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The organochlorine pesticide heptachlor disrupts the structure of model and cell membranes

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Abstract

Heptachlor is an organochlorine pesticide which is particularly toxic for aquatic life. A significant source of this pesticide for infants is breast milk, where its concentration is considerably higher than in dairy milk. Given the lipophilic character of heptachlor, lipid-rich cell membranes are a very plausible target for its interaction with living organisms. In order to evaluate its toxicity towards cell membranes, heptachlor was made to interact with human erythrocytes and molecular models of the red cell membrane. These consisted of multilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), which are types of phospholipids that are respectively located in the outer and inner monolayers of the erythrocyte membrane, and large unilamellar vesicles (LUV) of DMPC. Observations by scanning electron microscopy showed that 10 mM heptachlor produced various degrees of shape alterations to erythrocytes, which ranged from a few blebs in some cells to a great number of protuberances in others. On the other hand, experiments performed by X-ray diffraction on DMPC and DMPE indicated that the bilayer structure of DMPC was much more affected by heptachlor than that of DMPE. Measurements by fluorescence spectroscopy on DMPC LUV confirmed the X-ray diffraction results in that both the hydrocarbon chain and polar head regions of DMPC were structurally perturbed by heptachlor. The results obtained from the model studies could explain the shape changes induced to red cells by heptachlor. According to the bilayer hypothesis, they were due to the preferential interaction of heptachlor with the phosphatidylcholine-rich external moiety of the erythrocyte membrane. It is therefore concluded that toxic effects of this pesticide can be related to its capacity to perturb the phospholipid bilayer structure, whose integrity is essential for cell membrane functions.

Keywords: Heptachlor; Pesticide; Phospholipid bilayer; Erythrocyte membrane; X-ray diffraction; Fluorescence spectroscopy

1. Introduction

Organochlorine pesticides, including accompanying residues and metabolites, are ubiquitous in the environment because of their widespread use. Thus, the potential for human exposure and uptake is high [1]. Heptachlor, whose structural formula is shown in Fig. 1, is an organochlorine pesticide used for more

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; LUV, large unilamellar vesicles; DPH, 1,6-diphenyl-1,3,5-hexatriene; Laurdan, 6-dodecanoyl-2-dimethylaminonaphtalene; GP, general polarization; SEM, scanning electron microscopy

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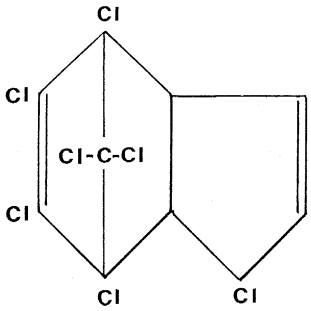


Fig. 1. Schematic formula of heptachlor.

than 40 years in the control of termites and soil insects. However, its approved use in several countries has been gradually withdrawn. Exposure of the general population is mainly through residues in food. A significant source of heptachlor for infants is breast milk, in which its level can be considerably higher than in dairy milk [2]. It has been shown to be toxic for aquatic life, being marine crustacean and younger life stages of both fish and invertebrates the most sensitive. In fact, oysters accumulate as much as 18 000 times the concentration in ambient water and 2800 to 21 300 times in estuary fish after a 96-h exposure [3]. In rats, at a dose of 20 mg/kg body weight, heptachlor caused a significant decrease in average body weight, disrupted oestrous cycles, decreased mating success and increased gestation length [4].

In general, the molecular mechanisms of pesticide action are poorly understood. However, the lipophilicity of most of them makes lipid-rich membranes a plausible target of their interaction with living organisms [5,6]. It has been suggested that some effects directly related to their toxicity could be due to changes in membrane fluidity as a primary pesticide effect [7]. Some of them are changes in the permeability of lipid bilayers for electrolytes and nonelectrolytes [8], inhibition of acetylcholinesterase by organophosphorous pesticides [9] or changes in lipid composition [10]. However, in spite of the implications that at least part of the toxic effects of pesticides could be due to the perturbation of the lipid phase of the membranes [11], a clear relationship between changes in fluidity evoked by pesticides and their chronic toxicity has not been established.

This paper describes the results of our studies on the interaction of heptachlor with the human erythrocyte membrane and models constituted by phospholipid multilayers and large unilamellar vesicles. These systems have previously been used in our laboratories to determine the interaction and perturbing effects on membranes by the pesticides DDT [12], pentachlorophenol [13,14], 2,4-D [15] and dieldrin [16]. The multilayers consisted in the phospholipids dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), which represent phospholipids that are respectively located in the outer and inner monolayers of the human erythrocyte membrane [17]. Given the lipophilic nature of heptachlor and the amphiphilic character of both phospholipids, their interactions were assayed in a hydrophobic medium as well as in water under a wide range of concentrations. The capacity of heptachlor to perturb the multilayer structure of DMPC and DMPE was determined by X-ray diffraction methods.

Fluorescent steady-state anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) and the general polarization of 6-dodecanoyl-2-dimethylaminonaphtalene (Laurdan) were determined in DMPC large unilamellar vesicles. DPH is one of the most used probes for the hydrophobic regions of phospholipid bilayers. Its fluorescence steady-state anisotropy provides a measure of the rotational diffusion of the fluorophore restricted within a certain region due to the phospholipid acyl chain order. On the other hand, Laurdan has a high sensitivity of excitation and emission spectra to the physical state of membranes [18]. With the fluorophore moiety located in a shallow position of the bilayer normal in the phospholipid polar head group environment, Laurdan provides information on dynamic properties in this zone of the bilayer [19,20]. Laurdan spectral shift quantification is done using the general polarization (GP) concept [21].

Finally, heptachlor was incubated with human erythrocytes which were later observed by scanning electron microscopy to detect shape changes induced by the pesticide.

2. Materials and methods

2.1. X-ray diffraction analysis of phospholipid multilayers

Synthetic DMPC (lot 80H8371, A grade, MW 677.9) and DMPE (lot 13H83681, A grade, MW

635.9) from Sigma and heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4-7-methanoindene) (99%, MW 373.3) from Supelco were used without further purification. About 3 mg of each phospholipid were mixed with the corresponding weight of heptachlor in order to attain DMPC:heptachlor and DMPE:heptachlor powder mixtures in the molar ratios of 10:1, 5:1, 2:1 and 1:1. Each mixture was dissolved in chloroform:methanol 3:1 v/v and left to dry. The resulting samples, under the form of crystalline powders, were introduced into special glass capillaries. They were diffracted in Debye-Scherrer cameras of 114.6 mm diameter and flat-plate cameras with 0.25 mm diameter glass collimators provided with rotating devices. The same procedure was followed with pure samples of each phospholipid and heptachlor. The aqueous specimens were prepared in glass capillaries mixing each phospholipid and the pesticide in the same proportions as described above. Each capillary was then filled with about 200 μl of distilled water. These specimens were X-ray diffracted 2 days after preparation in flat-plate cameras. Specimen-to-film distances were 8 or 14 cm, standardized by sprinkling calcite powder on the capillaries surface. Ni-filtered CuKα radiation from a Philips PW1140 X-ray generator was used. The relative reflection intensities were obtained from films by peak-integration in a Joyce-Loebl MKIIICS microdensitometer interfaced to a PC. No correction factors were applied. The experiments in water were performed at 17 ± 2°C, which is below the main transition temperatures of both DMPC and DMPE.

2.2. Fluorescence measurements on large unilamellar vesicles (LUV)

DMPC LUV suspended in water were prepared by extrusion of frozen and thawed multilamellar liposome suspension through two stacked polycarbonate filters of 400 nm pore size (Nucleopore, Corning Costar Corp.) employing nitrogen pressure at 10°C over the lipid transition temperature, to a final concentration of 0.3 mM. DPH and Laurdan were incorporated into LUV by addition of small aliquots of concentrated solutions of the probe in ethanol to LUV suspension in water and gently shaken by ca. 30 min. The final probe concentration was 0.5 μM. Fluorescence spectra and anisotropy measurements were performed in a Fluorolog spectrofluorometer from Spex and in a phase shift and modulation Greg-200 spectrofluorometer from ISS respectively, both interfaced to PC using ISS software. Measurements of LUV suspensions were made at 18°C employing 10 mm path-length square quartz cuvettes. Sample temperature was controlled by an external Cole Parmer bath circulator and measured prior and after each measurement using an Omega digital thermometer. Anisotropy measurements were done in the 'L' configuration using Glan Thompson prism polarizers in both exciting and emitting beams. The emission was measured with the aid of a WG-420 Schott high pass filter which showed negligible fluorescence. GP was evaluated by $GP = (Ib\text{-}Ir)/(Ib + Ir)$, where Ib and Ir are the intensities at the blue and red edges of the emission spectrum respectively. These intensities were measured at 440 and 500 nm, corresponding to the emission maxima of Laurdan in gel and liquid-crystalline phases respectively [21]. For both probes the excitation wavelength was set at 360 nm. Blank samples without probe were used to correct background light scattering. Heptachlor was incorporated as small aliquots from a concentrated aqueous suspension.

2.3. Scanning electron microscope (SEM) studies on human erythrocytes

Heptachlor was made to interact in vitro with erythrocytes by incubating blood samples of human healthy male adult donors not being treated with any pharmacological agent. For this purpose the samples were obtained by puncture of the ear lobule. One drop was aspirated with a plastic tuberculine syringe without needle containing 1 ml of saline solution (0.9% NaCl) at room temperature. This blood stock suspension was used to prepare the following samples in tuberculine syringes: (a) 0.1 ml of blood stock mixed with 0.9 ml of saline solution (control), and (b) 0.1 ml of blood stock mixed with saline heptachlor suspension in order to obtain concentrations equivalent to 1 mM and 10 mM. All the samples were incubated for 1 h at 37°C in an oven. Then, they were fixed with glutaraldehyde by adding one drop of each sample to plastic tubes containing 1 ml of 2.5% glutaraldehyde in saline solution, reaching a final fixation concentration of about 2.4%. After resting

overnight at 5°C, the fixed samples were washed two times with saline solution, placed directly on Al stubs, air-dried in an oven at 37°C for half to one hour and finally gold-coated for 3 min at 10-1 torr in a S 150 Edwards sputter device. The observations and photographic records were performed in an ETEC Autoscan scanning electron microscope.

3. Results

3.1. X-ray studies on phospholipid multilayers

The molecular interaction of heptachlor with multilayers of the phospholipids DMPC and DMPE were studied in both a hydrophobic and a hydrophilic medium. Table 1 shows the interplanar spacings and relative intensities of the reflections produced by DMPC, heptachlor and their 5:1, 2:1 and 1:1 molar mixtures after interacting and being recrystallized from chloroform:methanol 3:1 v/v solutions. Their respective diffractograms are compared in Fig. 2. The analysis of these results indicated that the X-ray pattern of DMPC was affected by increasing concentrations of the pesticide. In fact, at a DMPC:heptachlor molar ratio of 5:1, the reflection intensities of the lipid became weaker, many of them disappearing at the 1:1 ratio. However, several new reflections showed up in the 2:1 and 1:1 specimens, all of them corresponding to heptachlor's strongest reflections. On the other hand, the bilayer width of DMPC remained practically constant at about 55 Å. These facts indicated that one part of the pesticide interacted with DMPC, deeply penetrating into the phospholipid bilayer core perturbing its structure, while another part of heptachlor remained intact.

Table 2 and Fig. 3 show the results obtained when DMPC, heptachlor and their molar mixtures in the same ratios as above were immersed in distilled water. It was observed that water produced an expan-

Table 1
Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPC, heptachlor and of their 5:1, 2:1 and 1:1 molar mixtures [a–c]

DMPC:heptachlor									
DMPC		5:1		2:1		1:1		heptachlor	
do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel
54.5	490	54.6	170	54.5	170	54.5	143	–	–
27.2	9	27.1	3	27.2	2	27.1	1	–	–
18.3	4	18.4	2	18.3	1	18.2	1	–	–
13.7	14	13.8	6	13.8	6	13.8	3	–	–
9.30	7	9.29	3	9.29	1	9.30	2	–	–
8.30	5	8.29	3	–	–	–	–	–	–
–	–	–	–	7.92	2	7.92	5	7.90	5
–	–	–	–	7.00	2	7.03	3	7.04	12
6.29	13	6.28	4	–	–	–	–	–	–
–	–	–	–	6.13	12	6.13	13	6.13	15
–	–	–	–	4.85	4	4.85	4	4.85	5
4.66	5	4.64	3	4.62	3	4.62	3	4.62	2
4.29	38	4.27	7	4.27	6	4.27	5	–	–
4.13	100	4.14	51	4.13	40	4.13	28	–	–
3.88	18	3.89	4	–	–	–	–	–	–
–	–	–	–	3.82	2	3.82	2	3.82	2
–	–	–	–	3.47	2	3.48	3	3.47	4
–	–	–	–	3.37	3	3.37	5	3.36	11
–	–	–	–	3.06	3	3.06	4	3.06	7

[a] All the specimens were recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v).
[b] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams from Debye-Scherrer and flat-plate cameras with 8 and 14* cm specimen-to-film distances.
[c] Only the main observed reflections are included.

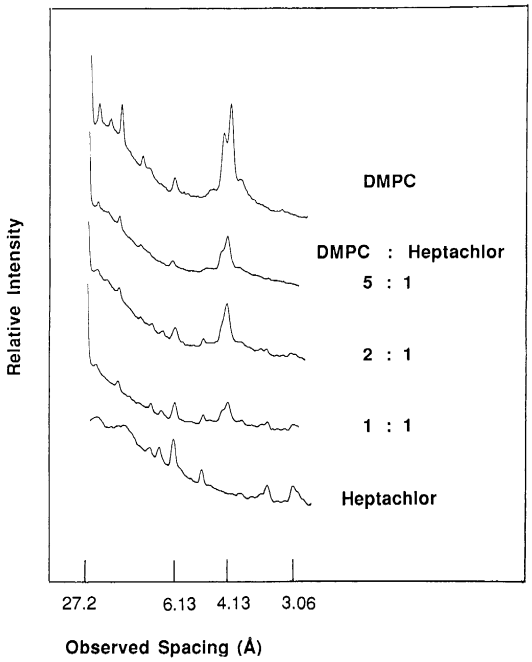


Fig. 2. Microdensitograms from X-ray diagrams of specimens recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v). Flat-plate cameras. Specimen-to-film distance 8 cm.

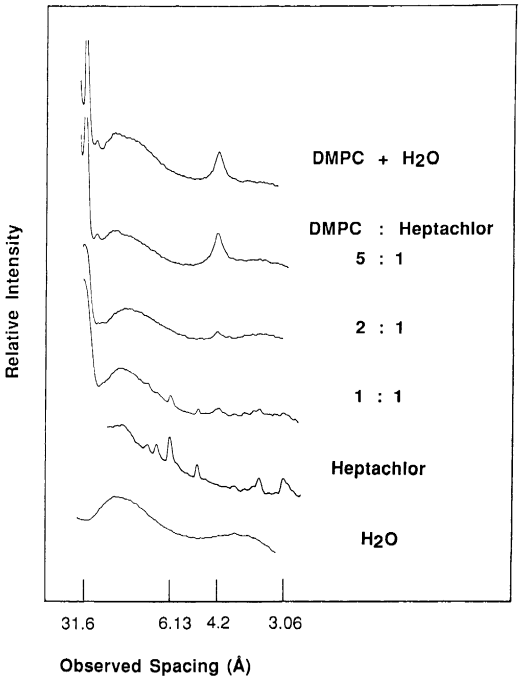


Fig. 3. Microdensitograms from X-ray diffraction diagrams of DMPC and aqueous suspensions of heptachlor. Flat-plate cameras. Specimen-to-film distance 8 cm.

Table 2
Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPC and its 5:1, 2:1 and 1:1 molar mixtures with heptachlor in water [a,b]

DMPC:heptachlor									
DMPC		5:1		2:1		1:1		heptachlor [c]	
do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel
63.4 *	172 *	63.4 *	24 *	–	–	–	–	–	–
31.6 *	75 *	31.7 *	38 *	–	–	–	–	–	–
21.2	8	21.2 *	6	–	–	–	–	–	–
–	–	–	–	–	–	–	–	7.90	5
–	–	–	–	–	–	–	–	7.04	12
–	–	–	–	–	—	–	–	6.13	15
–	–	–	–	–	–	–	–	4.85	5
–	–	–	–	–	–	–	–	4.62	2
4.2	100	4.2	84	4.2	11	4.2	9	–	–
–	–	–	–	–	–	3.82	1	3.82	2
–	–	–	–	–	–	3.47	2	3.47	4
–	–	–	–	–	–	3.36	3	3.36	11
–	–	–	–	–	–	3.06	2	3.06	7

[a] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams from Debye-Scherrer and flat-plate cameras with 8 and 14 * cm specimen-to-film distances.
[b] Only the main observed reflections are included.
[c] Data from dry specimens.

sion of the DMPC bilayer width from about 55 Å when dry to nearly 63 Å. The observed reflections were reduced to only the first three orders of the bilayer width and a relatively intense one of about 4.2 Å. The latter arose from the stiff and fully extended hydrocarbon chains organized with rotational disorder in an hexagonal lattice [22]. The increasing proportion of heptachlor in the mixtures produced a sharp diminishing of the phospholipid reflection intensities. Thus, in the 2:1 mixture only remained a considerable weak 4.2 Å reflection, whereas those in the low angle region were replaced by a central diffuse scattering. The X-ray diagram from the 1:1 ratio specimen showed practically only weak reflections arising from heptachlor. These results meant that the pesticide produced a high degree of perturbation to the DMPC whole bilayer structure, despite the fact that heptachlor is very insoluble in water.

Table 3
Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPE, heptachlor and of their 1:1 molar mixtures [a–c]

DMPE		DMPE:heptachlor 1:1		heptachlor	
do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel
51.4 *	850 *	51.4 *	545 *	–	–
25.3 *	3 *	25.3 *	1 *	–	–
17.1	5	17.1	3	–	–
12.7	3	12.8	1	–	–
–	–	–	–	7.90	5
–	–	7.05	4	7.04	12
–	–	6.15	15	6.13	15
5.96	11	5.98	2	–	–
5.72	8	–	–	–	–
5.22	7	–	–	–	–
5.03	4	–	–	–	–
4.78	19	4.82	8	4.85	5
4.66	14	4.64	5	4.62	2
4.23	5	4.24	4	–	–
4.04	100	4.05	51	–	–
3.92	12	3.93	8	–	–
3.80	60	3.81	26	3.82	2
–	–	3.50	3	3.47	4
–	–	–	–	3.36	11
–	–	–	–	3.06	7

[a] All the specimens were recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v).
[b] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams from Debye-Scherrer and flat-plate cameras with 8 and 14 * cm specimen-to-film distances.
[c] Only the main observed reflections are included.

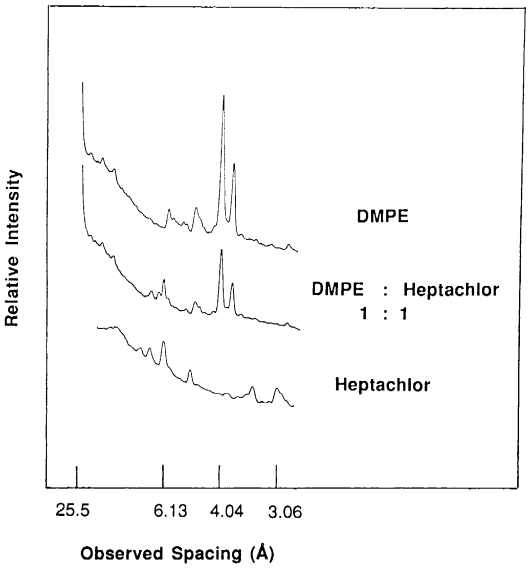


Fig. 4. Microdensitograms from X-ray diagrams of specimens recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v). Flat-plate cameras. Specimen-to-film distance 8 cm.

Table 3 and Fig. 4 respectively show the interplanar spacings and X-ray patterns obtained after DMPE was made to interact with heptachlor in the same way as described for DMPC in a hydrophobic medium. The perturbing effect of the pesticide upon the structure of DMPE multilayers was much milder than that observed in DMPC. In fact, at their 1:1 molar ratio only a decrease was observed in the lipid reflection intensities. On the other hand, several reflections from heptachlor were also present in this pattern. Finally, the results of the interaction of DMPE and heptachlor in the presence of water are presented in Table 4 and Fig. 5. As can be observed, the X-ray pattern of DMPE in the presence of water is very similar to the dry one shown in Fig. 4. On the other hand, at the 1:1 molar ratio, the pesticide only produced a moderate diminishing of DMPE reflection intensities. Moreover, most of heptachlor reflections also showed up in this pattern. These results undoubtedly reflect the lack of a significant interaction between the pesticide and DMPE bilayers.

3.2. Fluorescence measurements on large unilamellar vesicles (LUV)

The effect of heptachlor upon DMPC LUV was studied at the hydrocarbon chain and the hy-

drophilic/hydrophobic interface regions of the phospholipid bilayer. This was achieved through the evaluation of DPH steady-state fluorescence anisotropy (r) and Laurdan general polarization (GP). As it can be noticed in Table 5, increasing concentrations of heptachlor produced a monotonous decrease in the fluorescence parameters of both probes with a larger effect on Laurdan GP. The DPH steady-state anisotropy is primarily related to the rotational motion restriction due to the acyl chain packing order. Therefore, the observed decrease in this parameter can be explained by a high structural disruption of the bilayer hydrophobic region produced by the incorporation of heptachlor. On the other hand, the even larger effect of Laurdan GP indicated that the dynamics of the dipolar relaxation and/or the water penetration into the polar head group region was highly increased by the incorporation of heptachlor. These results quite agree with those obtained from the X-ray diffraction experiments on DMPC multilayers in an aqueous medium.

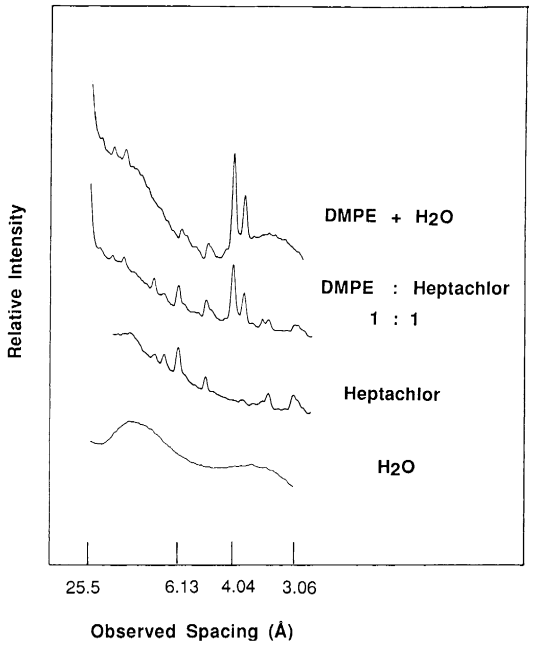


Fig. 5. Microdensitograms from X-ray diffraction diagrams of DMPE and aqueous suspensions of heptachlor. Flat-plate cameras. Specimen-to-film distance 8 cm.

Table 4
Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPE and its 1:1 molar mixture with heptachlor in the presence of water [a,b]

DMPE		DMPE:heptachlor 1:1		heptachlor [c]	
do (Å)	Io rel	do (Å)	Io rel	do (Å)	Io rel
51.3 *	1470 *	51.4 *	912 *	–	–
25.6 *	2 *	25.6 *	3 *	–	–
17.1	6	17.1	5	–	–
12.7	16	12.7	9	–	–
–	–	7.87	5	7.90	5
–	–	7.00	6	7.04	12
–	–	6.10	17	6.13	15
5.97	9	5.97	3	–	–
5.09	6	5.07	2	–	–
4.81	16	4.80	16	4.85	5
4.65	10	4.63	12	4.62	2
4.25	6	4.23	2	–	–
4.04	100	4.03	51	–	–
3.94	4	–	–	–	–
3.81	57	3.80	44	3.82	2
–	–	–	–	3.47	4
–	–	3.34	5	3.36	11
–	–	–	–	3.06	7

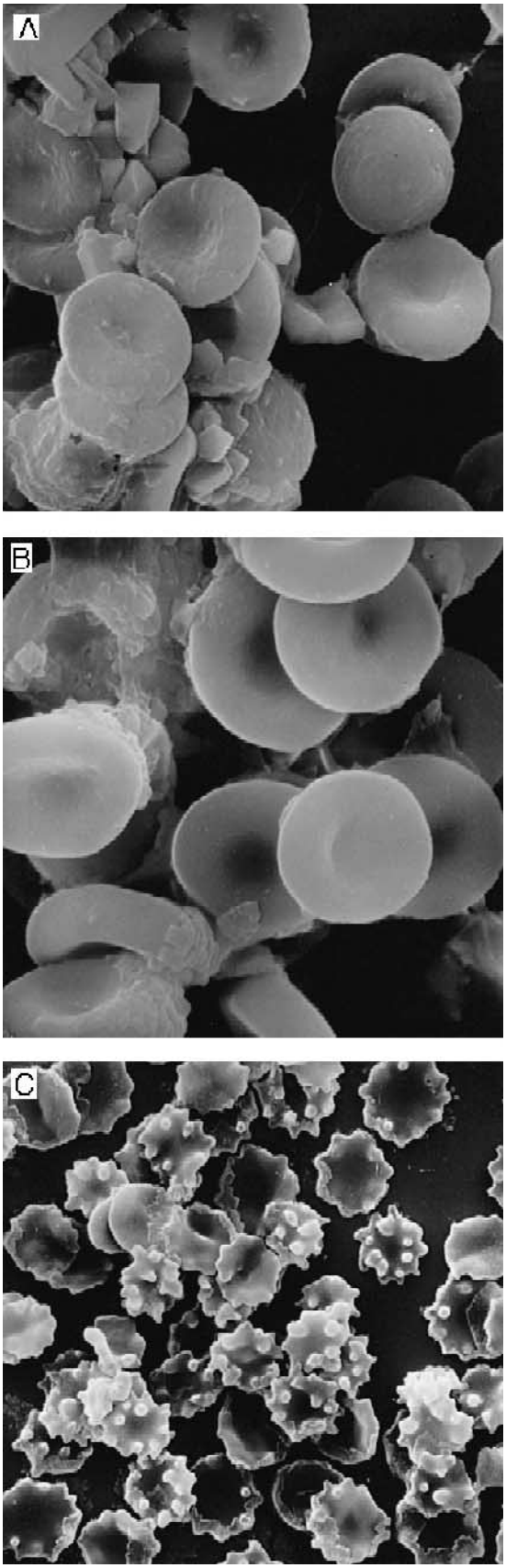
[a] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams from Debye-Scherrer and flat-plate cameras with 8 and 14 * cm specimen-to-film distances.
[b] Only the main observed reflections are included.
[c] Data from dry specimens.

3.3. Scanning electron microscopy (SEM) studies on human erythrocytes

Blood samples were incubated with heptachlor suspensions equivalent to 1 mM and 10 mM. The SEM examinations indicated that heptachlor 1 mM did not induce any significant change to the erythrocytes, which maintained their normal discoid biconcave shape (Fig. 6A,B). However, heptachlor 10 mM induced crenation to the erythrocytes, i.e., blebs or

Table 5
Effect of heptachlor on the anisotropy (r) of 1,6-diphenyl-1,3,5-hexatriene (DPH) and the general polarization (GP) of Laurdan embedded in large unilamellar dimyristoylphosphatidylcholine (DMPC) vesicles (probe: lipid ratio 1:600)

Heptachlor conc. (mM)	r DPH	GP Laurdan
0.00	0.271	0.513
0.01	0.250	0.394
0.10	0.110	0.084
1.00	0.063	–



protuberances were developed on the erythrocyte surface (Fig. 6C). As can be observed, the red cells showed various degrees of alterations, ranging from a few blebs in some cells to erythrocytes with a great number of protuberances resulting in an intense crenation phenomenon.

4. Discussion

The different type and degree of perturbation induced by heptachlor to DMPC and DMPE multilayers can be explained by differences in their packing arrangements and the structural effects of water upon them. Chemically they only differ in their terminal amino groups, being $^{+}NH_3$ in DMPE and $^{+}N(CH_3)_3$ in DMPC. Moreover, both molecular conformations are very similar in their dry crystalline phases [23]. In fact, both have their acyl chains mostly parallel and extended with the polar groups lying perpendicular to them. However, DMPE molecules pack tighter than those of DMPC. This effect, due to DMPE smaller polar group and higher effective charge, results in a very stable multilayer arrangement which is not significantly perturbed by the presence of water [24]. On the other hand, the gradual hydration of DMPC bilayers results in water occupying the highly polar interbilayer spaces. As a consequence, there is an increase in its bilayer width from about 55 Å when dry up to nearly 63 Å when fully hydrated at a temperature below that of its main transition. This situation facilitates the incorporation of heptachlor into DMPC bilayers and its penetration into the acyl chain region resulting in a profound structural perturbation of the lipid. The fluorescence spectroscopy experiments performed on DMPC large unilamellar vesicles completely confirmed these results. In fact, they showed that heptachlor indeed produced a high structural perturbation to DMPC hydrophobic acyl chain and polar head group regions.

The above results allow an interpretation about the molecular interaction of the pesticide heptachlor with the red cell membrane. According to the bilayer

Fig. 6. Scanning electron microscope (SEM) images of human erythrocytes. Control, 4000× (A); incubated with heptachlor 1 mM, 4500× (B) and 10 mM, 1600× (C).

couple hypothesis [25], the shape changes induced to erythrocytes by foreign molecules are due to a differential expansion of their two monolayers. Thus, speculated shapes (equinocytes) arise when the added compound locates into the outer monolayer, whereas cup shapes (stomatocytes) are produced when the insertion is in the inner monolayer. The fact that heptachlor produced the echinocytic shape is consistent with the results obtained from the experiments performed on phospholipid multilayers and vesicles. They clearly demonstrated that heptachlor strongly interacted only with DMPC, which is a type of phospholipid located in the external moiety of the erythrocyte membrane. Additional experiments performed on frog sympathetic synapses showed a significant decrease in the potential difference and short-circuit current responses after application of heptachlor. These results have been interpreted as an inhibition of the active transport of ions induced by the pesticide [26]. It can be concluded, therefore, that toxic effects of heptachlor are related to its perturbation of the phospholipid bilayer structure, whose integrity is essential for cell membrane functions.

Finally, the observed difference in heptachlor concentration required to induce structural changes in erythrocytes, LUV and phospholipid multilayers can be explained. In fact, whereas a concentration as low as 0.01 mM was able to affect the DMPC LUV structure, a minimum of 10 mM heptachlor was necessary to change the erythrocyte shape. The difference lay in the high cholesterol content (37 mol%) of the red cell membrane which increases its rigidity. In any case both concentrations are relevant to that of the pesticide in the environment. On the other hand, the specimens used in the X-ray experiments consisted in large aggregates of many phospholipid bilayers. Therefore, a relatively high proportion of the pesticide was required to reach and perturb their molecular arrangements.

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