Biochemical Pharmacology, Vol. 33, No. 14, pp. 2227–2230, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

INTERACTION OF THE CHLORINATED HYDROCARBON INSECTICIDE LINDANE OR DDT WITH LIPIDS—A DIFFERENTIAL SCANNING CALORIMETRY STUDY

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(*Received* 18 *October* 1983; *accepted* 4 *February* 1984)

Abstract—The interaction of DDT and lindane with glycosphingolipids and phospholipids was investigated by employing differential scanning calorimetry. The degree of perturbation produced by lindane is stronger than that of DDT and depends also on the lipid.

Interaction of chlorinated hydrocarbons with model and natural membranes was investigated extensively, in an attempt to elucidate the toxic effects of these compounds. Inasmuch as lipophilic compounds accumulate in the lipidic phase of the membrane en route to their target sites, they might exert an effect on the structure and function of the biomembranes. In natural membranes it was shown that DDT† or lindane influence various membrane-linked functions [1–7].

In model systems it was shown that chlorinated hydrocarbons bind to phospholipids [9–11], increase the permeability of liposomes to non electrolytes and modify the transport of cations mediated by ionophores [4, 14]. However, in spite of the implication in some reports that at least part of the toxic effects of insecticides could be due to the perturbation of the lipid phase of the membranes [2, 6] very little work was done on the influence of these compounds on the thermotropic properties of lipids and on the possible changes of lipid fluidity due to interaction. To our knowledge only two studies dealing with this aspect were reported [12, 13], demonstrating the perturbing effect of chlorinated hydrocarbons on liposomes from synthetic lipids (mainly DPL). With the use of fluorescent probes Buff and Berndt [12] found that DDT lowered the phase transition temperature and broadened the transition range of the phospholipids. Packham *et al.* [13] by employing DSC observed similar effects of DDT on the thermotropic behaviour of DPL.

The aim of the present work was to investigate the influence of two chlorinated hydrocarbons γ-lindane and DDT that differ considerably in both the structure and molecular size on the thermotropic properties of natural lipids. The lipids included in the present study were the glycosphingolipids galactocerebroside and ganglioside-GM_1, and the phospholipids phosphatidyl serine and dipalmitoyl lecithin. The results obtained show that both the structure of the chlorinated hydrocarbon and the structure of the lipid influence the interaction.

MATERIALS AND METHODS

Galactocerebroside (bovine brain), γ-lindane and DDT (Pesticide standard >98% pure) were purchased from Supelco Bellefonte, PA, USA. Phosphatidyl serine from bovine spinal cord, grade I monosodium salt was from Lipid Products, Nutfield, England, dipalmitoyl lecithin was from Dr. Berchtold Lab., Bern, Switzerland, GM_1 was isolated from bovine brain according to procedures published previously [15].

The chlorinated hydrocarbons were dissolved in methanol at a concentration of about 3 mg/ml. Appropriate volumes of the hydrocarbon solution were added to the lipid solution (in 2:1 chloroform:methanol or 1:1 chloroform:methanol) were mixed together, the solvents were driven off by a stream of nitrogen and the samples were kept under high vaccum for 3 hr. After an overnight storage at 4° the dry material (1–2.5 mg) was weighed into the aluminum pans of the instrument and an excess (at least three-fold) of salt solution 1.5×10^{-1} N NaCl in 10^{-2} N Tris. HCl buffer pH 7.3 was added and the pans were sealed. The lipid dispersions were incubated for 1 hr at temperatures above phase transition (DPL, 70°; PS, 50°, cerebroside, 80°; GM_1, 55°) and followed by DSC measurements. The calorimetric measurements were performed on a Du Pont 990 differential scanning calorimeter [19].

RESULTS AND DISCUSSION

DSC was used extensively for studying phase transitions of lipids and membranes and the influence of various drugs and modifiers on the thermotropic properties [16]. In the present work DSC was employed to investigate the interaction of chlorinated hydrocarbons with various lipids. γ-Lindane is a chlorinated cyclohexane, while DDT is an aromatic molecule comprised of two benzene rings. The two

† Abbreviations—DSC, differential scanning calorimetry; DPL, dipalmitoyl lecithin; PS, phosphatidyl serine; GM_1, galactosyl-*N*-acetylgalactosaminyl-(*N*-acetyl-neuraminyl) galactosylglucosylceramide; γ-lindane, hexachlorocyclohexane; DDT, 1,1,1-trichloro-2,2 bis (p-chlorophenyl) ethane.

compounds differ in the degree of their toxicity: human fatal dose of lindane is 150 mg/kg body and that of DDT is 500 mg/kg body.

Interaction with galactocerebroside. In Fig. 1 the thermograms of galactocerebroside interacting with lindane and DDT are presented. The traces A on both graphs are those of the pure lipid and its thermotropic properties (Table 1) are as published [17]. Progressive addition of the insecticides brings about a broadening of the transition peaks, a decrease in the melting temperature T_c, and a shift in the temperature of the maximum heat flow T_m with almost no change in the enthalpy of melting (Table 1). Moreover, the effects of lindane are much stronger than those of DDT at the same concentration (Fig. 1a, Table 1) and are more concentration-dependent, than those of DDT. The width of the peaks and the shift of T_m, increase with the concentration of lindane, shoulders or peaks appear at the higher concentrations employed (traces C and D) indicating phase separation of the lipid–insecticide complexes.

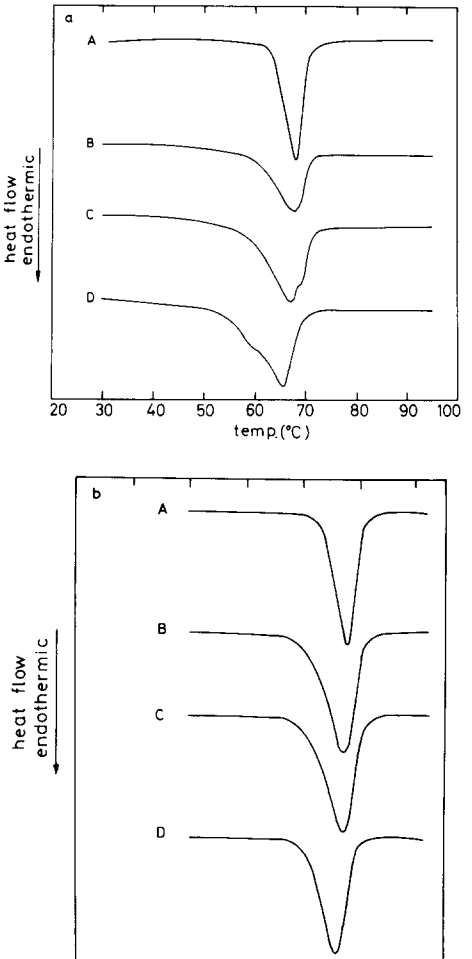

Fig. 1. Thermograms of galactocerebroside interacting with insecticides:

(a) lindane,	(b) DDT
A – lipid only – 1.3 mg	A – lipid only – 1.3 mg
B + 4.2 mole% – 2.6 mg	B + 5.6 mole% – 1.9 mg
C + 9.3 mole% + 2.0 mg	C + 12.1 mole% – 1.9 mg
D + 16.9 mole% – 1.8 mg	D + 21.9 mole% – 1.7 mg

sensitivity 0.1 milical/sec per inch except for a–B 0.2 milical/sec per inch, scan rate 5°/min.

Galactocerebroside is a neutral glycosphingolipid abundant in brain. The non polar molecules DDT or lindane are probably accumulating in the hydrophobic core of this lipid, thus decreasing the cooperativity of melting (broadening of the peak), and destabilizing the packing-downward shift of T_m. However, no significant change in the enthalpy of melting was apparent indicating that the insecticides do not affect the interactions between individual molecules.

Interaction with GM_1. Figure 2 presents the thermograms of the ganglioside GM_1 alone and interacting with lindane. The thermotropic behaviour of gangliosides is very complex as the peak is composed of at least two overlapping endotherms centered at about 15 and 35° [18, 19] and Fig. 2(A). Interaction with lindane causes a downward shift of T_m of the 35° peak (Fig. 2B and C). However, the main effect of interaction is a 40% decrease in the enthalpy of melting. The decrease is almost the same at the two concentrations of lindane investigated: 10 and 20 mole% which might suggest that the limiting solubility of lindane in the GM_1 gangliosides is around 10 mole%. Gangliosides in aqueous solutions form micelles, with very low critical micelle concentration [20], the apolar molecule lindane penetrating the hydrophobic core disturbs the structure and removes some molecules from melting.

Interaction with phosphatidyl serine. Phosphatidyl serine is a negatively charged glycerophospholipid, heterogenic with respect to the fatty acyl residues resulting in a broad peak, Fig. 3(A), so that no attempt was undertaken to measure the transition half width (Table 1). As the phase transition temperature of the phospholipid is low, the scanning was started at −10° the lowest temperature possible, without interference from the water melting. The effect of DDT on the thermograms of PS is very small, with a shift in T_m of about 1°, independent of the concentration of the insecticide indicating very limited solubility of the DDT in the lipid phase. On the other hand, lindane exerts stronger perturbing effect (Table 1, Fig. 3), the shift in T_m is concentration-dependent reaching about 5° in the case of the highest concentration of lindane employed (~20 mole%). Concomitantly a decrease in ΔH is detected amounting to about 60% at ~20 mole%, but it is possible that at least part of the decrease of ΔH results from the shift of the melting peak below 0° and therefore becoming undetected. Lindane perturbs the phosphatidyl serine bilayers by influencing the packing of the hydrocarbon chains (downward shift of T_m) and removing at least some of the molecules from melting (decrease of ΔH).

Interaction with dipalmitoyl lecithin. Dipalmitoyl lecithin is a synthetic lipid, with two equal fatty acyl residues. The phospholipid undergoes melting with high cooperativity (sharp peak) and its thermotropic properties are well established [16]. The interaction with DDT causes a downward shift of T_m, broadens the peak (the maximal transition half width is ~4.5°

Table 1. Summary of DSC data

Lipid	Insecticide	Insecticide (mole%)	T_c (°)	T_m (°)	Transition (half-width, °)	ΔH (kcal/mole)
Galactocerebroside	–	–	63.9	68.4	3.5	7.1[a]
Galactocerebroside	lindane	4.2	60.5	68	5.7	7.3
Galactocerebroside	lindane	11.6	58.8	66.4	7.0	6.8
Galactocerebroside	lindane	19.2	53.5;57.5	64.3	7.0	6.9
Galactocerebroside	DDT	5.6	61.4	67.7	5.3	7.4
Galactocerebroside	DDT	12.1	59.9	66.8	5.5	7.3
Galactocerebroside	DDT	21-9	60.3	66.3	5.7	6.7
Phosphatidyl serine	–	–	3.8	13.9	–	4.7[b]
Phosphatidyl serine	lindane	5.0	3.2	12.5	–	3.9
Phosphatidyl serine	lindane	11.0	4.1	11.5	–	2.9
Phosphatidyl serine	lindane	20.0	4.3	8.6	–	2.1
Phosphatidyl serine	DDT	5.6	4.0	12.9	–	3.7
Phosphatidyl serine	DDT	12.5	3.5	12.8	–	3.7
Phosphatidyl serine	DDT	21.8	3.6	12.7	–	4.6
DPL	–	–	40.7	42.0	1.5	10.9[c]
DPL	lindane	4.7	37.7	40.8	2.9	9.2
DPL	lindane	10.0	32.5;35.5	39.7	4.5	8.8
DPL	lindane	18.1	25.5;29	36.5	11	8.9
DPL	DDT	4.9	37.4	41.3	3.7	9.3
DPL	DDT	10.7	36.0	38.8	3.6	10.7
DPL	DDT	19.5	35.7	38.7	4.3	10.4

Mean of two or three independent experiments: (a) assuming molecular weight 800; (b) assuming molecular weight 750; (c) pretransition included.

at 20 mole%); however, almost no change in ΔH is detected (Table 1).

These data agree qualitatively with the results of Packham *et al.* [13]. However, the broadening of the DPL peak seen in this study is much smaller than the one reported by Packham *et al.* [13], also in our thermograms no shoulders on the main peak are seen. In the two investigations the pretransition appearing in pure DPL ~37° disappears upon addition of about 5 mole% DDT; however, in our study a very small peak is seen at ~30°, this peak is probably not due to a shifted pretransition as its size increases with the increase of DDT concentration. The small peak results probably from DPL–DDT rich phase.

Buff and Berndt [12] investigated the interaction

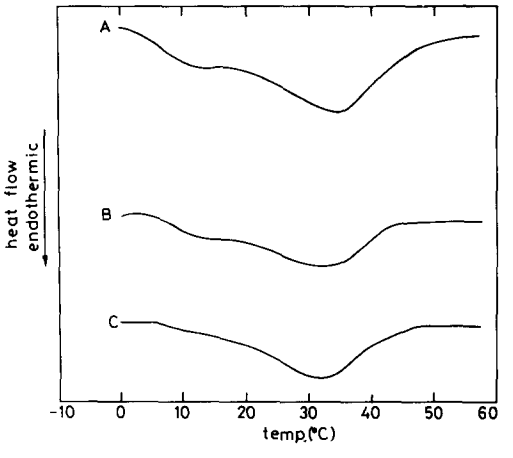

Fig. 2. Thermograms of gangliosides interacting with lindane:
A – lipid only – 1.5 mg
B + 10.7 mole% lindane – 1.4 mg
C + 21.9 mole% lindane – 1.2 mg
sensitivity 0.01 milical/sec per inch, scan rate 5°/min.

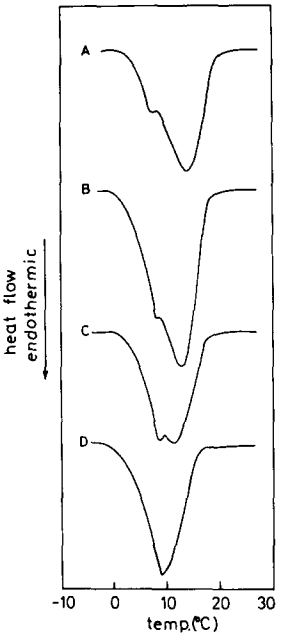

Fig. 3. Thermograms of phosphatidyl serine interacting with lindane:
A – lipid only – 0.8 mg
B + 5.0 mole% – 1.4 mg
C + 11.0 mole% – 1.3 mg
D + 20.0 mole% – 1.6 mg
sensitivity 0.02 milical/sec per inch except for A – 0.04 milical/sec per inch, scan rate 5°/min.

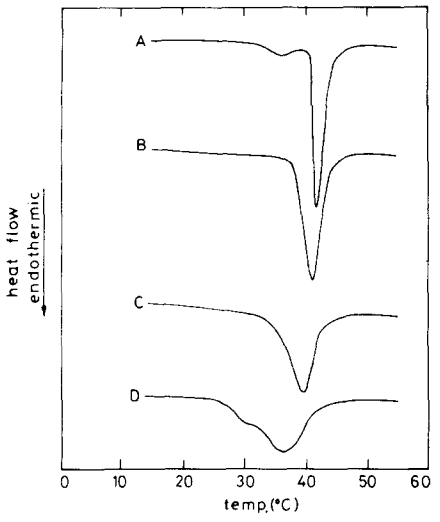

Fig. 4. Thermograms of dipalmitoyl lecithin interacting with lindane:
A – lipid only – 1.3 mg
B + 4.6 mole% – 1.6 mg
C + 10 mole% – 1.6 mg
D + 18.1 mole% – 1.9 mg
sensitivity 0.1 milical/sec per inch, scan rate 5°/min.

of DDT with DPL by employing fluorescent probes. They detected even smaller effect of DDT on the thermotropic properties of DPL than the one found in this study.

As in the case of other lipids investigated in the present study, lindane has much stronger perturbing effect on DPL than DDT (Fig. 4 and Table 1). The width of the peak increases by about 10° (at 20 mole% lindane); at 10 mole% a shoulder appears on the main peak turning into a new peak at higher concentrations, with another shoulder at still lower temperatures—indicating coexistence of phases containing various concentrations of lindane. When the experiment was performed after keeping the samples at 4° overnight (below the melting temperature of the lipid) multiple peaks disappeared and the total effect corresponded to that produced by 5–10 mole% lindane suggesting that this is the highest solubility of lindane in the gel phase of DPL. The saturating concentration of lindane in the gel and liquid phases of DPL as estimated from DSC measurements agree with those calculated by Omann and Lakowicz [11] from fluorescence quenching. Omann and Lakowicz's [11] data show also lower values for the saturating concentrations of DDT and this limiting concentration is almost independent of the state of the lipid.

CONCLUSIONS AND GENERAL DISCUSSION

In the present study we have shown that chlorinated hydrocarbons perturb the structure of the bilayer changing the ratio between the gel and liquid crystalline states of the lipid. The modified lipid phase might influence the microenvironment of the proteins, as recently it was shown that at least in some membranes crystalline lipid exists around physiological temperatures [16, 21, 22]. The degree of perturbation depends on the type of the lipid and on the type of the chlorinated hydrocarbon being higher for the small molecule lindane than this of the bigger molecule DDT. A question arises as to how the findings of the present investigation can be correlated with the toxic effects of the chlorinated hydrocarbons. The lowest concentration of DDT used (4 mole%) corresponds approximately to the concentration producing fatal dose of this compound, whereas the concentration of lindane employed is higher. With respect to the concentrations employed two points should be taken into consideration: (i) the chlorinated hydrocarbons are bioaccumulative, (ii) the effective local concentration at some specific site (e.g. nervous system) can be much higher.

The results presented in this study show that at least part of the toxicity of the chlorinated hydrocarbons is due to the interaction of these compounds with the lipids of the membranes perturbing their structure and influencing the ratio between the crystalline and liquid crystalline states.

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