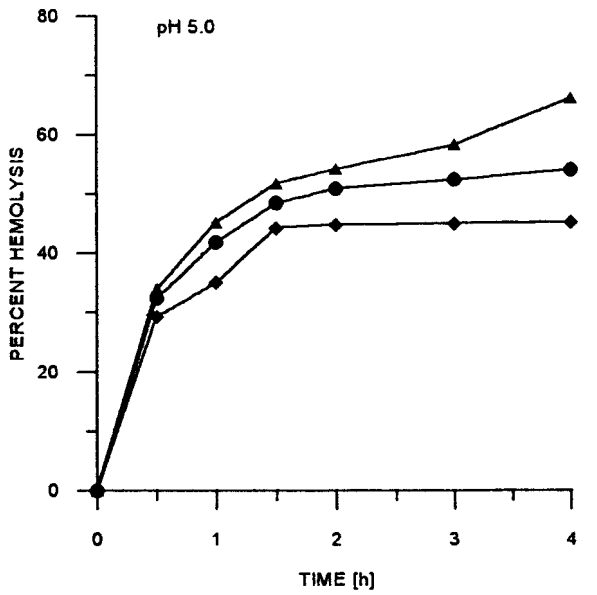


Figure 5 Haemolysis of TPhT chloride at pH 7.4 and 5.0. The standard deviations did not exceed 6%.

Figure 6 Haemolysis of DPhT chloride at pH 7.4 and 5.0. The standard deviations did not exceed 6%.

The resulting increase in polarity of this compound could be the reason for its weaker interaction with model lipid membranes.

The behaviour of diorganotin dichlorides in aqueous environment is more complicated. Whereas at neutral pH the hydroxo form dominates, in the vicinity of pH 5.0 various dicationic forms[40] of greater polarity occur that can strongly interact with model membranes in their polar region. Once again, the weakest interaction with membranes was observed for the methyl compound of the diorganotin dichloride series.

The general conclusions coming from these results are as follows.

(1) The differences between the interactions of

particular serious of organotins with model membranes can result from the preference of the respective organotins to localize at different regions of the lipid bilayer. It seems that triorganotins can position themselves at or in the hydrocarbon interior of the lipid membrane whereas diorganotins prefer to localize at the polar head region of the lipids. The differences in localization can be caused by significant differences in the lipophilicity of the compounds studied. This conclusion does not agrees with that drawn from fluorimetric data[39] and confirms that of Ambrosini *et al.*[32]

(2) In the most cases, changes in the solution pH did not significantly change the interaction of organotins with membranes implying that the same forms of compound exist at the pH range studied (5.0–7.4). The only exception was TMT chloride as shown in PLM experiments.

(3) A significant increase in the interaction of diorganotin dichlorides with erythrocytes was found in the case of the more acidic environment (pH 5.0). This could be due to the mentioned changes in diorganotin species existing at this pH and to the change in mechanical properties of the erythrocyte membranes caused by a changed membrane organization in comparison with that at physiological pH.[43]

No direct conclusions on the toxicity of compounds studied can be made on the basis of the results obtained. The discussion does not concern the possible interaction of tin organics with membrane proteins. It concerns modifications of the lipid phase of biological membranes which can induce further biological effects via lipid–protein interactions.

Acknowledgements This work was sponsored by the Polish Research Committee (KBN) grant no. 4 S401 024 07).

REFERENCES

1. P. J. Craig, Environmental aspects of organometallic chemistry. In: *Comprehensive Organometallic Chemistry. The Synthesis, Reactions and Structures of Organometallic Compounds*, Vol. 2, Wilkinson, G., Stone, F. G. A. and Abel, E. W. (eds), Pergamon, Oxford, 1982, pp. 979–1020.
2. O. Andersen and P. Grandjean, *Appl. Organomet. Chem.* **1**, 15 (1987).
3. H. G. Heumann, *Protoplasma* **136**, 37 (1987).
4. W. N. Aldridge and J. E. Cremer, *Biochem. J.* **61**, 406 (1995).
5. K. Fent, *Crit. Rev. Toxicol.* **26**, 1 (1996).
6. M. A. Nudelman, C. Carro and N. S. Nudelman, *Appl. Organomet. Chem.* **12**, 67 (1998).
7. A. J. Crowe, *Appl. Organomet. Chem.* **1**, 143 (1987).
8. A. J. Crowe, *Appl. Organomet. Chem.* **1**, 331 (1987).
9. A. Kumari, J. P. Tandon and R. V. Singh, *Appl. Organomet. Chem.* **7**, 655 (1993).
10. K. M. Attar, *Appl. Organomet. Chem.* **10**, 307 (1996).
11. P. J. Smith and L. Smith, *Chem. Br.* **11**, 208 (1975).
12. J. S. Thayer, *J. Organomet. Chem.* **76**, 265 (1974).
13. H. F. Krug, *Appl. Organomet. Chem.* **6**, 297 (1992).
14. M. Porvaznik, B. H. Gray, D. Mattie, A. G. Jackson and R. E. Omlor, *Lab. Invest.* **54**, 254 (1986).
15. B. H. Gray, M. Porvaznik, C. Flemming and H. L. Lanfong, *Cell Biol. Toxicol.* **3**, 23 (1987).
16. T. Hamasaki, H. Masumoto, T. Sato, H. Nagase, H. Kito and Y. Yoshioka, *Appl. Organomet. Chem.* **9**, 95 (1995).
17. T. Sato, H. Masumoto, H. Nagase, H. Kito and M. Niikawa, *Appl. Organomet. Chem.* **11**, 231 (1997).
18. J. O. Wieth and M. T. Tosteson, *J. Gen. Physiol.* **73**, 765 (1979).
19. T. Hamasaki, H. Nagase, T. Sato, H. Kito and Y. Ose, *Appl. Organomet. Chem.* **5**, 83 (1991).
20. M. T. Musmeci, G. Madonia, M. T. Lo Giudice, A. Silvestri, G. Ruisi and R. Barbieri, *Appl. Organomet. Chem.* **6**, 127 (1992).
21. J. M. Tsangaris and D. R. Williams, *Appl. Organomet. Chem.* **6**, 3 (1992).
22. G. Huang, S. Dai and H. Sun, *Appl. Organomet. Chem.* **10**, 377 (1996).
23. L. E. Khoo, N. K. Goh, L. L. Koh, Y. Xu, D. J. Whalen and G. Eng, *Appl. Organomet. Chem.* **10**, 459 (1996).
24. A. Fargasova, *Ecotoxicol. Environ. Safety* **37**, 193 (1997).
25. H. Kleszczynska, J. Hładyszowski, H. Pruchnik and Z. Przestalski, *Naturforsch. Teilc* **52**, 65 (1997).
26. R. B. Laughlin Jr, R. B. Johannesen, W. French, H. Guard and F. E. Brinckman, *Environ. Toxicol. Chem.* **4**, 343 (1985).
27. G. Eng, E. J. Tierney, G. J. Olson, F. E. Brinckman and J. M. Bellama, *Appl. Organomet. Chem.* **5**, 33 (1992).
28. H. Nagase, T. Hamasaki, T. Sato, H. Kito, Y. Yoshioka and Y. Ose, *Appl. Organomet. Chem.* **5**, 91 (1991).
29. H. Kleszczyńska, J. Sarapuk, S. Przestalski and M. Kilian, *Stud. Biophys.* **135**, 191 (1990).
30. J. Sarapuk, H. Kleszczyńska and B. Rózycka-Roszak, *Biochem. Mol. Biol. Int.* **44**, 1105 (1998).
31. A. Ambrosini, E. Bertoli, F. Tanfani and G. Zolese, *Phys. Lipids* **99**, 189 (1991).
32. A. Ambrosini, E. Bertoli and G. Zolese, *Appl. Organomet. Chem.* **10**, 53 (1996).
33. J. Kuczera, J. Gabrielska, T. E. Kral and S. Przestalski, *Appl. Organomet. Chem.* **11**, 591 (1997).
34. M. Langner and H. Kleszczyńska, *Cell. Mol. Biol. Lett.* **2**, 15 (1997).
35. H. Kleszczyńska, H. Pruchnik and S. Przestalski, *Mol. Biol. Int.* **44**, 305 (1998).

36. J. Sarapuk, J. Gabrielska and S. Przestalski, *Polish J. Environ. Stud.* **1**, 27 (1992).
37. W. R. Cullen, F. G. Herring and B. U. Nwata, *Appl. Organomet. Chem.* **11**, 369 (1997).
38. R. St-Louis, E. Pelletier and P. Marsot, *Appl. Organomet. Chem.* **11**, 543 (1997).
39. M. Langner, J. Gabrielska, H. Kleszczyńska and H. Pruchnik, *Appl. Organomet. Chem.* **12**, 99 (1998).
40. R. S. Tobias, The chemistry of organometallic cations in aqueous media. In: *Organometals and Organometalloids: Occurrence and Fate in the Environment*, Brinckman F. E. and Bellama J. M. (eds), American Chemical Society, *ACS Symp.* Ser. No. 82, Washington DC, 1978, pp. 130–148.
41. A. G. Davies and P. J. Smith, Tin. In: *Comprehensive Organometallic Chemistry*, vol. 2, Wilkinson G, Stone F. G. A. and Abel, E. W. (eds), Pergamon, Oxford, 1982, pp. 519–626.
42. B. R. Heywood, K. C. Molloy and P. C. Waterfield, *Appl. Organomet. Chem.* **3,** 443 (1989).
43. P. J. Raval, D. P. Carter and G. Fairbanks, *Biochim. Biophys. Acta* **983,** 230 (1989).

