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Relationship between structure and mutagenicity of dichlorvos and other pesticides

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Genetica. — *Relationship between structure and mutagenicity of dichlorvos and other pesticides.* Nota di GIORGIO MORPURGO (*), FRANCESCA AULICINO (**), MARGHERITA BIGNAMI (**), LUIGI CONTI (**) e ANNA VELCICH (**), presentata (***) dal Socio G. MONTALENTI.

RIASSUNTO. — Il Dichlorvos, un estere fosforico che presenta un gruppo vinilico come catena laterale, ha mostrato attività mutagena e ricombinogena in *Aspergillus nidulans*. Sono stati saggiati con risultati negativi sei esteri fosforici privi del gruppo vinilico per induzione di mutazioni puntiformi e crossing-over mitotico in *Aspergillus*. Tuttavia tre carbammati, Triallate, Diallate e Sulfallate, che posseggono un gruppo cloro allilico molto simile al gruppo vinilico presente nella molecola del Dichlorvos sono risultati potenti mutageni e ricombinogeni. Due altri carbammati privi del gruppo cloro allilico non mostrano alcuna attività genetica nei nostri tests.

Si conclude quindi che i gruppi clorinati sono responsabili delle attività mutagena e ricombinogena del Dichlorvos, Triallate, Diallate, Sulfallate. L'attività mutagena del Dichlorvos è stata studiata più attentamente: i vapori del prodotto puro e del suo derivato commerciale " Vapona " si sono dimostrati geneticamente attivi solo sui conidi in germinazione e inattivi sui conidi quiescenti. Si suggerisce in fine, che in vista delle recenti scoperte sulla mutagenicità e cancerogenicità del cloruro di vinile nell'uomo, i pesticidi che contengono gruppi vinilici e cloro allilici sono pericolosi per l'uomo.

INTRODUCTION

Dichlorvos is a widely used pesticide both in agriculture and in the human habitat. Dichlorvos has been found to be mutagenic with a variety of test systems (Ref. [2], [8], [15], [20]).

In spite of some negative results (Ref. [10], [24]) it is undoubtedly mutagenic; this fact is of great importance because the use of this pesticide is very widespread, especially for home insect control and a large part of the population may be exposed continuously to its action.

The aim of the experiments is 1) to increase the knowledge on the mutagenic action of Dichlorvos by testing its mutagenicity with new test systems which permit detection of induced crossing-over 2) to establish a relationship between the structural formula and the mutagenic properties of this drug.

Dichlorvos is (see Table I) a phosphoric ester with a vinyl group attached. In view of the recent findings that vinyl chloride is a strong mutagenic and carcinogenic agent (Ref. [4], [9], [18], [23]), one may wonder if it is the phosphoric ester moiety of the molecule, or the vinyl group, or both, responsible for the mutagenic action. Previous works (Ref. [8], [25], [26]) have concluded that the mutagenic action of Dichlorvos is due to the alkylating properties of the phosphoric ester moiety; however, the evidence is in our opinion rather scanty, considering both the genetic and the chemical data.

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We will go deeper into the matter in the discussion. The problem may be solved by comparing the mutagenic action of Dichlorvos with that of other pesticides that show some structural similarity to it in only one part of the molecule; the results are reported in the present paper.

MATERIALS AND METHODS

Strains.

Two strains of *Aspergillus nidulans* were used in the course of this work; strain 35 is haploid (pabaA1 , anA1 , yA2, methG1, nicA2, nicB8) and was used to test the occurrence of point mutations.

Strain P is diploid and was used to test somatic crossing-over and non-disjunction; the genetic constitution of chromosome I is: su adE20/+, riboA1/+, fpaA1/+, anA1/+, proA1/+, pabaA1/+, yA2/+, adeE20/+, biaA1/+. (Plate II).

This strain is also heterozygous for the following markers: methG1/+, nicA2/+, AcrA1/+, phenA2/+, pyroA4/+, lysB5/+, nicB8/+; these symbols are from Barratt *et al.* (Ref. [3]).

Media.

Czapek-Dox (Ref. [7]) and complete media (KH_2PO_4 1g; $MgSO_4$ 0.5 g; KCl 0.5 g; $FeSO_4$ 0.01 g; Corn Steep 10 g; methionine 0.05 g; hydrolysed yeast nucleic acid 0.3 g; Sogesil (SISS, Silicones, Milan) antifoam 0.3 g; yeast extract 3 g; biotin 0.01 g; H_2O 1000 g; agar 20 g) were used throughout this work.

Test of genetic activity.

Point mutations were tested as 8-Azaguanine resistance (Ref. [21]); crossing-over induction was tested by scoring the appearance of " fpa " (para-fluorophenylalanine)-resistant green colonies, homozygous for the fpaA1 marker (Ref. [5]).

Non-disjunction was scored by two different methods: the first one by measuring the induction in the spot test (Ref. [7]) of yellow " fpa "-resistant clones; the second by counting the yellow or dark green sectors (homozygous $yA2^+/yA2^+$) induced in the colonies grown in the presence of the drug under study, following the non selective technique described by Fratello *et al.* (Ref. [13]). In some experiments to score induction of mutants and recombinants we used a technique that gives an estimate of the concentration of the drug showing genetic activity. According to this method, already described in Beccari *et al.* (Ref. [5]) the haploid strain 35 and the diploid strain P were allowed to grow, from a dense plating of conidia, on a minimal medium supplemented with the nutritional requirements of the strains; the chemical under test was added at the maximum possible concentration that allows sporulation. Conidia were harvested and the frequency of mutants and recombinants was determined as 8-Azaguanine resistance and " fpa " resistance respectively.

TABLE I

Pesticides investigated. Mutagenic and recombinogenic activity observed with the spot test.

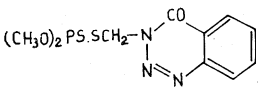
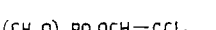
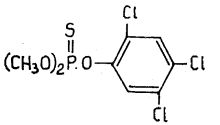
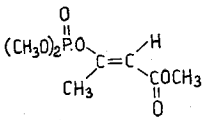
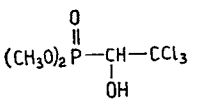
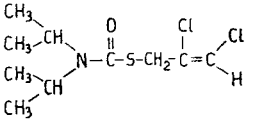
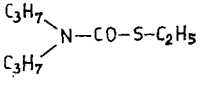
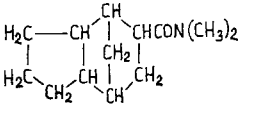
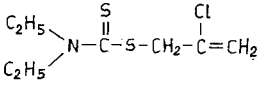
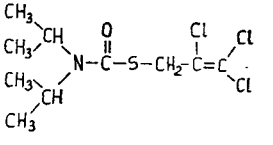
Common Names	Trade Name	Systematic Name	Chemical Structure	Conc.(*)	Point mutation	Conc.(*)	Somatic C. over
ORGANOPHOSPHORUS ESTERS							
Azinphos Methyl .	Gusathion M	S-(3,4-dihydro-4-oxobenzo-d-1,2,3-triazin-3-ylmethyl) dimethyl phospho-ro thiolothionate		2	—	2	—
Dichlorvos	Vapona	2,2-dichloro-vinyl dimethylphosphate		14	+	28–56	+++
Fenchlorphos . .	Nankor	dimethyl-2,4,5-trichlorophenyl phosphorothionate		2	—	2	—
Mevinphos	Phosdrin	2-methoxy-carbonyl-1-methylvinyl dimethylphosphate		2.4	—	2.4	—
Monocrotophos .	Azodrin	cis-1-methyl-2-methylcarbamoylvinyl phosphate		2	—	2	—
Parathion methyl .	Dalf	dimethyl-4-nitrophenyl phosphorothionate		2	—	2	—
Trichlorphon . . .	Dipterex	dimethyl 2,2,2-trichloro--1-hydroxyethyl phosphonate		2	—	2	+

TABLE I: *Continued*.

CARBAMATES							
Diallate (**) . . .	Avadex	S (2,3-dichloroallyl) diisopropylthio carbamate		2	+	0.2	++
EPTC	Eptam	S-ethyldipropyl thiolcarbamate		0,02	—	0.02	—
Noruron	Herban	3-(hexahydro-4-7-methanoindan-5-yl)-1,1-dimethylurea		0.2	—	0.2–2	—
Sulfallate (****). .	Vegadex	2-chloroallyl diethyldithiocarbamate		21,7–217	+++	108–217	+++
Triallate (***). . .	Avadex BW	S (2,3,3-trichloroallyl) diisopropyl thiocarbamate		230	+++	2.3–230	+++

Legend to Table I (*) Mg in the triangle. (**) Effective pH 4.5. (***) Effective pH 4.5, 7, 8.2. (****) Effective pH 4.5,7. Symbols: somatic c. over = somatic crossingover; + = positive; +++ = strongly positive.

Source of pesticides.

Azinphos methyl, Parathion methyl, Trichlorphon and EPTC were kindly provided by Dr. I Camoni (Istituto Superiore di Sanità); Fenchlorphos was purchased from Dow Chemical Co.; Mevinphos from SIPCAM (Società Italiana Prodotti Chimici per l'Agricoltura, Milano); Monocrotophos from Shell; Noruron from Hercules Powder Co.; Triallate from Monsanto; Diallate from Chemical Manufacture Laboratory USA; Sulfallate was kindly provided by Prof. V. Leoni (Università di Roma, Italy).

Results

The drugs are divided into two groups; 1) *organophosphorus compounds*: the phosphoric ester moiety of the molecule is very similar in different chemicals while other groups are widely different; 2) *carbamates*: in this group the structure is very different in the various chemicals while the carbamic moiety remains constant.

In Table I the tested compounds, their chemical structure, trade and common names, mutagenic and recombinogenic activity of the compounds are indicated.

It can be seen that only Dichlorvos, Trichlorphon, Triallate, Diallate and Sulfallate show some genetic activity. On Dichlorvos and Triallate mutagenic and recombinogenic activity have been studied quantitatively with a different method (see Materials and Methods) which permits evaluation of the concentration of the drug that shows genetic activity.

The results are shown in Table II. We also attempted to induce recombination in quiescent conidia by direct treatment with Dichlorvos; conidia were treated in aqueous suspension with Dichlorvos (18 mg/ml) and stirred for 1/2, 1 and 2 minutes at 36 °C.

After that time conidia were seeded on minimal medium and also on minimal medium plus para-fluorophenylalanine (fpa) to determine the survival rate and the frequency of induced crossing-over.

As we can see in Table III, even with high lethality, there was no increase in the frequency of induced crossing-over.

Therefore our conclusion is that Dichlorvos needs either cell division or a metabolically active cell, in order to induce genetic damage. In view of these findings we tried to induce mitotic crossing-over and point mutations in germinating conidia by exposure to the vapours of the commercial preparation of Dichlorvos (a " Vapona " strip). Conidia were seeded on minimal medium, in a glass Petri dish of 14 cm diameter, and allowed to grow for 4 hours at 37 °C in order to obtain their germination; after this time, in the middle of the dish (total volume = 308 cm^3) we put a fragment of Vapona strip (1 cm × 1 cm) weighing approximatly 2 g in a small plastic box, and then conidia were allowed to grow till sporulation. In these experimental conditions

TABLE II

Quantitative tests for mutagenic and recombinogenic activity.

Pesticide	N° Exper.	Concentr. mg/plate	Somatic recombination (colonies $\times 10^{-4}$) *		Mutagenesis (colonies $\times 10^{-6}$) **	
			Non treated	Treated	Non treated	Treated
Triallate	1	18.5	0.96	41.4	0.53	1.98
	2	18.5	1.04	24.4	0.53	1.38
	3	18.5	1.02	40.0		
Dichlorvos	1	2.8	1.48	5.45		
	2	2.8	0.73	6.24		

* Plated conidia in each experiment: 7×10^5.
** Plate conidia in each experiment: 45×10^6.

TABLE III

Treatment of conidia in suspension with dichlorvos.

	Conc. (mg/ml)	Time (sec.)	SRV %	Somatic Segregation ($\times 10^{-4}$ SRV conidia)	N° observed
Control . .	—	—	100	1.2	24
Treated . .	18	30″	61	0.5	7
		60″	10	0.6	1
		120″	6	0	0

TABLE IV

Recombination and mutation induced by "Vapona" Strip vapours.

	Exp.	Somatic Recombination (recombinants $\times 10^{-4}$) *		Mutagenesis (mutants $\times 10^{-6}$) **	
		Non treated	treated	Non treated	treated
Treatment on quiescent conidia	1	1.25	1.03	0.92	0.92
	2	1.14	1.02	1.32	1.16
	3	1.17	1.10	1.25	1.52
Treatment on germinating conidia	1	1.30	5.40	0.42	1.44
	2	1.40	3.60	0.50	1.34

* Plated conidia in each experiment: 7×10^5.
** Plated conidia in each experiment: 50×10^6.
Conidia were exposed nineteen hours to "Vapona" vapours.

Vapona vapours depress growth of the colonies, even though they do not kill conidia; conidia were then harvested and the frequency of mutants and recombinants detected. Results are shown in Table IV.

The frequency of mutation and somatic recombination of quiescent conidia exposed to the Vapona vapours for the same time on the selective medium did not increase.

In Plate I induction of colonies resistant to 8-Azaguanine by Triallate are shown.

Discussion

The data reported in Table I show that the presence of a phosphoric ester linkage is not sufficient to confer mutagenic and recombinogenic activity.

We tested a large variety of phosphoric esters and among them the only mutagen and recombinogen was Dichlorvos; Trichlorphon also weakly induces recombination but it is known (Ref. [12]) that Trichlorphon is spontaneously converted into Dichlorvos.

In our experiments Trichlorphon does not induce point mutations but the negative results are probably due to the fact that the induction of recombination is a much more sensitive test than the induction of point mutations.

Hence the phosphoric ester moiety of the molecule is not " per se " capable of inducing mutations with our systems. Therefore the genetic activity must be due either to the vinyl group or to the combination of the two groups. We have therefore tested some quite different compounds i.e. a series of carbamates: three of them, Triallate, Diallate and Sulfallate were found to be strongly mutagenic and recombinogenic while the others do not show any genetic activity. It is interesting to note that Triallate, Diallate and Sulfallate have a side chain structurally similar to vinyl chloride. It seems therefore that a chlorovinyl or a chloro allyl group attached to any type of molecule may confer on the compound a strong genetic activity if it can be spontaneously or metabolically split; it is then reasonable to conclude that in all four compounds the mutagenicity is due to the chlorinated group.

This conclusion is at variance with the conclusions of various authors on the causes of Dichlorvos mutagenicity. Both Bridges (Ref. [8]) and Wild (Ref. [25], [26]) conclude that Dichlorvos mutagenicity is due to the alkylating properties shown *in vitro* by the phosphoric moiety of the molecule.

The arguments of Bridges are based mainly on the genetic similarity of action in Dichlorvos and Methyl methan sulphonate (MMS); however he notes that in some respects (i.e. ratio between mutagenicity and DNA breakage) the two compounds are very different from each other. The reasons of Bridges are by no means conclusive.

The same final issues can be obtained from the paper of Green *et al.* (Ref. [14]) that notes a very different effect of Dichlorvos and MMS on the DNA of *E. coli* cells.

The conclusions of Wild (Ref. [25], [26]) are based mainly on the claim that the mutagenic activity of some phosphoric esters (i.e. bidrin, dimethoate, oxydemethon methyl, methyl parathion and malathion) parallels the alkylating activity tested *in vitro*. Vice versa, considering the work of Bedford and Robinson (Ref. [6]), there is no relationship between the "intensity" of the mutagenic and alkylating activity. Moreover, some compounds structurally and metabolically related to phosphoric esters and to Dichlorvos i.e. trimethylphosphate, dichloroethanol, dichloroacetaldehyde are mutagenic but *are not* alkylating agents (Ref. [6]). We must again conclude that the fact that Dichlorvos mutates through alkylating is far from demonstrated.

This conclusion obviously does not exclude the fact that some phosphoric esters, in some systems, show a weak mutagenic activity through alkylation of DNA mediated by the phosphoric moiety.

An abundant literature (Ref. [4], [9], [18], [22], [23]) has recently shown that vinyl chloride is beyond all doubt carcinogenic and mutagenic. Its mutagenicity has also been established by epidemiological studies (Ref. [11], [16]) on factory workers exposed to relatively high concentrations of the monomer and on communities which have PVC (polivinylchloride) production facilities.

The conclusion that the chlorinated group may be responsible for the mutagenic action of Dichlorvos and of the three herbicides is enhanced by the fact that Dichlorvos is decomposed in plants and also in water producing dichlorovinylalcohol, dichloroethanol, traces of dichloroacetic acid and dichloroacetaldehyde, which are very similar to the products of decomposition of vinyl chloride (Ref. [19]) and are known to be mutagenic. Vinyl chloride needs activation by the microsome system in order to be mutagenic while Dichlorvos is mutagenic without activation in all systems. This is due most likely to the spontaneous hydrolysis of the phosphoric ester bond with the direct production of the mutagenic intermediates.

Triallate also, in plants and animals, is split down near the sulfur atom (Ref. [1]) most likely producing allyl chloride, a compound very similar to vinyl chloride. Considering that:

1) Dichlorvos is mutagenic and recombinogenic also when the vapours of "Vapona" strip are tested

2) Vapona, Diallate, Sulfallate and Triallate are widely used and a large part of the population is exposed to these compounds

3) Diallate is a powerful carcinogen (Ref. [17])

4) Allyl chlorine groups or vinyl groups, which are most likely responsable for the mutagenic action of Dichlorvos, Triallate, Diallate and Sulfallate, are very similar to vinyl chloride, a known carcinogen and mutagen in man and in mammals also at very low concentrations, we think that the widespread use of the above mentioned compounds is hazardous because it releases into the organism compounds that have been already shown to be mutagens or carcinogens.

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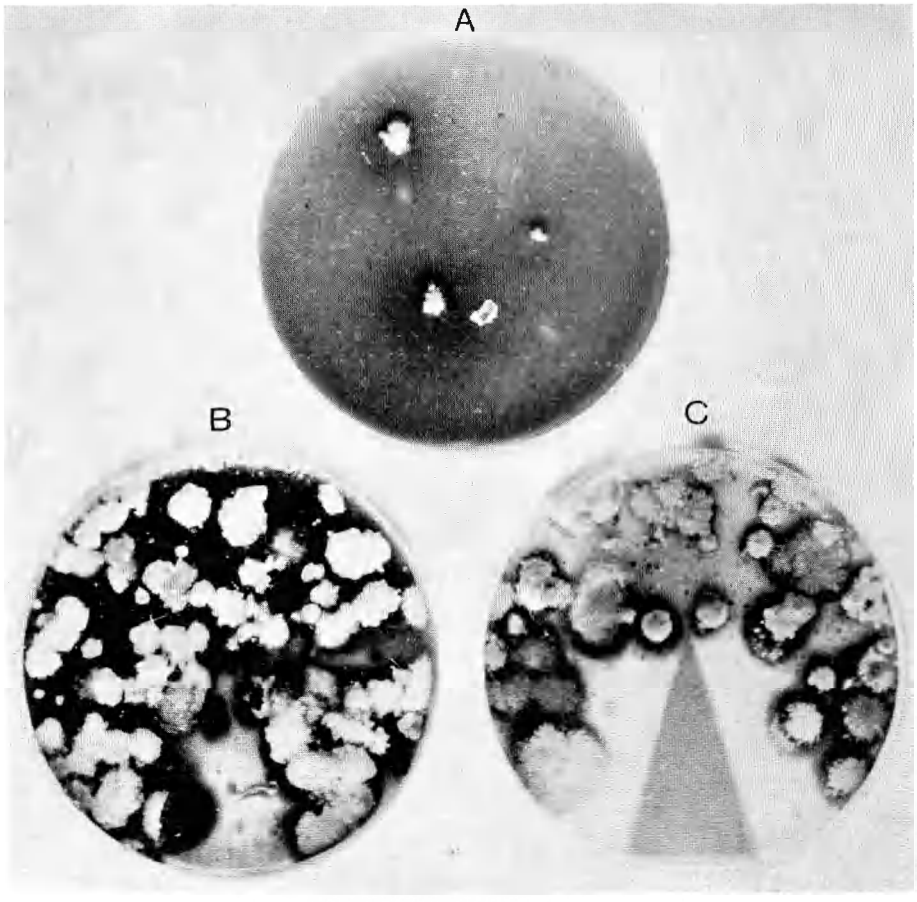

Fig. 1. – Induction of colonies resistant to 8-Azaguanine by Triallate (pH 7)
A = Control plate; B = The triangle was removed after two hours;
C = Triangle left till the end of the experiment.

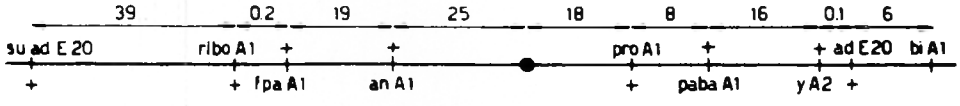

Fig. 2. – Genetic constitution of P strain (first chromosome).

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