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Mechanisms of interaction of pesticides with the lipid bilayer in cell membranes

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Abstract. The available information about the mechanisms of the interaction of pesticides having different chemical structures with the lipid bilayer in cell membranes is presented. It is shown that, depending on the degree of lipophilicity, the molecular dipole moment, and certain other characteristics of the pesticide compound, its interaction with the lipid bilayer generally takes place via one of the three main mechanisms involving the incorporation of the pesticide molecules in the hydrocarbon region within the bilayer, the adsorption of these molecules in the zone of the polar membrane phospholipids, or the incorporation of the amphiphilic pesticide compounds in both the non-polar and polar regions of the membrane. The bibliography includes 109 references.

I. Introduction

Various mechanisms of the action of pesticidal preparations on organisms are known at the present time. The principal site acted upon by pesticides is the cell membrane system. Pesticides can influence both the structure and the functions of membranes and are capable of damaging membrane systems directly (as a result of the physicochemical interaction with them) or indirectly (as a result of the physicochemical interaction with them) or indirectly (as a result of the blocking reactions involving the biosynthesis of specific membrane components).[1–5]

The direct interaction of pesticides with biological membranes is achieved as a result of the binding of the preparations to some type of component of the membranes, usually to the membrane proteins or lipids. The mechanisms of such interaction of pesticides with the lipid bilayer in cell membranes are considered in the present review.

The structural organisation and the principal functions of the lipid bilayer in cell membranes are fundamentally the same regardless of the purpose of the membranes. Therefore the nature of the direct interaction of pesticides with the lipid matrix of the membranes is as a rule independent of the particular membrane (plasma, chloroplast, mitochondrial, or some other membrane) with which the preparation interacts and is determined exclusively by the physicochemical properties as well as the overall types of organisation of lipids in the membranes.

Among the wide variety of the physicochemical properties of a pesticidal preparation, the nature of its interaction with the lipid bilayer is influenced decisively by its lipophilicity, which is usually characterised in terms of the partition coefficient of the pesticide between 1-octanol and water (K_{ow}). According to the value of K_{ow}, it is possible to differentiate three groups of pesticides — lipophilic, amphiphilic, and hydrophilic (Table 1).

The preparations of each group usually interact with the lipid bilayer via their own mechanism, which differs from the mechanisms of the interaction of the preparations of the other groups with the bilayer.

Table 1. The coefficients K_{ow} for certain pesticides.

Preparation	K_{ow}	Ref.
Lipophilic preparations		
Aldrin	7.5	6
Permethrin	7.44	7
Dieldrin	6.2	6
DDT	6.19	8
PCP† (undissociated form)	5.01	9
Methoxychlor	4.93	8
Parathion	3.761	10
Lindane	3.72	11
Dinoseb (undissociated form)	3.56	12
2,4-D (undissociated form)	2.90	13
Chlordime form	2.894	10
Malathion	2.838	10
Diurone	2.68	14
Metribuzin	1.70	14
Amphiphilic preparations		
Dodine	1.15	13
Benzyldimethyldecylammonium chloride	−0.08	13
Hydrophilic preparations		
2,4-D (dissociated form)	−0.24	13
Maleic hydrazide	−0.63	13

†Pentachlorophenol (translator).

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The nature of the interaction of the pesticide with the lipid matrix of the membranes depends not only on the lipophilicity of the preparation but also on its molecular properties, in particular on the molecular dipole moment, the size and shape of the molecule, and other steric factors.

II. Incorporation of pesticide molecules into the hydrocarbon region of bilayer lipid membranes

Most of the pesticide preparations are characterised by a high lipophilicity. Therefore the commonest way in which they interact with the lipids of cell membranes involves the incorporation of pesticide molecules into the hydrocarbon region of the lipid bilayer. It has been established that many organochlorine, organophosphorus, and pyrethroid insecticides, herbicides (derivatives of phenylureas), imidazole fungicides, and certain other compounds are incorporated into the non-polar region of the bilayer lipid membranes (BLM) (Table 2).

One of the most thoroughly investigated lipophilic pesticides is the organochlorine insecticide DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane]. DDT is known to be capable of accumulating in living organisms for a long time. The principal objects in which insecticides accumulate are the lipid systems of the organism, in the first place the lipid-rich cell membranes.[42-44] This has been convincingly demonstrated by experiments both *in vivo* and *in vitro*. Studies on models of lipid systems have confirmed the hypothesis that DDT accumulates in the lipid matrix of cell membranes.[11, 15, 16, 45, 46]

Experimental determination of the localisation of DDT in biological membranes have shown that the preparation is present in the inner hydrophobic component of the lipid bilayer. Both DDT and its analogues DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] and DDA [bis(*p*-chlorophenyl)acetic acid] give rise to distinct proton magnetic resonance (^{1}H NMR) spectra in CCl_4. They also have a distinct ^{1}H NMR spectrum in a suspension of lecithin liposomes in D_2O. On the other hand, in the absence of liposomes, the peak corresponding to the protons of the aromatic ring of chlorohydrocarbons in an aqueous medium was not observed.[17] The most distinct spectrum has been observed for DDA. The appearance in the presence of liposomes of signals in the high-resolution spectra, which strongly resemble the spectrum in CCl_4, shows that DDT and its analogues are incorporated into the lipid bilayer in such a way that the aromatic rings of their molecules are in a medium resembling CCl_4. Such a medium is evidently the liquid crystal phase within the bilayer. On the other hand, some inconsistency between the chemical shifts of the ring protons in CCl_4 and in a suspension of liposomes is evidence of the non-identity of the local micro-environment of the aromatic ring of the DDT molecules in the lipid and in the organic solvent.

Table 2. Examples of the incorporation of pesticidal compounds into various regions of the lipid bilayer.

Region of bilayer	Preparation	Ref.
Hydrocarbon region	DDT	15, 16, 11, 17
	Methoxychlor	16, 11
	Lindane	18, 19, 20, 11
	Parathion	21
	Malathion	22
	Diurone	23
	Permethrin	24
Region of polar groups	2,4-D (undissociated form)	25
	PCP (dissociated form)	26
	Triforine	27, 28
	Phytone	29
Polar and non-polar regions	Dodine	30-32
	Glyodin	33, 34
	Benzylalkylammonium chlorides	35, 36, 37, 38, 39, 40, 41

The incorporation of DDT into phosphatidylcholine liposomes containing carbazole-labelled phospholipids — β-[11-(9-carbazolyl)undecanoyl]-L-α-phosphatidylcholine and β-[3-(9-carbazolyl)propionyl]-L-α-phosphatidylcholine — as fluorescent probes led to the intense quenching of the fluorescence of the probes.[11] DDT and certain other chlorinated hydrocarbons are known to quench effectively the fluorescence of *N*-ethylcarbazole in homogeneous solutions.[20] Therefore the quenching, observed in liposomes, of the fluorescence of phosphatidylcholines containing a fluorescent carbazole fragment in the aliphatic acid chain must be interpreted as the result of the incorporation of the molecules of the quenching agent into the region of the hydrocarbon chains of the membrane lipids. Methoxychlor and DDE interact similarly with the lipid bilayer.[11, 47]

Yet another indication of the localisation of DDT in the hydrophobic part of the lipid bilayer is the decrease in the polarisation of the fluorescence of 12-(9-anthroyloxy)palimitic acid incorporated into lecithin liposomes in the presence of the insecticide, because in this case too the fluorophor is in the region of the hydrocarbon chains.[48]

There exist different views concerning the possible location of DDT within the hydrocarbon chains. For example, the displacement of DDT by cholesterol from the membranes of liposomes is regarded as an argument in support of the disposition of the extended DDT molecules alongside the planar cholesterol molecules.[15] Experiments with lecithin monolayers without cholesterol have shown that, for a 1:1 DDT:lecithin molar ratio, the minimum area occupied by the lecithin molecule in the monolayer increases from 50.8 Å (in the absence of the insecticide) to 56.3 Å, which is exactly equal to the minimum cross-sectional area of the DDT molecule.[49] This result permits the conclusion that DDT may form 1:1 complexes with lecithin as a result of the deposition of DDT molecules layer by layer on the surface of the lipid monolayer. One cannot rule out the possibility that the DDT molecules in the bilayer membrane can also be arranged in the vicinity of the ends of the aliphatic acid chains of the membrane lipids and occupy the space within the centre of the bilayer.

Finally, experiments designed to investigate the interaction of DDT with lecithin in CCl_4 by ^{1}H NMR spectroscopy have shown that lecithin causes an appreciable shift and broadening of the signal corresponding to the proton of the diphenylmethyl group of DDT and the latter in its turn appreciably alters the positions of the proton resonances of the choline group of lecithin.[50] These data are interpreted as an indication of the binding of the proton of the diphenylmethyl group of DDT (the acidity of which is due to the presence in the insecticide molecule of five chlorine atoms) to the electronegative oxygen atom of the phosphate group of lecithin. The formation of similar complexes with lecithin has been observed also in the study of analogues of DDT such as methoxychlor, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane, and DDA.[51]

The possibility of the formation of complexes of this kind by DDT owing to the presence of the highly polar H-C-C-Cl group has been confirmed in experiments with a series of inorganic compounds,[52, 53] but the significance of these structures in biochemical processes remains controversial. Apparently, the formation of complexes in organic solvents is consistent with the ability of the DDT molecules to assume an arrangement in membranes such that the proton of the diphenylmethyl group is close to the phosphate group of the lecithin molecule, while the benzene rings of the insecticide molecule are 'immersed' in the hydrocarbon region of the bilayer. Such location of the insecticide molecules near the polar groups of the lipid molecules, while most of the insecticide molecule is still in the region of hydrocarbon chains, appears extremely likely for the more polar compounds of the DDT series, for example for dicofol.[54, 55]

A similar mechanism of the interaction with lecithin based on ^{1}H NMR data has been proposed also for the vinyl organophosphorus insecticides dichlorofos and azodrin.[56] It has been suggested tentatively that the insecticide–dipalmitoyllecithin complex is formed as a result of the interaction of the acid proton of the vinyl group of the insecticide molecule with the oxygen anion of the phospholipid molecule. However, as in the case of DDT, the toxicological importance of this interaction is not clear.

However, evidently the ability to form hydrophobic complexes with lecithin is characteristic also of imidazole antibiotics — miconazole and chlotrimazole, which are effective fungicides.[57] When unsaturated lecithins are added to a suspension of the fungi *Candida albicans*, which are sensitive to imidazole preparations, the fungicidal effect of both antibiotics virtually disappears. Saturated lecithins do not exhibit a similar protective action. Furthermore, it has been reported that these imidazole fungicides interact preferentially with liposomes consisting of unsaturated phospholipids.[58] These data as well as analysis of the UV spectra of the fungicides in the presence of lecithin indicate the possibility of the formation of miconazole and chlotrimazole complexes with unsaturated lecithin. The inter-action, responsible for the formation of complexes of this type probably leads to the incorporation of the molecules of the above antibiotics into the hydrophobic component of the lipid bilayer of the fungal membranes with a high content of unsaturated phospholipids.[57, 59]

The conclusion that molecules of imidazole fungicides are incorporated into the hydrocarbon component of the membrane has been confirmed by the results of conformational analysis.[60] It was observed that the molecules of the two dichlorophenyl groups of miconazole and the piperazine fragment of the molecule of kitaconazole are localised in the hydrophobic component of the lipid bilayer. On the other hand, part of the fungicide molecule (the imidazole ring of miconazole and the dichlorophenyl group of kitaconazole) are located in the region of the polar groups of the phospholipid molecules, which makes the pattern in the interaction of imidazoles with the lipid bilayer similar to the mechanism of incorporation of fungicidal surfactants into the lipid component of the membranes. The interaction of miconazole with the polar region of the cell membranes has been confirmed by the dependence of the effectiveness of its action on the permeability of lecithin liposomes and their surface charge[58] and also by the protective effect of magnesium ions when the plasma membranes of the fungi *Candida albicans* are damaged by high concentrations of miconazole. It may be that the protective effect is a consequence of the competition between Mg^{2+} and miconazole for common binding sites on the membrane.[61]

Various isomers of hexachlorocyclohexane (HCCH), namely the α-, β-, γ-, (lindane), and δ-isomers, are readily incorporated into the hydrocarbon component of cell membranes. They all effectively quench the fluorescence of carbazole-labelled phospholipids incorporated into the aliphatic acid chain liposomes.[11, 19, 20] Fluorescence data make it possible to estimate the diffusion coefficient of the quenching agent in the membranes of the liposomes, which, for example, in the case of lindane, is on average 10^{-6} cm^2 s^{-1}.[19] Comparison of this coefficient with the diffusion coefficients of other molecules in the hydrophobic part of the membranes (about 5×10^{-8} cm^2 s^{-1} for phospholipids, 10^{-7} cm^2 s^{-1} for pyrene, and 8×10^{-6} cm^2 s^{-1} for oxygen) confirms that lindane is localised in the hydrocarbon part of the lipid bilayer. The diffusion coefficient of the insecticide corresponds to an intermediate molecular size (between the molecular sizes of pyrene and oxygen). Experiments designed to investigate the incorporation of ^{14}C-labelled lindane into the lecithin liposomes have shown that the insecticide molecule is located within the lipid bilayer between the hydrocarbon chains of the phospholipids.[62]

Diurone, a well known inhibitor of the photosynthetic electron transport, is also localised in the hydrophobic component of the membranes of phospholipid liposomes.[23]

The study of the ^{1}H NMR spectra made it possible to determine the disposition of the phosphonothioate insecticide derived from bis(5-chloro-2-hydroxyphenyl)methane (GA-41).[63] The decrease in the spin–lattice relaxation time of the protons of the methyl group linked to the phosphorus atom of the insecticide molecule when the insecticide is transferred from an aqueous solution to a suspension of lecithin liposomes indicates the incorporation of GA-41 into the lipid bilayer. The absence of an effect by the insecticide on the spin–lattice relaxation time of the protons of the choline group of lecithin permits the conclusion that GA-41 is located in the region of the hydrocarbon chains of the membrane lipids. Certain insecticides (permethrin, cypermethrin, fenpropathrin, and fluvalinate) decrease the fluorescence and the lifetime of the excited state of 1,6-diphenylhexatriene incorporated into the liposomes comprising dipalmitoylphosphatidylcholine.[24] The 1,6-diphenylhexatriene molecule has an extended shape and is in all probability located within the bilayer parallel to the hydrocarbon chains of the lipids. The reason for the observed effects is evidently the disturbance of the packing of the membrane lipids in the presence of pyrethroids and also the quenching of the fluorescence of the probe as a result of the collisions with the insecticide molecules. One can therefore postulate that pyrethroids are localised in the hydrocarbon part of the membranes next to the molecules of 1,6-diphenylhexatriene. The molecules of certain herbicidal inhibitors — uncoupling agents, which also affect the anisotropy of the fluorescence of 1,6-diphenylhexatriene are probably also at least partly located in the same region of the membranes.[3, 64, 65]

Apart from the results of experiments specially carried out in order to elucidate the positions of the pesticide molecules in the lipid bilayer, there exist a series of experimental data indicating indirectly the incorporation of pesticides into the hydrophobic region of cell membranes. Thus the pronounced dependence of the partition coefficient of the pesticide between the membrane medium and water (K_{mw}) on the fluidity of the membranes, observed for parathion and malathion as well as DDT and various isomers of HCCH, indicates the incorporation of the preparations into the inner hydrophobic component of the cell membranes.[16, 18, 21, 22, 62]

The decrease in K_{mw} with decrease in the fluidity of the membrane lipids, observed in experiments with organophosphorus and organochlorine pesticides, is in all probability a reflection of the more general dependence of the degree of incorporation of lipophilic pesticidal preparations into the lipid bilayer on its fluidity. This is expressed, in particular, by the dependence of K_{mw} on parameters influencing the fluidity of membranes such as temperature, length and degree of unsaturation of aliphatic acid chains of the membrane phospholipids, and the concentration of cholesterol in the membrane. Table 3 presents typical values of K_{mw} for certain lipophilic pesticides.

A change in temperature has different effects on the incorporation of pesticides into membranes made up of phospholipids in the liquid crystal and gel states. In the liposomes comprising egg lecithin, which has largely unsaturated aliphatic acid chains and undergoes a phase transition in the range from −15 to −5 °C,[66, 67] an increase in temperature from 10 to 40 °C leads to a monotonic decrease in the partition coefficient of the ^{14}C-labelled DDT,[15] lindane,[62] parathion,[21] and malathion[22] between the membrane medium and water. Experiments involving the use of fluorescent probes have shown that, with increase in temperature in the same range, there is a significant decrease also in the partition coefficient of lindane[18,20] and one of its analogues (the α-isomer of HCCH[18]) between dioleoyl-phosphatidylcholine liposomes and water (the gel–liquid crystal phase transition temperature for dioleoylphosphatidylcholine is −22 °C).[68] A similar decrease in K_{mw} on

Table 3. The coefficients K_{mw} for certain lipophilic compounds and their analogues.

Preparation	Membrane lipid[a]	T/ °C	K_{mw}	Ref.
DDT	EL	40	260000	15
	DPPC	41	234000	15
DDE	PM	37	300000	47
Methoxychlor	PM	37	70000	47
	DPPC	25	28300	11
Lindane	EL	40	1300	62
	DPPC	41	2700	62
Malathion	EL	40	70	22
	DPPC	41	244	22

[a] The following abbreviations have been adopted: EL = egg lecithin, DPPC = dipalmitoylphosphatidylcholine, PM = plasma membranes of the mouse liver mitochondria.

raising the temperature has been observed for phospholipids with saturated aliphatic acid chains but only above their phase transition temperatures, where these lipids also exist in the liquid crystal state. Thus the incorporation of all the pesticides mentioned into dimyristoyl-, dipalmitoyl-, and distearoyl-phosphatidylcholine liposomes diminishes on raising the temperature in the region above the phase transition temperatures of the lipids.[15,21,22,62]

On the other hand, the incorporation of pesticidal preparations into the hydrocarbon component of membranes comprising phospholipids in the 'solid' state increases appreciably on raising the temperature. This effect has been observed in dimyristoyl-, dipalmitoyl-, and distearoyl-phosphatidylcholine liposomes for DDT, lindane, parathion, and malathion.[15, 18, 21, 22, 62]

The temperature variation of K_{mw} at temperatures both above and below the phase transition temperatures of the membrane lipids shows that the maximum value of K_{mw} should be observed directly in the phase transition. Experiments with synthetic phospholipid bilayers have clearly confirmed this conclusion.[15, 18, 21, 22, 62] The attainment of the maximum value of K_{mw} at the phase transition temperature of the membrane lipid is evidently associated with the fact that the incorporation of pesticides into the lipid bilayer takes place most effectively at the interface between the two phases coexisting in the membrane during the phase transition.

Comparison of the values of K_{mw} at temperatures equidistant from the phase transition temperature shows that pesticidal preparations are usually incorporated more readily into the more 'liquid' membranes. In other words, an increase in the fluidity of the membranes usually promotes the incorporation of pesticides into them. This effect has been clearly noted in experiments designed to investigate the incorporation of parathion, malathion, and lindane into dipalmitoyl- and distearoyl-phosphatidylcholine liposomes[21, 22, 62] and of DDT, 2-(2,4-dichlorophenyl)-1-chloro-2-(4-chlorophenyl)ethylene (DDE), and methoxychlor into dimyristoyl- and dipalmitoyl-phosphatidylcholine liposomes.[16] The preferential incorporation of pesticide molecules into bilayers with a higher fluidity makes it possible to explain the positive value of K_{mw} at temperatures below the phase transition temperature of the membrane lipid by the fact that, on raising the temperature in this region, there is an increase in the number of liquid crystal domains in the 'solid' phase, which promotes an enhanced incorporation of pesticides into the bilayer. On the other hand, the incorporation of certain insecticides into dimyristoyl-phosphatidylcholine liposomes takes place more effectively in the gel state of the membrane lipid. This serves as yet another confirmation of the fact that the incorporation of pesticides into lipid membranes is determined not only by the fluidity of the latter but also by other factors, in particular by the geometry of the molecules of the phospholipids and pesticides.

The facilitation of the incorporation of pesticides into membranes with increase in the fluidity of the membrane lipids is manifested also by the fact that, for example, DDA is hardly incorporated, according to ^{1}H NMR data, into dimerystoyl-lecithin liposomes below the phase transition temperature of this lipid,[17] while experiments with mouse liver mitochondria have shown that the inhibition, observed in the presence of DDT, of the valinomycin-induced absorption of potassium by the mitochondria is independent of temperature in the range up to 20 °C, but increases sharply on further heating of the suspension.[69] Furthermore, the insecticide increases the release of endogenic potassium from the mitochondria, although the effect is observed only at temperatures above 10 °C (the temperature corresponding to the phase transition of the mitochondrial lipids) and increases on raising the temperature.

Evidently this effect of DDT on the transport of potassium through the mitochondrial membranes is due to its incorporation into the membranes, which occurs much more effectively when the membranes are in the liquid crystal state. Finally, comparison of the ^{1}H NMR spectra of DDE obtained in D_2O in the presence of dipalmitoyl-lecithin at different temperatures permits the conclusion that DDE is also incorporated preferentially into the more 'liquid' part of the bilayer.[70]

Since the fluidity of the lipid membranes depends in many respects on the length of the hydrocarbon chains of the membrane lipids, it is natural to expect that the incorporation of pesticides into the lipid bilayer will also depend on this length. Indeed, experiments have shown that an increase in the length of the aliphatic chains of phosphatidylcholines in the range corresponding to 14–18 carbon atoms as a rule diminishes significantly the partition coefficients of insecticides between liposomes and an aqueous solution.[15,19,21,22,62] For example, in the case of DDT an increase in the length of the hydrocarbon chain by one methylene group is accompanied by a decrease in K_{mw} approximately by 50%.[15] The dependence of K_{mw} on the length of the hydrocarbon chain of phosphatidylcholines proves to be linear under these conditions.[15,22] As a result of such significant hindrance of the incorporation of pesticides into the membranes with increase in the length of the aliphatic acid chains of the membrane lipids, in mixtures of phospholipids having chains of different lengths the preparations may be distributed preferentially in the membrane domains consisting of lipids with shorter chains, as happens, for example, in the interaction of parathion with liposomes comprising a mixture of dimyristoyl- and distearoyl-phosphatidylcholines.[21]

The incorporation of pesticides into the hydrocarbon region in lipid membranes is greatly facilitated by the increase in the degree of unsaturation of the membrane phospholipids. Thus at 20 °C the transition from dipalmitoyllecithin to dioleoyllecithin liposomes increases K_{mw} for lindane by a factor of 2.7,[18] while K_{mw} for DDT increases by a factor of 6.3.[16] With increase in temperature, the promoting influence of the degree of unsaturation of the lipids on the incorporation of pesticides into the bilayer increases still further.[11] Furthermore, an increase in the degree of unsaturation of the membrane phospholipids of human erythrocytes, for example, by their preincubation with dioleoylphosphatidylcholine liposomes, greatly intensifies (by 60% after incubation for 4 h) the outflow of potassium from the erythrocytes induced by DDT.[71] This result can also serve as confirmation of the more effective incorporation of pesticides into membranes with an increased fluidity. Finally, the marked dependence of the fluidity of cell membranes on their cholesterol content is also reflected in the characteristic features of the incorporation of pesticides into the hydrocarbon component of the membrane. The increase in the cholesterol content from 0 to 50 mol% leads to almost complete elimination from the membranes of lecithin liposomes of lindane,[62] parathion,[21] and malathion[22] and appreciably reduces the content of DDT.[15]

The inclusion of cholesterol (20 mol%) in dipalmitoylphosphatidylcholine liposomes diminishes the partition coefficients of lindane in the membrane/water system from 3800 to 800.[59] Furthermore, the decrease in the cholesterol content in the erythrocyte membranes in the presence of DDT[71] is in all probability a consequence of the increased concentration of the insecticide in the membranes. Evidently, the cause of all the observed effects is that cholesterol displaces the insecticides from the membrane by occupying their place in the region of the aliphatic acid chains of the membrane lipids.

In the experiments designed to investigate the incorporation of pesticides into biological membranes, it has been observed that all the preparations investigated (parathion, lindane, malathion, DDT) are incorporated into cell membranes (and, incidentally, also into the liposomes comprising the total lipids of these membranes) much less effectively than into the membranes of the liposomes made up of the individual lipids.[15,21,22,62]

For all the preparations investigated, K_{mw} diminishes with increase in the content of cholesterol in membranes of different types (of the sarcoplasmic reticulum, mitochondria, microsomes, myelin, and erythrocytes). This confirms yet again the appreciable role of the fluidity of the lipid bilayer in the incorporation of pesticides into the membranes, but in this case this applies to biomembranes. However, when the dependence of K_{mw} on the cholesterol content in membranes is extrapolated, the total displacement of lindane, parathion, and malathion from the biomembranes occurs no longer for 50 mol% of cholesterol but at much higher concentrations of the latter. This result may indicate a definite role of the membrane proteins in the incorporation of insecticides into cell membranes, which consists in the facilitation of the entry of the molecules of the preparations into the membranes tentatively as a result of the formation of defects at the lipid/protein interfaces. On the other hand, the results of the determination of K_{mw} for certain organochlorine insecticides in various biological membranes with the aid of carbazole-labelled probes do not indicate any significant role of the membrane proteins in the incorporation of insecticides into the membranes,[47] so that the question of the influence of the proteins on this process still remains open.

III. Adsorption of pesticide molecules in the region of polar groups

The adsorption of pesticide molecules in the region of the polar groups of the membrane phospholipids is characteristic of the interaction of hydrophilic preparations and lipophilic compounds with the lipid bilayer.The molecules of hydrophilic substances are then distributed in the bilayer in a way designed to ensure the retention of the possibility of the interaction with the aqueous medium, while lipophilic molecules, which have a high dipole moment, are immersed in the lipid matrix somewhat deeper and much closer to the hydrocarbon chains of the phospholipids.

On the basis of a wide variety of experimental data, one may assume that individual phenoxyacetic and phenolic preparations as well as certain pesticides with different chemical structures are adsorbed in the region of the polar groups of membrane lipids (Table 2).

The commonest phenoxyacetic acid herbicide, namely 2,4-dichlorophenoxyacetic acid (the 2,4-D preparation) is effectively incorporated into the membranes only in the undissociated form, which is extremely lipophilic (Table 1). Experiments with lecithin BLM have shown that the undissociated form of the herbicide increases the rate of transport of lipophilic cations through the membrane and inhibits the transport of lipophilic anions, acting as a typical dipolar modifying agent.[25] Evidently the cause of the observed effect is that 2,4-D makes the potential within the bilayer more negative. In all probability, the herbicide molecules are adsorbed in the region of polar groups (closer to the hydrocarbon part of the membranes) in such a way that the vectors of their dipole moments are directed towards the aqueous medium, altering thereby the dipolar potential of the bilayer. The acid anions are probably adsorbed on the membrane surface itself.[72]

Such disposition of the 2,4-D molecules in the membranes is probably due to their interaction with the ammonium and/or phosphate groups of the polar part of the lecithin molecule. Evidence in support of such interaction comes from the sharp increase in the diffusion transport of 2,4-D from one aqueous phase to another via pentanol[73] and model lipid membranes formed on a millipore filter[74] in the presence of lecithin and also from the increase in the adsorption of 2,4-D by the parenchyma of the potato tubers under the influence of this lipid.[75] The influence of lecithin on the transport of the herbicide through lipid membranes increases with decrease in the pH of the medium, which may indicate a more effective interaction of the phospholipids with the undissociated form of 2,4-D.[74] Furthermore, the addition of lecithin to carbon tetrachloride in contact with an aqueous phase greatly diminishes the amount of 2,4-D present in the aqueous phase, which indicates a strong interaction of the latter with lecithin.[76,77] Tentatively, this interaction takes place via the formation of a hydrogen bond between the herbicide and the phosphate group of lecithin or, in the case of the dissociated form of 2,4-D, of an ionic bond with the ammonium group of the choline residue. Evidently, the carboxy-group of the 2,4-D molecule is located in the membrane close to the choline group of the lecithin molecule, while the benzene ring is immersed somewhat deeper in the bilayer.[74]

It may be that a similar mechanism of the binding to lecithin molecules operates in the incorporation of benzoic acid molecules into cell membranes, in particular those of 2,3,6-trichlorobenzoic acid when they are absorbed from solution by segments of the oat mesocotyls.[78] The carboxy-group of the acid molecule can interact with the nitrogen atom of the choline group of the phospholipid. One cannot rule out the possibility that these processes constitute the basis of the adsorption of 2-methyl-4-chloro-phenoxyacetic acid (the 2M-4C preparation) on plasma membranes observed in experiments with the leaves of various plants.[79]

Triforine and dinitro-*o*-cresol can be located, in all probability, like other lipophilic pesticidal compounds, in the region of the polar groups of the membrane phospholipids. Like 2,4-D, the systemic fungicide triforine increases the rate of transport through the lecithin BLM of lipophilic caitons, in the given instance the valinomycin-K^+ complexes, and diminishes the permeability of the membranes in the presence of the lipophilic tetraphenylborate anions.[80] Furthermore, the fungicide imparts to the BLM, formed in solution in KCl at physiological pH, a weak cationic selectivity.[28] The nature of the dependence of the cationic-anionic selectivity of the membranes on the pH permits the conclusion that, being a lipophilic compound, triforine is incorporated into the lipid matrix of the membranes and lowers the positive potential of the inner component, but is located in the vicinity of the polar groups of the lecithin molecules and not in the hydrocarbon region. Apparently the mechanism of the incorporation of triforine into the lipid membranes is in many respects similar to the mechanism of the inclusion of the undissociated form of 2,4-D in the lipid bilayer. Furthermore, measurements of the fluorescence of 1-anilino-naphthalene-8-sulphonate incorporated into erythrocyte membranes have shown that 2-methyl-4,6-dinitrophenol (the herbicide DNOC) is located in the polar component of the lipid bilayer.[81]

On the other hand, the herbicide dinoseb, a structural analogue of DNOC, partially penetrates into the hydrophobic region of the membrane by virtue of the intensified interaction with the hydrocarbon chains of the lipids resulting from the appearance of the alkyl side chain in the dinitrophenol molecule.[81] Evidently, when the dinitrophenol preparations are incorporated into the bilayer, their benzene ring is located between the polar groups of the phospholipid molecules, the hydroxy-group being apparently oriented in the direction of the

aqueous phase, while the alkyl chain, if present in the herbicide molecule, is directed towards the hydrocarbon component of the bilayer parallel to the aliphatic acid chains of the lipid molecules.[82] The results of ^{31}P and ^{13}C NMR studies permit the conclusion that the molecules of certain acylanilide (propanil, karsil) and phenylcarbamate (propham chlorpropham) herbicides, which also contain a benzene ring and a hydrocarbon side chain, are incorporated similarly into the lipid bilayer.[89]

Certain hydrophilic pesticidal preparations are likewise adsorbed in the region of the polar groups. Thus, when the readily water-soluble fungicide phytone is introduced into an aqueous solution on one side of a bilayer lipid membrane comprising brain phospholipids, it induces the appearance of a transmembrane potential difference on the membrane.[29] Since phytone is the complex between $CuCl_2$ and ε-caprolactam $[Cu(CLM)_2]^{2+}$ $[CuCl(CLM)_2]_2$ (CLM = caprolactam), a single crystal of which has an ionic structure, the generation of potentials on the membrane is evidently associated with the adsorption of the charged form of the fungicide on it. By virtue of the hydrophilicity of the preparation, it is probably adsorbed at the lipid/water interface.

Finally, according to the measurements of the electrophoretic mobility of lecithin liposomes, the ionised form of pentachlorophenol (PCP) may be adsorbed in the layer of lipid membranes adjoining the aqueous medium.[25,26] Apparently, the adsorbed PCP anion is capable of forming a hydrogen bond with a water molecule. On the other hand, the undissociated form of PCP is located in the hydrocarbon region of the membranes. The adsorption of the neutral and ionised forms of PCP in egg lecithin liposomes constitutes competitive processes. The adsorption centre consists of six lecithin molecules.[84] The undissociated form of the preparation is bound to the liposomes more effectively than the dissociated form (the corresponding association constants are 55000 litre mol^{-1} for the dissociated form and 279 litre mol^{-1} for the undissociated form).[85] It is of interest that the binding to the membrane alters the pK_a of PCP, increasing it from 4.75 in a homogeneous solution to 5.97 on a phosphatidylcholine membrane formed in 0.1 M KCl.[26]

The different nature of the interaction of the dissociated and undissociated forms of pesticides such as 2,4-D and PCP with the lipid membranes suggests a dependence of the effectiveness of their incorporation into the membranes on the pH of the medium. Indeed, the results of numerous studies have demonstrated an increase in the incorporation of 2,4-D and some of its analogues into various membrane systems with increase in the acidity of the solution employed.[13,74,86] This leads to an increase in the degree of adsorption of the herbicide,[73,75,87-90] the intensification of its influence on various processes associated with the membranes,[74,82,89-96] and an increase in its toxicity.[97] Thus 2,4-D and 2,4,5-triphenylacetic acid (the 2,4,5-T preparation) are adsorbed much more effectively by lecithin liposomes in a medium at pH 3 than in more alkaline solutions (pH 5.5 or 9.8).[86] On reduction of the pH from 6.5 to 4.5, the maximum concentration of the absorbed 2,4-D in the protoplasts isolated from the roots of the rye plant increases from 4.02 to 32.13 μM.[87] These data confirm yet again that 2,4-D is incorporated into the membranes mainly in the undissociated form. Indeed, it has been found, for example, that the permeability of the cell membranes of the roots of the barley plant to the dissociated form of the herbicide is smaller by a factor of 5.5×10^4 than to the undissociated form.[13]

A similar increase in incorporation into the cell membranes and the associated increase in the degree of absorption and toxicity with decrease in the pH has been observed for PCP,[98,99] and dinoseb.[12] On the other hand, the biological activity and the incorporation into the membranes of basic preparations, the molecules of which are protonated with decrease in pH, are appreciably reduced in acid solutions. The imidazole fungicide imasalil ($pK_a = 6.53$), the antifungal activity of which falls sharply as the pH is reduced from 0.7 to 5.2,[59] as well as the formamidine acaricide chlordimeform (its uncoupling activity increases with increase in the pH)[100] may serve as examples. The preferential incorporation into the lipid bilayer of the unionised compounds has been confirmed, in particular, by the appreciable weakening of the effect of chlorphenprop-methyl on the leakage of aminoacids from the tissues of *Avena fatua* when it is hydrolysed to chlorphenprop — the corresponding acid with the carboxy-group charged at physiological pH.[101]

The effectiveness of the adsorption of pesticidal preparations by cell membranes is also influenced by the molecular structure of the pesticides. This influence is clearly demonstrated, for example, by phenoxyacetic acids — when the latter are chlorinated, the effectiveness of their permeation into bean leaves increases.[102] As already mentioned, this is caused by the increase in the lipophilicity of compounds of the given series on chlorination.

The fluidity of the lipid bilayer also exerts a definite influence on the adsorption of pesticides in the polar component of the membrane. Thus, with increase in the cholesterol content in lecithin liposomes, the amount of uncharged 2,4-D in 2,4,5-T molecules bound to the membranes diminishes,[86] while the degree of adsorption of PCP on egg lecithin liposomes increases in the phase transition of the lipid from a gel to a liquid crystal.[84]

IV. Interaction of amphiphilic pesticidal compounds with the lipid bilayer

In the interaction of amphiphilic pesticidal compounds with the lipid bilayer, the polar component of the pesticide molecule is located in the region of the polar groups of the membrane lipids, while the non-polar component is incorporated into the hydrocarbon part of the membranes. This mechanism is characteristic of the interaction of various ionogenic surfactants with lipid membranes. Some of these substances (dodine, glyodin, quaternary ammonium salts) are effective fungicides (Table 2).

The cationic fungicides dodine and glyodin, which have long hydrocarbon chains and small polar components (the latter comprise the guanidine and imidazole basic groups in dodine and glyodine respectively), are readily incorporated into the cell membranes of different fungi. The fungicides then interact with the negatively charged sections of the membrane surfaces, neutralising their surface charge.[33] Thus dodine rapidly diminishes to zero the negative charge on the surfaces of intact conidia, cell walls, and protoplasts in the spores of *Neurospora crassa*. In the case of the protoplasts, this takes place at a fungicide concentration in solution of only 0.35 μM.[32] In other words, the process involving the incorporation of dodine into the cell membranes is extremely rapid and effective.[38] A further increase in the fungicide concentration in solution renders the surface charge on the membranes positive. Apparently dodine is bound to the membranes as a result of electrostatic interaction with the basic charge-generating components on the surfaces of fungal spores — the phosphate groups of the polar fragment of membrane lipids and the carboxy-groups of the aminoacid residues of the membrane proteins.

This hypothesis is consistent also with the decrease in the binding of the fungicide in the presence of divalent metal ions (apparently owing to competition for common binding sections) and also with suppression of the dissociation of the carboxy- and phosphate groups present on the surface of the membrane.[31] The van der Waals interaction of the fungicide hydrocarbon chains and the membrane phospholipids also plays a significant role in the retention of the dodine molecules in the lipid bilayer because these chains are evidently arranged parallel to one another in the bilayer.

Glyodin apparently also interacts in the same way with the cell membranes of microorganisms.[34] In the general case, the electrostatic interaction of virtually all the amphiphilic pesticidal compounds with the ionogenic groups of the membrane lipids and the van der Waals interaction with their non-polar components determine the detergent nature of the binding to the lipid bilayer.

A similar mechanism of the interaction with biomembranes has been proposed also for the morpholine fungicide tridemorph, which has a long alkyl chain.[103] The induction by the fungicide of the outflow of the electrolyte from the zoospores of *Plasmopora halstedii* and the *Corynebacterium* bacteria only a few minutes after treatment also supports this mechanism.[104,105] On the other hand, experiments on the determination of the electrophoretic mobility of the protoplasts of the micelia of *Neurospora crassa* and *Botrytis fabae* as well as the spores of *N. crassa* in the presence of this fungicide have not revealed any kind of influence on tridemorph on their surface charge.[106] In all probability, these results show that the long-chain molecules of tridemorph are incorporated into the lipid bilayer of the cell membranes in the same way as those of dodine and disturb the integrity of the bilayer but do not necessarily influence the surface charge of the membranes.

It may be that cationic imidazole fungicides — clotrimazole, miconazole, and econazole — are also capable of interacting with the lipid bilayer via the mechanism of the interaction of the amphiphilic detergents. These cationic fungicides are, on the one hand, lipophilic preparations, but they contain at the same time an imide nitrogen atom, which readily combines with a proton on acidification of the medium. This hypothesis is based on the increase in the effectiveness of the interaction of the above fungicides with lecithin liposomes when a negative charge is imparted to the surface of the latter (by incorporating diacetyl phosphate into their membranes) and also on the decrease in the effectiveness of the interaction when a positive charge is induced in the liposomes (with the aid of stearylamine).[58]

Quaternary ammonium salts with fungicidal activity constitute a large group of amphiphilic pesticides which interact effectively with the lipid bilayer. The disruptive effect of these cationic surfactants on the membranes, exerted via the usual detergent mechanism, has been known for a fairly long time,[107] but a systematic study of its characteristic features has been carried out comparatively recently. The detergent mechanism of the incorporation of the fungicidal ammonium salts into the lipid matrix of the membranes has been established in experiments with various compounds — benzylalkylammonium chlorides[108] and bromides[32] and nitro-vinylalkylammonium,[36,38,40] alkoxymethylenemorpholinium,[35] and nitrovinylbenzylmorpholinium[38,39] chlorides.

The experiments showed that the effectiveness of the incorporation of these preparations into the lipid bilayer depends significantly on the characteristics of the polar and non-polar fragments of their molecules. Thus, among the three benzylalkylammonium fungicides investigated, the preparation containing in the polar component of the molecule two electronegative groups at once — nitrovinyl and benzyl — exerts the greatest influence on the haemolysis of erythrocytes and the conductivity of bilayer lipid membranes.[39,40] The removal of one of these groups (nitrovinyl) from the molecule leads to some weakening of the effect of the fungicide on the membrane; an even greater effect is shown by the removal of both the electronegative fragments. Evidently the increased electronegativity of the polar component of the fungicide molecule intensifies its electrostatic interaction with the positively charged choline group in the polar component of the membrane lipids and ensures thereby a deeper penetration not only by the polar component but also by the entire molecule of the preparation into the lipid bilayer. The more effective incorporation of the hydrocarbon chain of the fungicide into the membrane inevitably leads in its turn to an increase in the degree of disorder in the internal part of the bilayer and hence to a stronger effect on the barrier properties of the membranes.

A similar dependence of the degree of incorporation into the lipid bilayer on the electronegativity of the polar component of the molecule has been observed also in the series of benzylalkylmorpholinium chlorides.[39] On the other hand, when the ammonium residue in the fungicide molecule is replaced by the morpholinium residue, the fungicidal activity of the preparations diminishes somewhat, which may be associated with the increase in the size of the polar part of the molecule.[38,39] It may be that the unduly large size of the polar components of the molecules of the quaternary ammonium salts weakens their interaction with the polar groups of the phospholipid molecules, which leads to a less deep incorporation of the fungicide molecules into the bilayer and hence to a weaker perturbation of the latter.

Furthermore, when quaternary ammonium salts are incorporated into the cell membranes, the membrane characteristics such as the surface charge of the lipid bilayer and the effectiveness of the interaction of the molecules of the membrane phospho-lipids with one another change in all probability.[36-39] These data also show that the positively charged polar part of the fungicide molecule is located at the membrane/water interaface, disturbing thereby the interaction of the polar groups of the membrane lipids.

Finally, the degree of interaction of benzylalkylammonium fungicides with the lipid matrix of cell membranes depends on the length of the hydrocarbon chain of the molecule of the preparation. In the series of alkoxymethylenetrimethylammonium chlorides, there is an optimum chain length, which influences the transport of Ca^{2+} and SO_4^{2-} through the liposome membranes. This length corresponds to 12-16 carbon atoms.[37] Approximately the same optimum length of the hydrocarbon chain (10-14 carbon atoms) is observed in the series of alkoxymethylenetrimethylammonium chlorides and also among alkylguanidine acetates — dodine analogues.[35] Evidently, the existence of an optimum hydrocarbon chain length is associated with the fact that compounds with chains much longer than those of the membrane phospholipids form fewer structural defects in the lipid bilayer than preparations with the optimum chain length. On the other hand, in the case of compounds with a much shorter chain length the hydrophobic interaction of the chain with the hydrocarbon tails of the membrane lipids may be too weak to affect the packing of the membranes.[37]

In conclusion, it is essential to note that, apart from amphiphilic pesticides, preparations such as metribuzin and D$_5$[*N*-butyl-*N*-(3,4-dichlorophenyl)-*N*-methylurea] — an analogue of the herbicide neburon — evidently possess the ability to form transbilayer structures. They apparently produce ionic channels in the lipid bilayer, resembling the dimeric channels of gramicidin A.[109]

Thus, depending on the degree of lipophilicity, the molecular dipole moment, and other characteirstics of the pesticide, its interaction with the lipid bilayer of cell membranes as a rule takes place via one of the three mechanisms described above. The type of the biological activity of the pesticide hardly affects the overall pattern of its interaction with the membrane lipids. Furthermore, it is usually impossible to observe correlations between the biological activity of the preparations and the effectiveness of their interaction with the lipid bilayer. These factors suggest that the membranotoxic activity of pesticides does not usually operate at the level of membrane lipids. On the other hand, the incorporation of pesticides into the lipid matrix of the membranes can frequently make a significant contribution to the usual toxicity of the pesticides as a result of the infringement of the barrier properties of the bilayer (for example, in the case of pesticide-protonophores) or of the allosteric effect on the operation of the membrane proteins when the properties of their lipid microenvironment are altered (for example, in the case of DDT and pyrethroids).

V. Conclusion

The studies carried out in recent years have made it possible to establish the mechanisms of the toxic activity of many organochlorine, organophosphorus, and carbamate insecticides, phenylurea, triazine, and phenolic herbicides, fungicidal azoles, pyrethroid compounds with insecticidal activity, and also a whole series of other pesticidal preparations.

The knowledge of the mechanisms of the action of pesticides makes it possible to increase appreciably their effectiveness, to modify specifically their structure and properties, and to synthesise new highly effective preparations. The increase in the effectiveness of pesticides resulting from the determination of the biochemical mechanisms of their action is achieved by improving the existing technologies for the treatment of harmful organisms. The specific synthesis of new pesticides is carried out taking into account the characteristic features of the interaction of the pesticide molecules with their molecular targets in sensitive organisms. Furthermore, the data obtained in the study of the mechanisms of the action of pesticides can be used to develop compounds with antidotal properties and compounds which diminish the toxicity of pesticides to warm-blooded organisms. Finally, studies of this kind are frequently of fundamental importance. The best known illustration of this is the determination of the mechanism of the action of the herbicide diurone, which has promoted the development of research into photosynthetic electron transport.

Various mechanisms of the toxic action of pesticides on the organisms sensitive to them are now known. However, in all cases the principal site where pesticides act is the system of cell membranes. Pesticides are capable of influencing both the structure and the functions of the membranes and can damage the membrane systems either directly or indirectly. The direct interaction of pesticides with biological membranes takes place via the binding of the preparations to certain components of the membranes, usually to the membrane proteins or lipids. An indirect effect of pesticides on membranes occurs when the biosynthesis of the proteins, lipids, or pigments of the cell membranes is affected and leads initially to changes in the protein-lipid or pigment composition of the membranes and then, as a consequence, to the breakdown of their structure and functioning.

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