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The effect of some phenyltin compounds on the thermotropic phase behaviour and the structure of model membranes

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The effects of diphenyltin dichloride (DPhT), triphenyltin chloride (TPhT) and tetraphenyltin (TTPhT) on the thermotropic phase behaviour of phosphatidylcholine bilayers were studied. All the phenyltin compounds investigated affected phase transitions differently. TTPhT broadened the main phase transition but it left the transition temperatures and enthalpy unchanged. TPhT reduced the transition temperatures and the enthalpies while DPhT showed a dual effect on the pretransition and the main transition. At low concentrations DPhT reduced the temperatures of the transitions slightly and at higher concentrations it increased them.

Based on differential scanning calorimetry (DSC) and also ^{1}H NMR and ^{31}P NMR measurements, it is suggested that DPhT induces interdigitated gel phase formation and TPhT induces hexagonal phase formation. TTPhT seems to affect the structure only a little. The toxic activity of DPhT and TPhT seems to be connected with their ability to induce changes in the membrane structure. **Copyright © 2000 John Wiley & Sons, Ltd.**

Keywords: diphenyltin dichloride; triphenyltin chloride; tetraphenyltin; model membranes; phase transitions; inverted hexagonal phase; interdigitated gel structure; DSC; ^{1}H NMR; ^{31}P NMR

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INTRODUCTION

Although organometallic compounds exhibit toxic activity, they are widely used in many fields of industry, for example in the production of plastics, wood protection agents, textiles and leather, and in agriculture as pesticides (fungicides, bactericides and herbicides).[1–3] The toxicity depends on both the chemical structure and the number of organic groups attached to the tin atom.[4] In particular, triorganotin compounds are more toxic than diorganotin ones.[5] This may be linked to the differences in their interactions with membranes. It has been reported that di- and tri-phenyltin compounds are differently localized in the lipid bilayer: triphenyltin is probably localized in the hydrocarbon core of the bilayer while diphenyltin is on the surface of the bilayer,[5,6] although opposite localization of these compounds has also been suggested.[7]

Depending on its localization in the lipid bilayer, a compound affects the dipalmitoylphosphatidylcholine (DPPC) phase transitions differently. For example, proteins that adsorb at the lipid–water interface without penetrating into the bilayer interior either have no effect or increase the main phase transition temperature (T_m), and also produce a large increase in the enthalpy of the main phase transition (ΔH_m).[8] In contrast, proteins that adsorb at the lipid–water interface, with partial penetration of the lipid bilayer, produce a substantial decrease in both T_m and ΔH_m, indicating a marked fluidization effect.

The toxicity of organometallic compounds may result from their different localization in the lipid bilayer and also from subsequent different alternation in the membrane structure. The alternation may lead to the formation of non-lamellar phases, of which the most common, in biological lipid systems, is the inverted hexagonal (H_{II}) phase.[9] For some time also, interdigitated gel ($L_{\beta 1}$)

structures in phospholipid membranes have attracted much attention.[10–21] It has been stated that in the presence of polymyxin, acetylcholine, ethanol, ethylene glycerol and other small organic molecules, multilamellar vesicles (MLVs) of phosphatidylcholine (PC) can form $L_{\beta I}$ phases.

Knowledge of the way that organometallic compounds interact with model membranes is not only interesting from a scientific viewpoint, but it may also help in understanding of the mechanism of their interaction with biomembranes and consequently in finding better protection against them.

In this work we have studied the effect of phenyltin compounds with two phenyl groups [diphenyltin dichloride (DPhT)], three phenyl groups [triphenyltin chloride (TPhT)] or four phenyl groups [tetraphenyltin (TTPhT)] attached to the tin atom on phase behaviour and on the structure of dipalmitoylphosphatidylcholine (DPPC) model membranes. We applied differential scanning calorimetry (DSC), which is widely used to study the effects of cholesterol, anaesthetics, drugs, proteins and various small molecules on phase transitions of phospholipids. In addition, we used ^{1}H NMR and ^{31}P NMR; NMR is one of the most powerful techniques that have been applied to study biological and model membranes.[22,23] This paper is an extension of work published as preliminary results in a short communication.[6]

MATERIALS AND METHODS

Chemicals

DPhT, TPhT and TTPhT were purchased from Alfa (Karlsruhe, Germany). The compounds were used without further purification. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids, Birmingham, A. Egg-yolk lecithin was prepared in our laboratory using Singleton's method.[24] The lecithin was checked for purity by thin-layer chromatography.

Sample preparation

MLVs for DSC were prepared from DPPC and appropriate amounts of di- or tri-phenyltin chlorides or tetraphenyltin. The compounds were dissolved in chloroform. The mixture was evaporated to form a thin film on the flask wall. Traces of chloroform were removed with a stream of dry nitrogen. Then distilled water was added and the flask and its contents were heated to 60 °C in a water bath. The lipid film was dispersed by agitating the flask on a vortex mixer to give a milky suspension of liposomes. The final lipid concentration was 25 mg cm^{-3}. The lipid suspension was loaded into the sample cell of a DSC microcalorimeter (Metller Toledo Thermal Analysis System D.S.C. 821^{e}.) Scan rates of 0.5 °C min^{-1} and 2 °C min^{-1} were employed but because no differences were seen between the results obtained, we used the latter scan speed. The measurement was repeated for at least three independent sample preparations. Different times of incubation at a temperature of 4 °C (from 48 h to one week) were applied to the samples, and then the time was chosen after which no changes in the temperature of the main transition were observed.

Small unilamellar vesicles (SUVs) for ^{1}H NMR were prepared from egg-yolk lecithin and appropriate amounts of DPhT, TPhT and TTPhT. The compounds were dissolved in chloroform and then the mixture was evaporated to form a film on the flask wall. Traces of chloroform were removed with a stream of dry nitrogen. Then D_2O was added. The lipid film was dispersed by agitating the flask on a vortex mixer to give a milky suspension of liposomes.

Liposomes with diphenyltin dichlorides, triphenyltin chlorides and tetraphenyltin were formed by sonicating egg lecithin dispersion in D_2O at 0 °C for 0.5 h with a 20 kHz sonicator.

^{1}H NMR spectra were recorded on an Avance Bruker DRX 300 spectrometer at 300.13 MHz. Samples were enclosed in 5 mm diameter NMR tubes. Signals were acquired using a 6173 Hz spectral window, 10.6 μs pulse width and 2.65 s acquisition time. Digital resolution was 207.272 7 Hz cm^{-1} or 0.69061 ppm cm^{-1}. The compounds were dissolved in 99.98% D_2O. The residual water signal was used as a chemical shift reference. All experiments were performed at 300 K.

^{31}P NMR spectra were recorded on an Avance Bruker DRX 300 spectrophotometer at 121.51 MHz. The same samples were used as for ^{1}H NMR. Signals were acquired using a 38 535.645 Hz spectral window, 2.0 μs pulse width and 0.85 s acquisition time. Digital resolution was 364.4843 Hz cm^{-1} or 3.00 ppm cm^{-1}. Chemical shift values are given relative to 85% H_3PO_4.

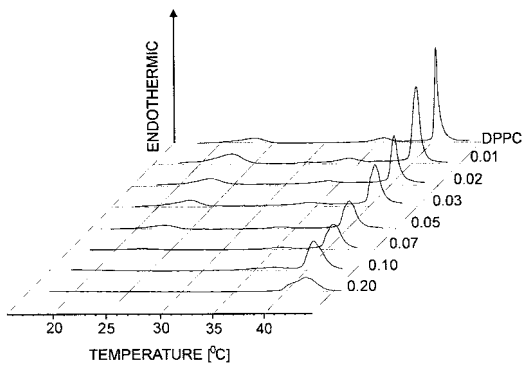

Figure 1 DSC heating curves of MLVs with increasing DPhT/DPPC molar ratio. The curves were normalized for the amount of DPPC.

RESULTS

Differential scanning calorimetry

The phenyltin compounds studied affect phase transitions of DPPC in different ways.

Diphenyltin dichloride (DPhT)

From Figs 1–3, at very low concentrations (up to about 0.03 molar ratio) DPhT hardly affects either the main phase transition temperature or the enthalpy. At molar ratios from about 0.03 to 0.05, DPhT decreases both T_m and ΔH_m, then increases them. At higher concentrations T_m remains practically unchanged, whereas ΔH_m decreases rapidly. Such a dual effect of DPhT on the main phase transition resembles that of substances which induce gel phases with interdigitated hydrocarbon chains ($L_{\alpha I}$).[18] This may indicate that DPhT is one such substance.

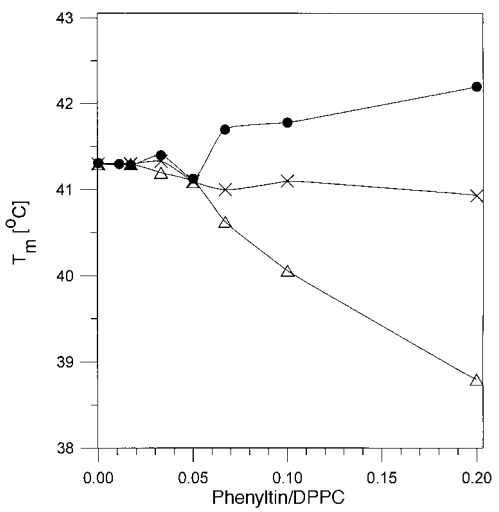

Figure 2 Main phase transition temperatures (T_m) as a function of phenyltin concentration: ●, DPhT; △, TPhT; ×, TTPhT.

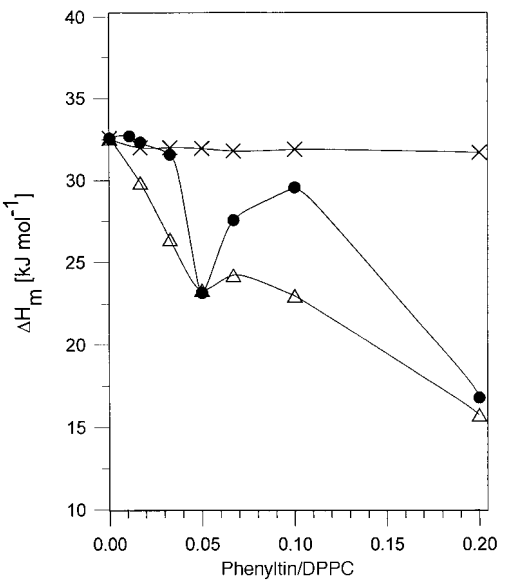

Figure 3 Main phase transition enthalpies (ΔH_m) as a function of phenyltin concentration: ●, DPhT; △, TPhT; ×, TTPhT.

As in the case of T_m, DPhT also shows a dual effect on the pretransition temperature, T_p (Figs 1 and 4). At very low concentrations DPhT decreases T_p, then increases it. The pretransition enthalpy decreases as the concentration of DPhT increases. At a molar ratio of about 0.10, DPhT abolishes the pretransition.

DPhT scarcely affects the subtransition temperature (Fig. 4) but it significantly decreases the subtransition enthalpy (Fig. 5). This seems to suggest that domains with interdigitated chains do not undergo subtransition. The subtransition is suggested to be associated with rotational disordering of the acyl chains.[25] It seems rather difficult for this process to occur in an ordered phase such as a gel phase with interdigitated chains.

Triphenyltin chloride (TPhT)

TPhT affects the main phase transitions of DPPC differently from DPhT. From Figs 2, 3 and 6, T_m and ΔH_m decrease progressively, and the main transition broadens, with increasing TPhT/DPPC

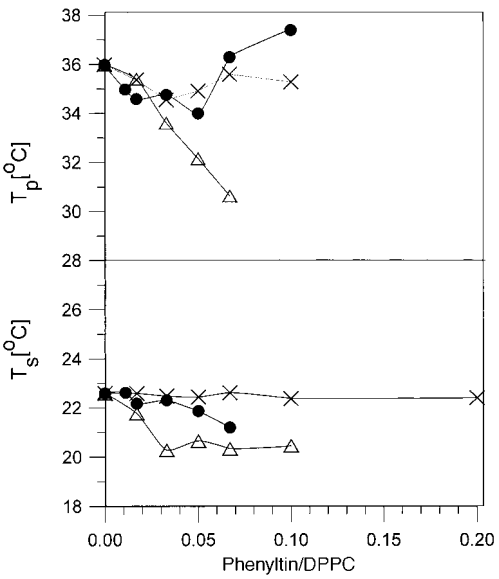

Figure 4 Pretransition and subtransition temperatures (T_p, T_s) as a function of phenyltin concentration: ●, DPhT; △, TPhT; ×, TTPhT.

molar ratio. Thus, TPhT changes T_m and ΔH_m analogously to proteins partially penetrating lipid bilayer.[8] This may suggest that TPhT is located at the surface of the lipid bilayer region and partially

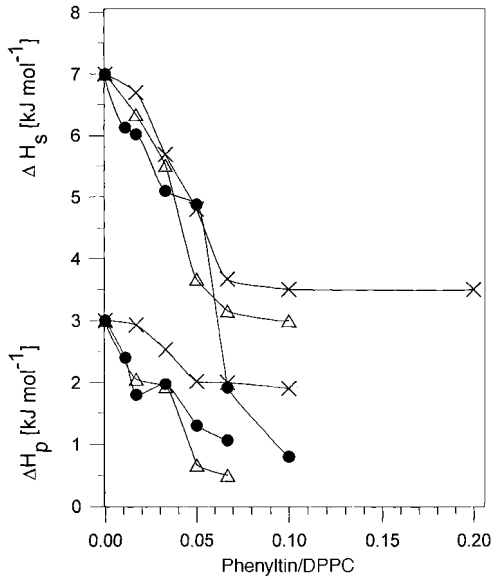

Figure 5 Pretransition and subtransition enthalpies (ΔH_p, ΔH_s) as a function of phenyltin concentration: ●, DPhT; △, TPhT; ×, TTPhT.

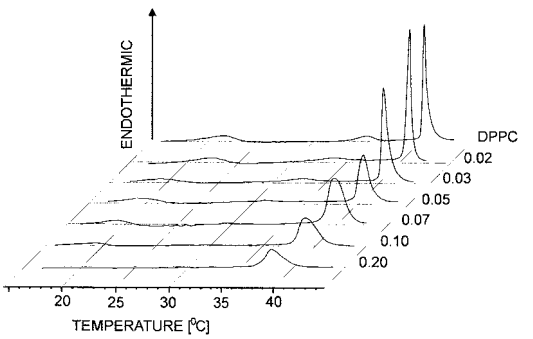

Figure 6 DSC heating curves of MLVs with increasing TPhT/DPPC molar ratio. The curves were normalized for the amount of DPPC.

penetrates into the hydrocarbon region. TPhT also affects the pretransition in a different way from DPhT. As in the case of T_m, TPhT decreases T_p and ΔH_p (Figs 4 and 5) with increasing concentration. Above the molar ratio 0.05, DPhT and TPhT shift T_p in opposite directions. The pretransition temperature is mainly sensitive to changes in the interfacial region,[26] so any changes in the interfacial region should be reflected in T_p. Thus, changes in T_p by DPhT and TPhT in opposite directions confirm that these compounds are located differently in the interfacial region. Since TPhT is probably located at the surface of the lipid bilayer and partially penetrates the lipid bilayer, so DPhT seems to be near an apolar/polar interface, where a substance that induces a gel phase with interdigitated chains would be expected to be located.[20]

TPhT decreases the subtransition temperature a little more (Fig. 4) and the subtransition enthalpy (Fig. 5) much less than DPhT.

Tetraphenyltin (TTPhT)

TTPhT broadens the main phase transition but hardly affects T_m and ΔH_m (Figs 2, 3 and 7), while the pretransition is affected only a little (Figs 4 and 5). Above a molar ratio of 0.1, the pretransition is abolished. TTPhT decreases subtransition enthalpy (Fig. 5) but hardly changes T_s (Fig. 4). The subtransition is not abolished within the investigated concentration range.

Nuclear magnetic resonance spectroscopy

In order to confirm DSC results concerning the location of phenyltin compounds within the phos-

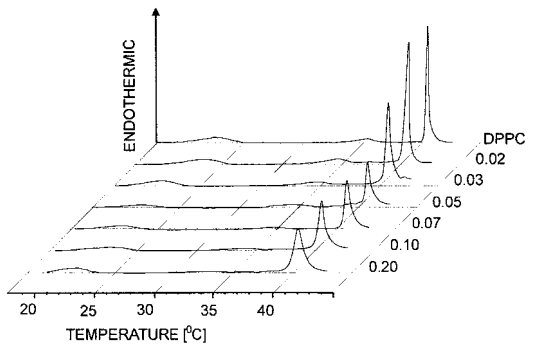

Figure 7 DSC heating curves of MLVs with increasing TTPhT/DPPC molar ratio. The curves were normalized for the amount of DPPC.

pholipid bilayer, ^{1}H NMR and ^{31}P NMR measurements were performed. We used the same sonicated samples for both ^{1}H NMR and ^{31}P NMR measurements. The spectra of sonicated egg-yolk PC (lecithin) dispersions for molar ratios of 0.07 and 0.20 are shown in Figs 8 and 9. At a molar ratio of 0.20, only PC vesicles with TPhT were studied because, in the presence of DPhT and TTPhT, precipitates (not detectable by ^{1}H NMR and ^{31}P NMR) were formed. In the absence of phenyltin compounds the narrow linewidth of the ^{1}H NMR choline $—N(CH_3)_3$ resonance (Fig. 8) and the narrow ^{31}P NMR signal (Fig. 9) characteristic of sonicated vesicles[27] were obtained. As in the case of DSC, DPhT and TPhT affect the ^{1}H NMR and ^{31}P NMR spectra in opposite directions. The ^{1}H NMR choline $—N(CH_3)_3$ resonance is more affected by TPhT than by DPhT, whereas the ^{31}P NMR spectrum of lecithin vesicles is more affected by DPhT than by TPhT. DPhT and TPhT shift the ^{1}H NMR chemical shift of $—N(CH_3)_3$ a little upfield (Table 1). The linewidth of the choline $—N(CH_3)_3$ group increases much more after addition of TPhT than of DPhT (Fig. 8 and Table 2). DPhT and TPhT also shift the ^{31}P NMR signal upfield; in the case of DPhT the signal is much more shifted and broadened than in the case of TPhT (Table 3).

TTPhT affects neither the ^{1}H NMR nor the ^{31}P NMR spectra.

DISCUSSION

DSC results suggest that DPhT induces interdigitated gel phase formation. As was mentioned

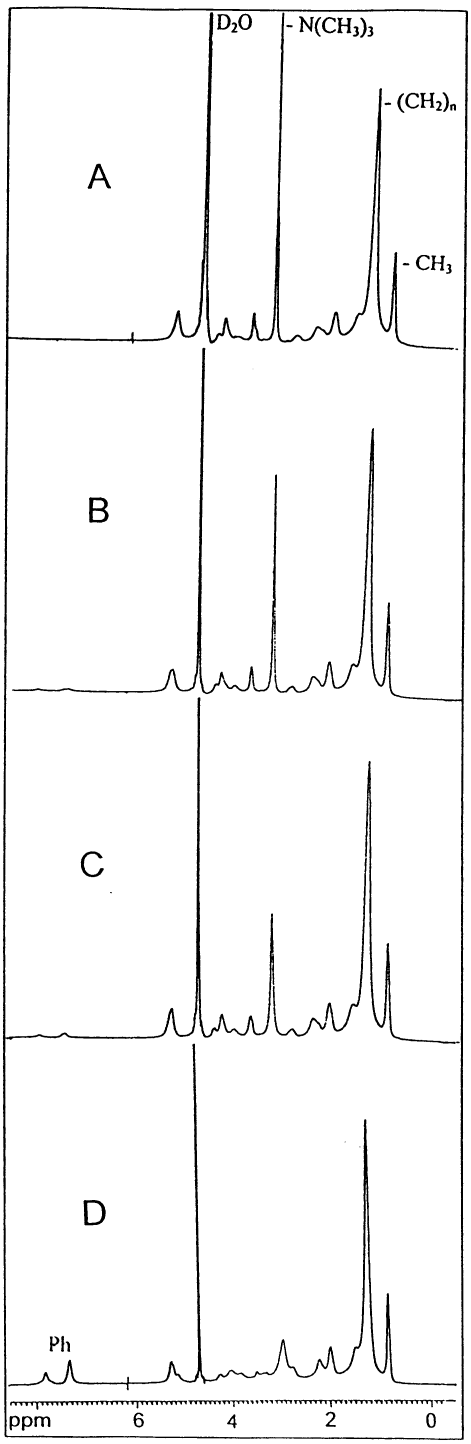

Figure 8 ^{1}H NMR spectra of egg-yolk lecithin liposome suspension in the absence (A) and presence of DPhT (B) or TPhT (C). The molar ratios of DPhT/DPPC and TPhT/DPPC were 0.07 (curves B, C) and 0.2 (curve D), respectively.

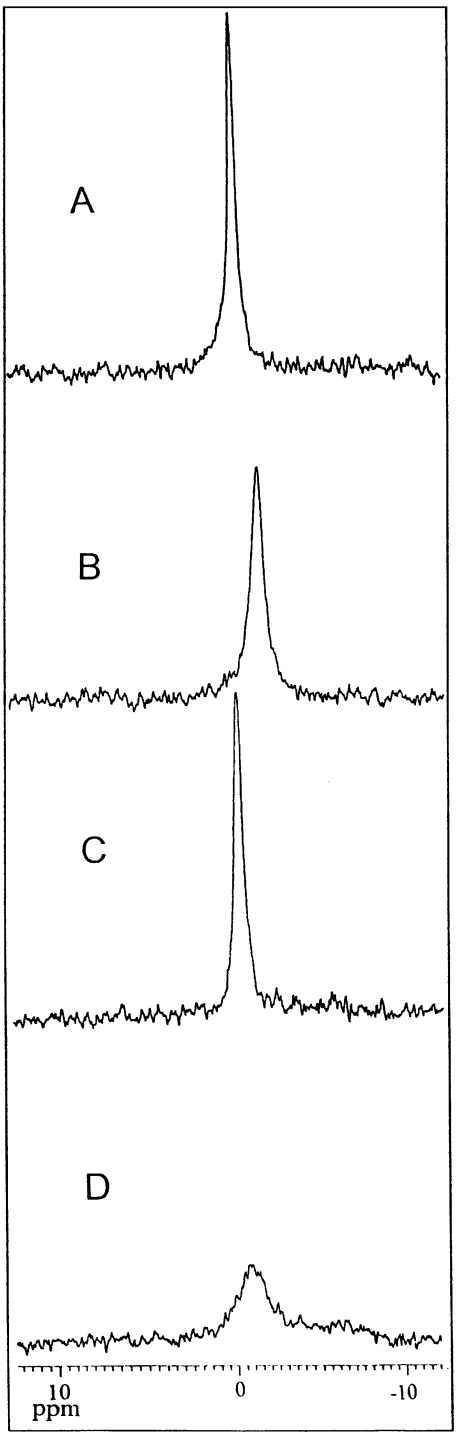

Figure 9 ^{31}P NMR spectra of egg-yolk lecithin liposome suspension in the absence (A) and presence of DPhT (B) or TPhT (C, D). The molar ratios of DPhT/DPPC and TPhT/DPPC were 0.07 (curves B, C) and 0.2 (curve D), respectively.

Table 1 ^{1}H NMR chemical shifts (ppm) in D_2O for lecithin dispersion with and without DPhT and TPhT

		Phenyltin/lecithin				
		DPhT		TPhT		
Group	Egg lecithin	0.03	0.07	0.03	0.07	0.20
C_6H_5	—	7.94	7.92	7.92	7.89	7.81
		7.29	7.35	7.38	7.39	7.33
$N(CH_3)_3$	3.19	3.14	3.12	3.29	3.15	2.94
$(CH_2)_n$	1.22	1.21	1.22	1.23	1.21	1.23
CH_3	0.83	0.83	0.83	0.84	0.83	0.84

before, in the interdigitated gel phase a compound is expected to be near polar/apolar interface. A substance in such a location should show a small effect on the ^{1}H NMR choline —$N(CH_3)_3$ resonance. Any changes in the structure of the bilayer due to interdigitation should be reflected in the ^{31}P NMR spectrum, since this spectrum is very sensitive to any alteration in the bilayer structure. A significant shift and broadening of the ^{31}P NMR spectrum after addition of DPhT may suggest that DPhT induces the formation of oriented bilayers or bilayer fragments.[28] The bilayers may be formed from interdigitated vesicles because they do not interdigitate in a stable way: they rupture and coalesce into viscous gels.[21] At higher DPhT concentrations this process probably leads to the formation of the above-mentioned precipitate, which appeared viscous. Possibly, the precipitate could also be formed in the case of MLV studied by DSC. The formation of the precipitate may explain the marked decrease in ΔH_m observed for higher molar ratios of DPhT/DPPC. However, in the case of a higher molar ratio of TTPhT/DPPC, the precipitate also formed, but in this case the precipitate looked quite different and was probably undissolved TTPhT.

DSC results suggest that TPhT is at least partially embedded in the lipid bilayer. This is in agreement

Table 2 ^{1}H NMR half width (Hz) in D_2O for lecithin dispersion with and without DPhT and TPhT

		Phenyltin/lecithin				
		DPhT		TPhT		
Group	Egg lecithin	0.03	0.07	0.03	0.07	0.20
$N(CH_3)_3$	6.0	8.4	8.4	12.6	16.0	36.0

Table 3 ^{31}P NMR spectrum of egg lecithin vesicles in D_2O with and without DPhT and TPhT

		Phenyltin/lecithin				
		DPhT		TPhT		
	Lecithin	0.03	0.07	0.03	0.07	0.20
Chemical shift (Hz)	−0.33	−1.26	−1.55	−0.36	−0.44	−0.67
Half width (Hz)	60.70	97.18	103.30	66.77	72.84	224.60

with NMR studies. The location of a molecule at the surface of a lipid bilayer with partial penetration into the hydrocarbon region should result in increased packing in the bilayer and a consequent decrease in the mobility of the headgroups. This in turn broadens and shifts the choline —$N(CH_3)_3$ proton resonance.[22] This effect was just observed after addition of TPhT (Fig. 8). Besides, embedding a molecule into the lipid bilayer should increase the size of the vesicles. This may explain the small increase in the vesicle ^{31}P NMR linewidth after addition of TPhT (Table 3), since there is a simple relationship between the size of the vesicle and the linewidth of the ^{31}P NMR resonance.[23,29] At a TPhT/DPPC molar ratio of 0.20, the chemical shift of the ^{1}H NMR choline group, as well as the ^{31}P NMR spectrum, is significantly shifted upfield and the linewidths are broadened. Also, the DSC curve is much shifted. Besides the ^{31}P NMR lineshape is changed: a low-field shoulder appears. This may suggest that on inverted hexagonal phase (H_{II}) is formed. Inverted hexagonal phases are known to be involved in the molecular mechanism of membrane aggregation and bilayer destabilization.[30] This may explain the marked fluidization effect observed in ^{1}H NMR (Fig. 8) and DSC (Fig. 6). Figures 2 and 3 suggest that at a molar ratio of about 0.05 the character of the TPhT interaction with the lipid bilayer has changed. Apparently, at that concentration formation of non-lamellar domains begins. TTPhT broadens DSC curves only a little, while ^{1}H NMR as well as ^{31}P NMR spectra of lecithin vesicles are not affected, we conclude that TTPhT has a small affect on the structure of the membranes. Also, haemolysis of red blood cells caused by TTPhT is slight.[7] Possibly, haemolytic toxicity of DPhT and TPhT[7,31] is connected with the changes they cause in the membrane structure.

The results suggest that toxicity, for instance haemolytic toxicity, of phenyltin compounds may be related to structural changes they induce in the bilayers. This is in agreement with the suggestion that the interdigitated gel phase as well as the inverted hexagonal gel phase may play an important role in regulating many functions of biomembranes.[16,32]

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