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Interaction of ethylazinphos with the physical organization of model and native membranes

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Abstract

The interaction of ethylazinphos with the physical organization of model and native membranes was investigated by means of fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and of its propionic acid derivative (DPH-PA). Ethylazinphos shifts the phase transition midpoint to lower temperature values and broadens the phase transition profile of bilayers reconstituted with dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines (DMPC, DPPC, DSPC), as detected by DPH and DPH-PA. Additionally, both probes detect significant effects of ethylazinphos in the fluid phase of the above lipid bilayers. The insecticide perturbations are more pronounced in bilayers of short-chain lipids, e.g., DMPC, in correlation with the higher partition in these membranes. On the other hand, the insecticide increases to some extent the ordering promoted by cholesterol in the fluid phase of DMPC, but high cholesterol concentrations ($\geq$ 30 mol%) almost prevent insecticide interaction, as revealed by DPH and DPH-PA. In agreement with the results in models of synthetic lipids, the increase of intrinsic cholesterol in fluid native membranes depresses the partition values of ethylazinphos and consequently its effects.

Keywords: Ethylazinphos; Cholesterol; Fluorescent probe; Membrane organization; Partition coefficient

1. Introduction

Chemical insecticides have been widely used in the control of insect pests since world war II [1]. Unfortunately, non target organisms, including man himself, are also affected by these compounds [1,2]. Therefore, exact knowledge of their toxic effects is imperative, in order to design compounds with improved efficiency and biological selectivity. However, the molecular mechanisms of acute toxicity of insecticides are, in general, poorly understood. Well-defined biochemical actions have been assigned only to organophosphorus and carbamate compounds, which are powerful inhibitors of acetylcholinesterase [3,4]. Additionally, insecticides can also cause chronic toxicity [5–8] and, again, the molecular mechanisms of these effects are far from clear, in spite of research efforts in recent years.

Owing to their lipophilic character, insecticides accumulate in membrane lipid domains where they may induce physical and chemical perturbations, and, consequently, alterations of the native properties of biomembranes. Partition studies have ascertained that common insecticides, namely, parathion, malathion, lindane, DDT and DDE, incorporate in model and native membranes with partition coefficients following the order: DDT $\approx$ DDE > > lindane > parathion > malathion [9,10]. Cholesterol strongly decreases the partition, but the sequence is maintained [9,10]. Furthermore, insecticides induce perturbations of membrane permeability and enzyme dynamics [11–13]. Among these compounds, the most powerful toxicant, parathion, was also the most effective in inducing membrane perturbations. On the other hand, the least toxic, malathion, affects basic membrane mechanisms to a lesser extent. The other insecticides have intermediate effects and intermediate toxicities. Since basic membrane mechanisms are greatly influenced by the physical state of lipids [14–17], the primary effects of insecticides may be related to physi-

Abbreviations: Ethylazinphos, *O,O*-diethyl-s-[4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl]-phosphorothioato; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPH-PA, 3-(*p*-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; egg-PC, egg yolk phosphatidylcholine.

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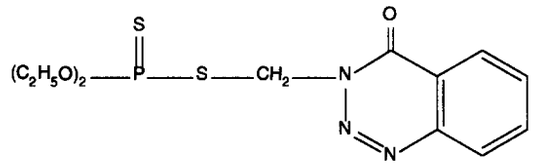

Fig. 1. Structure of ethylazinphos.

cal changes in lipid domains. Additionally, partition studies [9,10] indicate that membrane fluidity and insecticide structure are main parameters affecting insecticide incorporation. These findings and the order gradient across the width of the bilayer [18] suggest different distributions of the insecticides and, consequently, different physical effects across the bilayer thickness. Our recent work has been directed along these lines, using convenient fluorophores that probe the outer regions of the bilayer or the hydrophobic core. Thus, the effects of lindane, DDT, DDE, parathion and malathion on the fluidity of model and native membranes have been extensively studied and their localizations, along the bilayer thickness, have been tentatively suggested [19–26]. In the sequence of these studies, we now report the effects of the organophosphorus compound ethylazinphos (Fig. 1) on the physical state of model and native membranes, attempting to relate biophysical and biochemical perturbations with toxicity. To better evaluate the molecular interaction of ethylazinphos with membrane constituents, its partition has been determined.

2. Materials and methods

2.1. Materials

Cholesterol, DMPC, DPPC, DSPC, egg-PC and DPH were obtained from Sigma Chemical Co., St. Louis, MO, USA. DPH-PA was purchased from Molecular Probes, Eugene, OR, USA. Ethylazinphos (Fig. 1) was obtained from Supelco, Bellefonte, PA, USA. All these compounds were of the highest commercially available quality.

2.2. Methods

2.2.1. Preparation of membranes for fluorescence studies

Model membranes were prepared as described elsewhere [19]. Briefly, solutions of pure phospholipids in $CHCl_3$ were prepared in round bottom flasks and the solvent was evaporated to dryness. The resulting lipid film on the wall of the round-bottom flask was hydrated with an appropriate volume of 50 mM KCl, 10 mM Tris-maleate (pH 7), and dispersed under N_2 atmosphere by handshaking in a water bath 7–10°C above the transition temperature of the phospholipids. Phospholipid-cholesterol bilayers were obtained by supplementing original phospholipid solutions with appropriate amounts of cholesterol. Native membranes, namely, erythrocytes, sarcoplasmic reticulum and mitochondria were prepared as described elsewhere [27]. In all cases, the final nominal concentration of membrane lipids was 345 μM. Model and native membranes were briefly sonicated in a low energy water sonifier. This procedure does not distort the transition of lipid bilayers, but disperses aggregates, facilitating the readings of fluorescence and decreasing the scattered light. Sonication was applied for a controlled period of time to avoid the turbidity decreasing below 0.15 *A* units at 600 nm.

2.2.2. Preparation of membranes for partition studies

Multilamellar vesicles and native membranes were prepared by the methods described above, except that the buffer contained 50 mM NaCl and 10 mM Hepes (pH = 7.4). Hepes was chosen since it does not absorb in the UV range of interest, where ethylazinphos absorbs.

Large unilamellar vesicles were prepared from multilamellar vesicles by standard extrusion techniques [28]. Thirteen repeated extrusions were performed through two stacked polycarbonate membranes of 200 nm pore size. The diameters and the size distributions of vesicles were determined by dynamic light scattering using a Coulter model N4MD. The auto-correlation function was analyzed using Coulter software, applying the cumulant method and multi-exponential fit method. The average diameter of the vesicles varied between 180–230 nm, depending on the lipid used.

2.2.3. Incorporation of probes and ethylazinphos into membranes

DPH in tetrahydrofuran and DPH-PA in dimethylformamide were injected (a few μl) into membrane suspensions (345 μM in total lipid) to give a final lipid/probe molar ratio of about 200. The mixture was initially vigorously vortexed for 10 s, then ethylazinphos was added from concentrated ethanolic solutions (50 mM). It was ascertained that added concentrations of the insecticide were within the solubility range. The mixture was allowed to equilibrate in the dark for 18–20 h. The solvents by themselves (a few μl) had no detectable effects upon the assays.

At the ratio used (1:200), the probes induce minimal perturbation of the membrane bilayer geometry [29]. Therefore, data reported by these probes are considered as reliable as other data with other probes.

2.2.4. Fluorescence measurements

Fluorescence spectra were recorded in a Perkin-Elmer spectrofluorometer, model MPF-3, provided with a thermostated cell holder. The excitation was set at 336 nm and the emission at 450 nm. The excitation and emission slits were 4 and 6 nm, respectively. The temperature of the sample was checked with an accuracy of ± 0.1°C, using a thermistor thermometer. The degree of fluorescence polar-

ization (P) was calculated according to Shinitzky and Barenholz [30] from the equation:

$$P = \frac{I_{\parallel} - I_{\perp} G}{I_{\parallel} + I_{\perp} G}$$

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of the light emitted with its polarization plan parallel (||) and perpendicular ($\perp$) to that of exciting beam. A high degree of polarization represents a high structural order or low membrane fluidity. The term 'fluidity' is used here as being inversely proportional to the polarization of diphenylhexatriene probes. This fluidity is related but not identical to the physical definition of fluidity. G is the grating factor and is given by the ratio of vertically to the horizontally polarized emission components when the excitation light is polarized in the horizontal direction [31].

When data are expressed in terms of the anisotropy factor, essentially the same conclusions and relationships are achieved. To be consistent with previous articles with other insecticides [19–26], polarization has been preferred in this work.

All the fluorescence measurements were corrected for the contribution of light scattering by using controls with membranes, but without added probes.

2.2.5. Determination of partition coefficients

The partition coefficients of ethylazinphos were determined by means of derivative spectrophotometry as previously described for the anticancer drugs tamoxifen and hydroxytamoxifen [32], with appropriate modifications. The period of equilibration of ethylazinphos with membrane suspensions in buffer was 30 min, at required temperatures, before recording of spectra. The concentration of ethylazinphos was 2.5 μM. Large unilamellar vesicles have been used, since the incorporation of the insecticide into multilamellar vesicles is subject to significant kinetic effects and the true equilibrium may not be reached [33].

The present method explores a red shift in the absorption spectra of ethylazinphos when it is removed from the buffer to the lipid phase. The characteristic absorbance peaks in buffer and in the lipid are 229 nm and 232 nm, respectively. To determine ethylazinphos incorporated in the lipid phase, the second derivative spectra were recorded (Fig. 2). The overall change in absorbance (A), after addition of ethylazinphos, was measured as the vertical distance between the baseline and the minimum value of the peak of the second derivative spectrum. The height of the peak and, consequently, the second derivative absorbance (A) increases with the increase in the lipid concentration. Thus, at high lipid concentrations, A approaches its maximum (A_{max}) which corresponds to a complete incorporation of ethylazinphos. A plot of A versus lipid volume (V_L), assuming the lipid specific volume 0.984 μl/mg [34], yields a curve (Fig. 2, inset a). A_{max} was determined from a double-reciprocal plot (Fig. 2, inset b). The calculation of the amount of ethylazinphos in the membrane correspondent to each lipid concentration is $Q_L = (A/A_{max}) \cdot Q_T$, where Q_T is the total amount of added ethylazinphos.

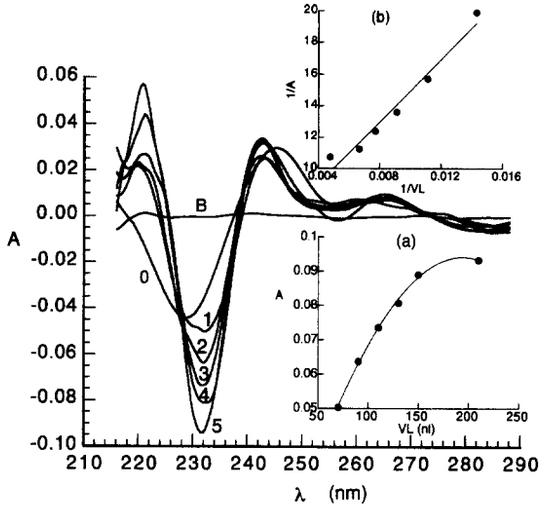

Fig. 2. Second derivative absorption spectra of ethylazinphos (2.5 μM) in sarcoplasmic reticulum membranes at different lipid concentrations. The nominal lipid concentrations of sarcoplasmic reticulum in suspensions was 0 (curve 0), 54 (1), 63 (2), 72 (3), 81 (4), 210 μM (5). Inset a depicts the relationship between the second derivative absorbance (A) and the membrane lipid volume (V_L). The double-reciprocal plot of these data is represented in inset b. The equation for the linear fit has the form of $y = 5.0631 + 991.7x$ with a correlation coefficient, $R = 0.983$ ($R^2 = 0.966$), meaning that 97% of the dependent variable variation is explained by the independent variable.

The present methodology is useful up to lipid concentrations that allow good signal to noise ratios of the second derivative signal. Lipid concentrations beyond the last useful point (outlier in inset b) are therefore avoided as a consequence of high light scattering that degrades the signal to noise ratio. As can be concluded from the correlation coefficient ($R^2 = 0.966$), 97% of $1/A$ variation is explained by $1/V_L$.

The partition coefficient is expressed by $K_p = C_L/C_W$ [35], where C_L and C_W are molar concentrations of ethylazinphos in the lipid and aqueous phases, respectively. The total amount of added ethylazinphos is $V_T \cdot C_T = V_L \cdot C_L + V_W \cdot C_W$ or $Q_T = Q_W + Q_L$, were V, C and Q state for volume, concentration and ethylazinphos amount, respectively, and the subscripts T, L and W state for total, lipid phase and aqueous phase, respectively.

Rearrangements give $K_p = (Q_L/V_L)/(Q_W/V_W)$. Replacing $Q_W = Q_T - Q_L$, it follows that $[K_p \cdot (Q_T - Q_L)]/V_W = Q_L/V_L$ or $1/V_L = [(K_p\ Q_T)/V_W] \cdot (1/Q_L) - (K_p/V_W)$ which represents a linear function of $1/V_L$ versus $1/Q_L$ with an ordinate intercept (b) given by $-K_p/V_W$ and, therefore, K_p may be calculated as: $K_p = -b \cdot V_W$.

The normal and second derivative spectra were recorded in a Perkin-Elmer Lambda 6 spectrophotometer and the automatic baseline correction mode was used for all spectra recordings.

3. Results and discussion

3.1. Pure phospholipid bilayers

The relative perturbations induced by ethylazinphos across the thickness of phospholipid bilayers were evaluated with two structural probes: DPH, which locates and probes the hydrophobic core of the membrane [30] and DPH-PA, which anchors close to the interfacial region by its charged propionic group [36] and probes the outer bilayer regions. DPH and DPH-PA polarizations report the rotational diffusion of the probes which strongly depends on the bilayer fluidity [19,25]. It should be emphasized that the probes by themselves, at the used ratios (1:200, relatively to lipids), induce minimal perturbations of the lipid geometry [29] and, therefore, can be used to report alterations promoted by xenobiotics used at much higher stoichiometric ratios relatively to bilayer phospholipids. In the present case, it was ascertained that the probes do not facilitate the interaction of ethylazinphos since the insecticide does not induce any significant effects at 10 μM, i.e., at a ratio fivefold higher relatively to the probes.

The effects of ethylazinphos (0–100 μM) on the fluorescence polarization of DPH and DPH-PA embedded into bilayers of DMPC are displayed in Fig. 3. Ethylazinphos broadens the transition profiles, i.e., expands the temperature range at which fluid and gel domains coexist and shifts the phase transition midpoint (T_m) to lower temperature values. As detected, for example, by DPH, T_m is shifted by 1.6, 2.0 and 3.2°C for insecticide concentrations of 25, 50 and 100 μM, respectively. Shifts detected by DPH-PA, although not identical, are, however, similar (Fig. 3B). Shifting and broadening of the transition profiles of pure lipid bilayers have been observed for other organophosphorus compounds, namely, parathion [25] and malathion [26]. According to Jain and Wu [37], shifting and broadening of the transition profile means a localization of the perturber in the vicinity of the cooperativity region, which regulates to a large extent the sharpness of the thermal transition. Although long distance effects of the perturber can take place in a different region from its localization, the strongest effects are certainly exerted locally. Thus, ethylazinphos possibly localizes, preferentially, somewhere close to this region of the bilayer, i.e.,

Table 1
Partition coefficients of ethylazinphos in DMPC bilayers at the midpoint temperature of thermotropic phase transition (T_m) and above and below this temperature

Type of bilayer	Temperature (°C)	Partition coefficients of ethylazinphos
DMPC	20	157.74± 87.37
	24 (= T_m)	739.80±123.86
	30	240.83± 59.10
	37	218.57± 30.95

Values are given as means (±S.D.) of four experiments.

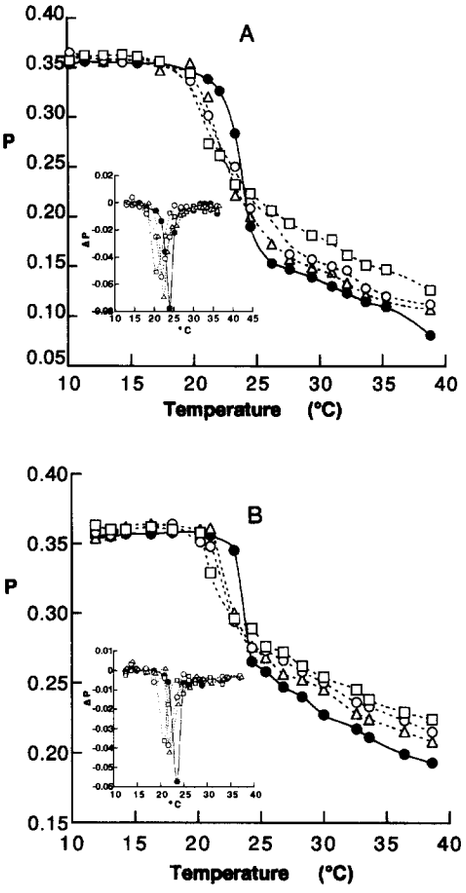

Fig. 3. Fluorescence polarization (P) of DPH (A) and DPH-PA (B) in DMPC bilayers, in the absence (solid symbols and lines) or in the presence (open symbols and dashed lines) of increasing concentrations of ethylazinphos (△, 25 μM; ○, 50 μM; □, 100 μM). These correspond to insecticide:lipid stoichiometries of 0.072, 0.145 and 0.299, respectively. As in other subsequent figures, the insets represent differential plots of the data in the main plots.

the region of C_1–C_9 atoms. Data of Fig. 3 also indicate that, for temperatures 10°C below the phase transition midpoint, DPH, as well as DPH-PA, detect a slight increase in P promoted by ethylazinphos. A significant effect is, however, detected by both probes in the fluid phase. These effects are not correlated with the insecticide partition since it is identical in the gel and fluid phases, with a maximum at the phase transition range, as illustrated in Table 1. Thus, the pronounced effects of ethylazinphos in the fluid phase are related with the bilayer physical organization. Therefore, the effects of ethylazinphos are less apparent in a condensed than in a fluid bilayer. Parathion also behaves similarly in bilayers of DMPC [25]. Furthermore, the effects of parathion, either in the gel phase or in the fluid phase, are stronger than those observed for ethylazinphos, in correlation with the higher toxicity of parathion [2]. On the other hand, the effects of malathion in the gel and fluid phases are very limited [26], in correlation with its very low partition [9] and toxicity [2].

Ethylazinphos–lipid interactions in the temperature range of the transition differ from those occurring in stabilized fluid and gel phases. As previously suggested for parathion [25], in the gel and fluid phases of the lipid, ethylazinphos reaches to a lesser extent the hydrocarbon core of the bilayer, suggesting that the outer bilayer regions are probably more important than the hydrophobic core in determining the extent of interactions. Hydrogen bonding or dipole–dipole interactions may take place between the insecticide phosphate group and the headgroups of the phospholipids with a consequent decrease in the headgroup spacing and an increase in interchain interactions. Consequently, condensing effects are detected by DPH-PA and DPH in the outer regions of the bilayer and in the hydrophobic core, respectively. The more pronounced effects of parathion [25] are certainly related with its particular structure. The insertion in the bilayer putatively occurs with reduced spacing of phospholipid headgroups resulting in increased interchain interactions. On the other hand, the strong perturbations in the transition range indicate that other interactions take place. In the transition range, the lateral density fluctuations are particularly important and structural defects between gel and fluid domains occur [38], increasing the free volume in the upper and hydrophobic region of the membrane and, consequently, the partition of the insecticide (Table 1). The phospholipid chains must conform to accommodate the larger amount of insecticide, inducing weakening of lipid–lipid interactions, resulting in a shifting of phase transition midpoint to lower temperatures.

The effects of ethylazinphos (50 μM) on the thermotropic behavior of DPPC (Fig. 4) and DSPC (Fig. 5) are qualitatively similar to those observed for DMPC. However, the effects of ethylazinphos are stronger in DMPC as compared with those in DPPC and DSPC in agreement with the higher partition coefficients in DMPC (Fig. 6). In native membranes, probably without gel phase, but composed of discrete lipid domains differing in composition and fluidity [39,40], ethylazinphos will preferentially associate with more fluid domains.

Figs. 4 and 5 also show that the pretransitions of DPPC and DSPC are removed by the insecticide, an effect similar to that of cholesterol [41], which localizes in the cooperativity region [42]. This finding further supports the hypothesis that ethylazinphos interferes with the cooperativity region abolishing the molecular changes that characterize the pretransition.

3.2. Phospholipid–cholesterol bilayers

Ethylazinphos induces limited physical perturbations in DMPC bilayers enriched with cholesterol (Fig. 7), at temperatures below the phase transition of DMPC, as detected either by DPH or DPH-PA. Above the phase transition of DMPC, both probes detect perturbations promoted by ethylazinphos, which gradually fade with the increase in

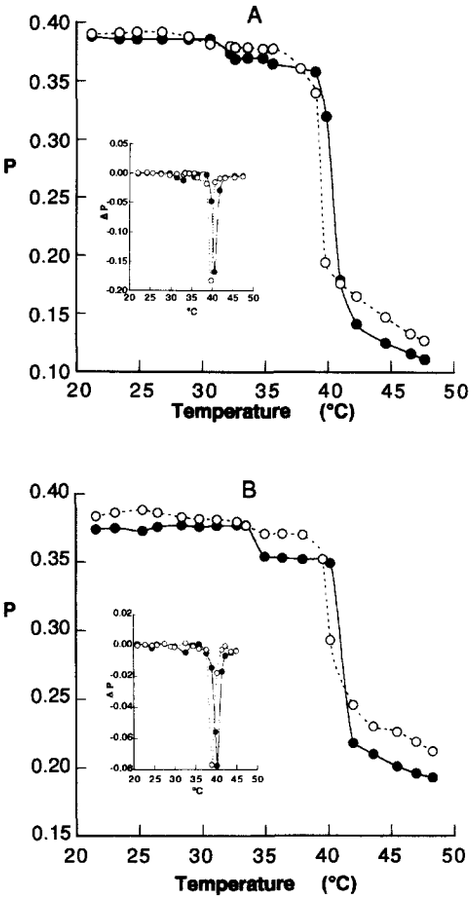

Fig. 4. Fluorescence polarization (P) of DPH (A) and DPH-PA (B) in DPPC bilayers in the absence (solid symbols and lines) or in the presence (open symbols and dashed lines) of 50 μM ethylazinphos, corresponding to a molar stoichiometry of 0.145 relative to lipids.

cholesterol concentration. Thus, for high cholesterol concentrations ($\geq$ 30 mol%) the effects practically vanish, as illustrated in Fig. 7. Identical but stronger effects have been described, previously, for parathion [25]. On the other hand, the effects promoted by the organophosphorus compound malathion are weaker [26] than those of ethylazinphos in correlation with its low partition [9] and toxicity [2]. Previous partition studies [9] also indicate that cholesterol determines to a large extent insecticide incorporation. Therefore, the partitioning of parathion, malathion and lindane, in egg-PC membranes, at 24°C, decreases linearly with the increase of cholesterol content, and a complete exclusion of the insecticides is observed for cholesterol concentrations of about 50 mol% [9]. Cholesterol also depresses the partitioning of ethylazinphos (Fig. 6), and the results of Fig. 7 suggest a strong exclusion of the insecticide from the bilayer for high cholesterol concentrations ($\geq$ 30 mol%), since a 55% exclusion is already observed at 10 mol% cholesterol (Fig. 6).

The present (Fig. 7) and previous studies [19,20,25,26] suggest that cholesterol changes the membrane organization in such a way that the free volume for insecticide

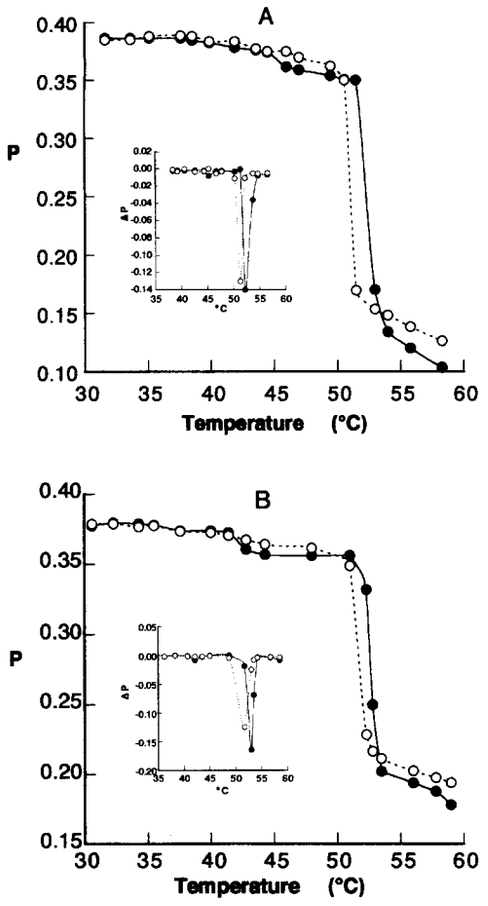

Fig. 5. Fluorescence polarization (P) of DPH (A) and DPH-PA (B) in DSPC bilayers in the absence (solid symbols and lines) or in the presence (open symbols and dashed lines) of 50 μM ethylazinphos, i.e., an insecticide/lipid stoichiometry of 0.145.

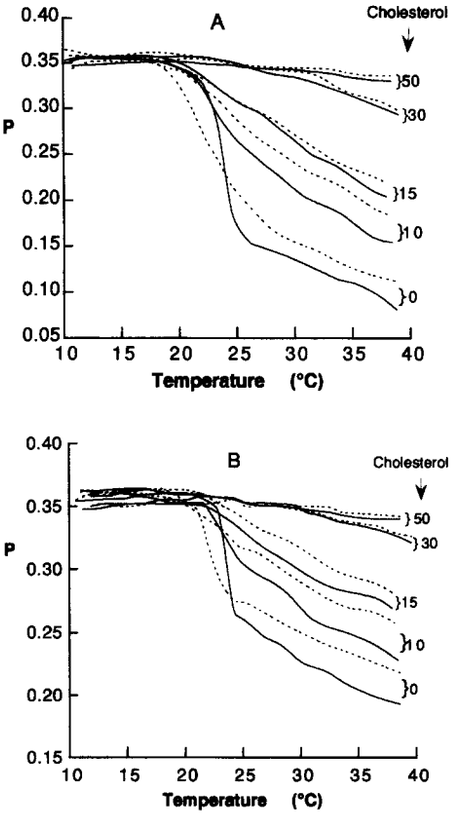

Fig. 7. Fluorescence polarization (P) of DPH (A) and DPH-PA (B) in DMPC/cholesterol bilayers, in the absence (solid lines) and presence (dashed lines) of 50 μM ethylazinphos (0.145 stoichiometry relative to lipids). The numbers adjacent to the curves represent mol% of cholesterol incorporated in DMPC bilayers. Each curve was drawn across 15–17 experimental points which were removed for the sake of clarity.

incorporation and interaction decreases, since insecticide and cholesterol compete for similar sites in the bilayer. On the basis of theoretical and experimental approaches [43,44], the way by which cholesterol affects the physical state of lipids and, consequently, the interaction of the insecticides with the bilayer, can be discussed as follows. Fluid phospholipid membranes can exist in a liquid-disordered phase, at very low cholesterol concentrations, a liquid-ordered phase, at high cholesterol concentrations, or the two phases, at intermediate sterol concentrations. Consequently, the liquid-ordered phase induced by the packing of cholesterol with lipids in the cooperativity region [42] would prevent insecticide incorporation and interaction. Only, the liquid-disordered phases, with low cholesterol, allow the interaction of the insecticide. Ethylazinphos, as well parathion [25] and malathion [26], upon interaction with these cholesterol-poor (or free) regions potentiate the condensing effect of cholesterol. Apparently, this is a general characteristic of organophosphorus compounds that may interact with the polar region of the phospholipids [25,26].

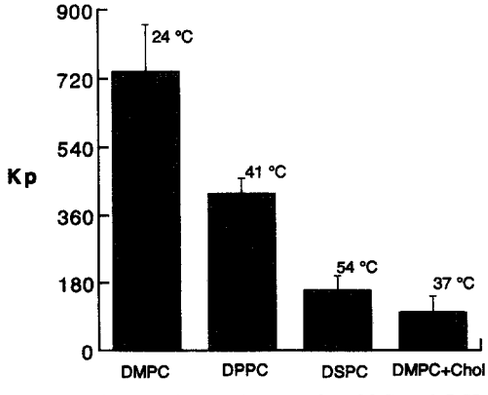

Fig. 6. Partition of ethylazinphos in DMPC, DPPC and DSPC at the midpoint temperature of the thermotropic phase transition, i.e., at 24, 41 and 54°C, respectively. Also represented the partitioning of the insecticide in DMPC bilayers with 10 mol% cholesterol, at 37°C. Error bars represent standard deviations for four independent experiments.

3.3. Native membranes

Studies of ethylazinphos were also extended to native membranes to gather pertinent information with biochemical significance. Several representative native membranes,

differing in intrinsic cholesterol, were chosen, namely, mitochondria, (mostly inner membrane), sarcoplasmic reticulum and erythrocytes. Although most native membranes are in the fluid state [45], over the temperature range of our studies (0 to 40°C), the degree of fluidity depends on the temperature and on cholesterol content (Fig. 8). Therefore, membranes of mitochondria almost devoid of cholesterol are relatively more fluid than those of sarcoplasmic reticulum with 6 mol% of cholesterol; in turn, these are significantly more fluid than erythrocyte membranes, where cholesterol accounts for 37 mol%. Thus, according to classical observations [46], the degree of order in fluid membranes [45] increases with cholesterol, as revealed by DPH and DPH-PA (Fig. 8, solid lines). The effects of ethylazinphos, over the temperature range from 10 to 40°C, are qualitatively similar to those described for models in the fluid state (Fig. 7), since the effects of ethylazinphos fade out with the increase in intrinsic cholesterol (Fig. 8, dashed lines). Partitioning of ethylazinphos also decreases with the increase of relative content of cholesterol (Fig. 9), similar to the observations in model membranes. Possibly as consequence of low partition values and high light scattering of erythrocyte ghosts, reliable data could not be obtained with this material. According to data in model membranes, cholesterol-poor membranes, e.g. mitochondria and sarcoplasmic reticulum, may be more vulnerable to the interaction of ethylazinphos. Consequently, these highly functional membranes may be putative target sites for insecticide action. Similar conclusions have been previously drawn for parathion [25] and malathion [26]. However, the perturbations induced by the organophosphorus compounds either in native membranes or in the models follows a sequence in correlation with their effects in membrane linked functions [11–13] and with their toxicities [2]: parathion > ethylazinphos > > malathion.

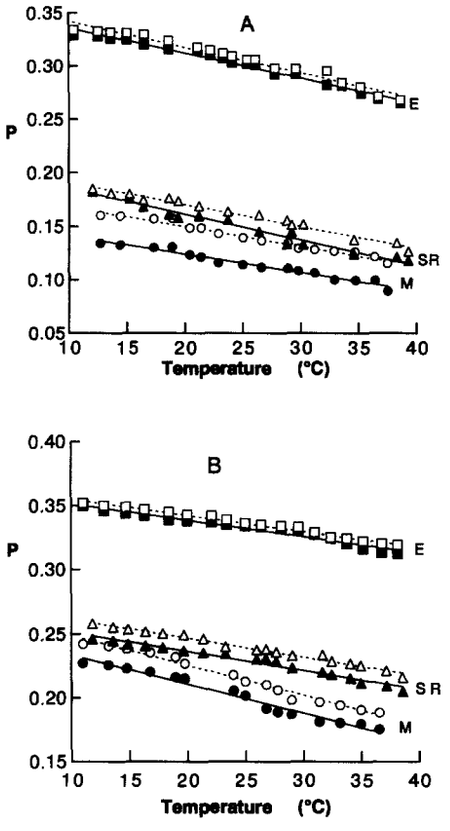

Fig. 8. Fluorescence polarization (P) of DPH (A) and DPH-PA (B) in several native membranes differing in intrinsic cholesterol, as a function of temperature, in the absence (solid symbols and lines) and in the presence (open symbols and dashed lines) of 50 μM ethylazinphos (insecticide:lipid stoichiometry of 0.145). Cholesterol/phospholipid molar ratios for mitochondria (M), sarcoplasmic reticulum (SR) and erythrocytes (E) are 0, 6 and 37 mol%, respectively. Regression lines were calculated by means of the least squares method. The correlation coefficients vary from −0.978 to −0.999. As in other previous figures, error bars are not represented, since for most experimental points they are encompassed by the size of the symbols.

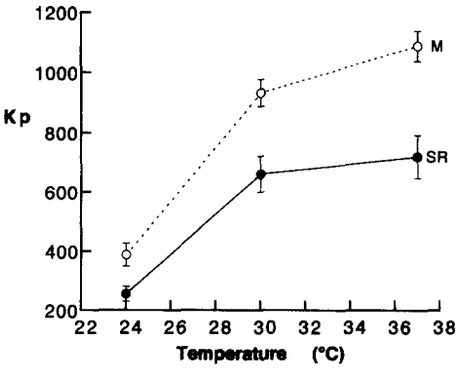

Fig. 9. Partition of ethylazinphos into fluid native membranes of mitochondria (M) and sarcoplasmic reticulum (SR), as a function of temperature. Each point represents the average of at least three independent measurements (vertical lines indicate ±S.D.).

3.4. Concluding remarks

The reported data may be particularly useful in any studies of xenobiotic kinetics and dynamics pertinent to the toxicology of ethylazinphos.

The present data for ethylazinphos and previous data for parathion [25] and malathion [26] suggest that these organophosphorus compounds and, possibly, the organophosphorus insecticides in general interact with the cooperativity region and the bilayer surface by alignment of the molecular structures containing a hydrophobic portion and a polar phosphate group. These interactions promote physical perturbations sensed across all the thickness of the bilayer, mainly at the outer regions. These physical perturbations may significantly affect membrane permeability [11,12] and protein functions [13,47–51]. Additionally, protein functions can be affected by interaction of the phosphate group of the insecticide with enzyme sites located at the membrane surface, as in the case of acetylcholinesterase [3,4].

Acknowledgements

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