Table III. Toxicity to Houseflies (Roberds) by Topical Application of Halogenated Ethyl and Vinyl Tertiary Esters of Dimethyl Phosphate

General formula. $(CH_3O)_2P(O)OR$

Compound	R Group	Micrograms per Fly		Relative Toxicity[a]	
		LD_{50}	LD_{90}	LD_{50}	LD_{90}
III	$CHClCCl_3$	0.147	0.325	1.0	1.0
IV	$CH{=}CBr_2$	0.123	0.243	1.2	1.3
I	$CH{=}CCl_2$	0.076	0.100	1.9	3.3
V	$CHBrCBr_3$	0.068	0.163	2.2	2.0
VI	$CHClCBr_2Cl$	0.059	0.093	2.5	3.5
II	$CHBrCBrCl_2$	0.048	0.082	3.0	4.0

[a] Based on value of 1.0 for compound III.

Table IV. Toxicities Toward Houseflies and Rats of Halogenated Ethyl and Vinyl Esters of Dimethyl Phosphate

General formula. $(CH_3O)_2P(O)OR$

Compound	R Group	LD_{50}, Mg./Kg.		LD_{50} Ratio, Rat/Fly
		Rat, oral	Fly[a], topical	
II	$CHBrCBrCl_2$	250	2.7	93
V	$CHBrCBr_3$	184	3.8	48
IV	$CH{=}CBr_2$	253	6.8	37
I	$CH{=}CCl_2$	80	4.2	19
VI	$CHClCBr_2Cl$	49	3.3	15
III	$CHClCCl_3$	14	8.2	2

[a] Average fly weight = 18 mg.

over the range 1 to 3 for LD_{50} values and 1 to 4 for LD_{90} values.

Comparison of Rat and Fly Toxicities

A comparison of the toxicities of these compounds toward rats and flies can be made by comparing the values for the rat-fly LD_{50} ratios (Table IV). The higher the figure the more specificity the compound has for flies and the more favorable should be its use as an insecticide. The compounds are listed in descending order of these ratios. The compounds containing only bromine give more favorable ratios than those having only chlorine, and the mixed bromo-chloro compound having the bromine on the 1-carbon atom of the ethyl group has a better ratio than its bromo-chloro isomer. This order is similar to that found for the relative toxicities toward rats. The variation in ratios showing a gradation of from 2 to 93 represents a 45-fold difference in relative rat-fly toxicities.

One interesting point is the relative toxicity of the mixed bromo-chloro compounds to rats. The two compounds (II and VI) have similar LD_{50} values for flies but differ by a factor of 5 in their LD_{50} values for rats. This large difference in rat toxicity could be due to differences in the rate of absorption of the compounds from the intestinal tract or in the chemical stability of the compounds in the digestive tract, or to differences in specificity of these compounds toward various enzymes found in the rat but not common to the fly. Thus these compounds may be of potential use in comparing the enzyme systems of rats and flies in relation to inhibition by organic phosphorus compounds.

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INSECTICIDE POTENTIATION

Effect of EPN on in Vivo Metabolism of Malathion by the Rat and Dog

J. B. KNAAK and R. D. O'BRIEN[1]

University of Wisconsin, Madison, Wis.

The phenomenon of "potentiation" in organophosphates, where certain combinations of compounds display an unexpectedly high toxicity, was first described in 1957 (*5*) and has been frequently confirmed (*4*). One of the best combinations for potentiation is EPN, *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothionate, with malathion, *S*-[1,2-bis-(ethoxycarbonyl)ethyl] *O,O*-dimethyl phosphorodithioate, and one of the most susceptible animals is the dog.

The low toxicity of malathion to the mammal is due to its extensive degradation by carboxyester hydrolysis (*6*). Cook *et al.* (*3*) showed that EPN in vitro inhibited the degradation of malathion by liver in vitro, and Murphy and DuBois (*4, 7*) found that it inhibited the degradation by liver in vitro of malaoxon (the oxidized form of malathion which is the actual toxicant). One would, therefore, suspect that potentiation results from an increased level of malaoxon at some target site as a result of reduced destruction of malathion and/or malaoxon. However, Seume and O'Brien (*9*) showed for several rat tissues in vitro that EPN inhibited the oxidation as well as the degradation of malathion, so that EPN led to reduced levels of malaoxon in spite of increased levels of malathion. They suggested that EPN produced potentiation by increasing the persistence of malaoxon at some target site, rather than by raising its short-term concentration. In support of this, they showed that with the rat EPN in vivo or in vitro

[1] Present address, Pesticide Research Institute, London, Ontario, Canada

The mechanism by which pretreatment with EPN increases the subsequent toxicity of malathion was investigated. In both the rat and dog EPN resulted in a marked shift in the initial detoxification site of the malathion molecule from the carboxyester to the thiolophosphate bond. The percentage of the administered malathion excreted as metabolites in urine was increased by EPN in the dog but unchanged in the rat. Malathion levels in rat tissues were increased by EPN, whereas malaoxon levels in rat blood were reduced. Potentiation appears to result from an increased persistence rather than an increased concentration of malaoxon in the tissues.

Table I. Compounds and Their Order of Elution from Columns

Name Used in Text	Chemical Name	Formula	Source	Pair of Solvents Which Elutes
A. Celite columns				
Malathion	*S*-(1,2-bisethoxycarbonyl) ethyl *O,O*-Dimethyl phosphorodithioate	$(MeO)_2P(S)SCH(CH_2COOEt)COOEt$	See methods	Iso-octane–methanol
Malaoxon	*O,O*-Dimethyl *S*-(1,2-biscarboethoxy) ethyl phosphorothiolate	$(MeO)_2P(O)SCH(CH_2COOEt)COOEt$	American Cyanamid Co. and G. D. Thorn	Same
B. Ion exchange columns				
Dimethyl phosphate	Dimethyl phosphate	$(MeO)_2POOH$	Victor Chemical Co.	250 ml. 0.01*N* HCl 250 ml. 0.1*N* HCl
Malathion monoacid	*O,O*-Dimethyl *S*-(1-carboethoxy 2-carboxy) ethyl phosphorodithioate	$(MeO)_2P(S)CH(CH_2COOH)COOEt$	American Cyanamid Co.	50 ml. 0.01*N* HCl + 200 ml. 99% MeOH 50 ml. 0.1*N* HCl + 200 ml. 99% MeOH
Malathion diacid	*O,O*-Dimethyl *S*-(1,2-biscarboxy) ethyl phosphorodithioate	$(MeO)_2P(S)CH(CH_2COOH)COOH$	American Cyanamid Co.	Same
Dimethyl phosphorothioate	*O,O*-Dimethyl phosphorothioate	$(MeO)_2POSH$	American Cyanamid Co.	60 ml. 0.1*N* HCl + 190 ml. 99% MeOH 60 ml. 1*N* HCl + 190 ml. 99% MeOH
Unknown A	...	...	...	Same
Unknown B	...	...	...	Same
Desmethyl malathion	*O*-Methyl *O*-hydrogen *S*-(1,2-biscarboethoxy)ethyl phosphorodithioate	$MeOP(S)(HO)SCH(CH_2COOEt)COOEt$	See methods	60 ml. 1*N* HCl + 190 ml. acetone 30 ml. concd. HCl + 30 ml. H_2O + 190 ml. acetone
Dimethyl phosphorodithioate	*O,O*-Dimethyl phosphorodithioate	$(MeO)_2PSSH$	American Cyanamid Co.	Same

produced a shift in the nature of the degradation products of malathion in vitro, from primarily products of carboxyester hydrolysis to primarily products of phosphorothiolester cleavage. This confirmed the view of Cook *et al.* (*3*) that the EPN acted by inhibition of a carboxyesterase.

In the present study the EPN-malathion metabolic relationship has been examined in vivo. From the foregoing studies one would anticipate that EPN would produce a reduction in total excretion of malathion metabolites, a shift in the nature of the metabolites from primarily products of carboxyester cleavage to primarily products of phosphorothiolester cleavage, and an increase in malathion levels and malaoxon persistence in various tissues. These effects should perhaps be more pronounced in the dog than the rat, because the dog is more susceptible to potentiation.

Methods and Materials

Synthesis. Radioactive malathion was prepared as described by Krueger and O'Brien (*6*). The yield was 50% and the specific activity was about 15 mc. per gram.

Desmethyl malathion (Table I) was synthesized by a procedure suggested by R. W. Young, American Cyanamid Co. Equimolar malathion and potassium *O,O*-dimethyl phosphorodithioate were refluxed in acetone for 4 hours. The acetone was evaporated off and the resulting mixture was partitioned between water and chloroform. The water phase was chromatographed on the ion exchange column (see below) and gave two peaks, one which cochromatographed with known dimethyl phosphorodithioate and the other which was assumed to be desmethyl malathion. This identification is only tentative, because difficulties in extraction and purification have so far prevented the isolation of the pure compound.

Treatment and Handling of Animals. Radioactive malathion and nonradioactive EPN were administered intraperitoneally in 10% ethanol and 90% propylene glycol. Usually with the rats, 10 mg. of EPN was dissolved in 4 ml. of the ethanol-glycol mixture and 50 mg. of EPN was dissolved in 6 ml. of the ethanol-glycol mixture. Doses and EPN-malathion intervals are de-

scribed in the "Results" section. Thirty-four 150-gram male rats (Holtzmann Co., Madison, Wis.) were treated and held individually in metabolism cages, allowing for the separate collection of urine and feces during a 48-hour period. Feces were discarded and urine samples were collected periodically. Sample volumes and radioactivity were determined and then the samples were quick-frozen and stored for subsequent metabolite analysis.

The two adult female mongrel dogs used had been handled and trained for use in physiological experiments. The dogs were held separately in metabolism cages for the 72-hour duration of the experiment. They were fasted 12 hours prior to and 12 hours after malathion administration. The doses were administered intraperitoneally in 5 ml. of 10% ethanol and 90% propylene glycol. Ten-milliliter blood samples were drawn periodically from the femoral vein by syringe using heparin as an anticoagulant. Urine samples were taken by catheter to ensure total samples up to 72 hours.

Cholinesterase Determinations. For rat brain studies, rats were treated with nonradioactive malathion and EPN according to the schedule given in Table II. The brains were removed, homogenized in cold 0.9% sodium chloride, and diluted with saline to a final concentration of 0.75 mg. per ml. Warburg flasks were prepared containing 1.25 ml. of the homogenate, 1 ml. of 0.033*M* phosphate buffer at pH 7.2, and 0.25 ml. of 1.25% sodium bicarbonate, while 0.2 ml. of 2% acetylcholine bromide was placed in the side arm. Manometric assay was made at 38° C. under 5% carbon dioxide for 30 minutes.

For dog blood studies, the blood was cooled in ice immediately and centrifuged to remove plasma, and the erythrocytes were washed twice with cold 0.9% sodium chloride. The original blood volume was restored by using distilled water. Warburg flasks were prepared containing 1 ml. of hemolyzed cells, 1 ml. of 0.033*M* phosphate buffer at pH 7.2, and 0.5 ml. of 1.0% sodium bicarbonate, while 0.2 ml. of 3% acetyl-β-methylcholine was placed in the side arm. Cholinesterase activity was measured manometrically at 38° C. under 5% carbon dioxide in nitrogen for 30 minutes.

Extraction of Metabolites. For studies on the whole rat, the animals were decapitated and each was homogenized separately in 3 liters of chloroform with a 4-quart Waring Blendor. After blending 5 minutes, dry ice was added to the brei to freeze the protein out of suspension. This allowed the chloroform to be filtered off readily. The chloroform extracts were concentrated by evaporation and extracted with water adjusted to pH 7 with sodium bicarbonate. The protein fraction was blended with 400 ml. of 0.001*N* hydrochloric acid and filtered, and a fraction of the filtrate was readjusted to pH 7 and extracted with chloroform. The activity of the two aqueous fractions was summed to give chloroform "inextractables," and of the two chloroform fractions to give "extractables."

Blood was collected from decapitated rats in iced vessels using sodium citrate as an anticoagulant. The dog blood used was a portion of that taken for cholinesterase assays. The whole blood was extracted three times with equal volumes of chloroform. Fifteen milliliters of water and of chloroform were added, and the mixture was shaken and centrifuged at 2000 r.p.m. The chloroform fractions were combined, as were the water fractions, and counted. In a separate experiment it was shown that none of the radioactivity in the chloroform was removed by extraction with water.

Urine Analysis. The hydrolysis products of malathion in urine were separated by ion exchange columns modified from those described by Plapp and Casida (*8*). The pairs of solvents used are given in Table I. Five to 10 ml. of urine adjusted to pH 1 and containing dissolved known compounds for cochromatography were used on a 2.5 × 20 cm. column, with a flow rate of 4 ml. per minute. Fractions of 12 ml. each were collected and analyzed for total radioactivity and for phosphorus according to the method of Allen (*1*).

Blood Analysis. Partition chromatography with 1.25 × 20 cm. Celite columns (*2*) was used to separate malathion and malaoxon derived from blood. The chloroform extracts from blood were evaporated to dryness and dissolved in the iso-octane–methanol mixture, added to the column, and eluted without pressure. Twenty 5-ml. fractions were collected. Identification was based on cochromatography with known compounds.

Table II. Per Cent Cholinesterase Inhibition of Rat Brain in Vivo

	Time of Sacrifice,[a] Hours		
	0.5	1.0	4.0
a. EPN	36.5	6.5	8.5
b. Malathion	10.0	22.0	6.0
c. EPN + malathion	68.0	17.0	15.0

[a] Zero time taken as time of injection of malathion (b and c) or 2 hours after injection of EPN (a). In (c), EPN-malathion interval was 2 hours. Ten rats as controls, 3 rats for each treatment.

Table III. Effect of EPN on Nature of Metabolites in Whole Rat and in Rat Blood

	% as Chloroform-Extractables[a]	% of Chloroform-Extractable[b] as Malaoxon
Whole rat		
No EPN	18	...
With EPN	49	...
Blood		
No EPN	0.52	16.4
With EPN	1.76	0.0

EPN-malathion interval 3 hours. Animals killed 0.5 hour after malathion. Results for blood and for whole rat are from different animals. Results means of 3 (whole rats) or 2 samples of blood each from 2 rats.

[a] Remainder as chloroform-inextractables.

[b] Remainder as malathion.

Results

The Rat. Doses of 5 mg. of EPN per kg. plus 25 mg. of malathion per kg. were lethal to rats when the EPN-malathion interval was 1 hour. Doses of 2.5 mg. of EPN per kg. followed by 25 mg. of malathion per kg. were not lethal to the dog or rat and were selected for use throughout. On varying the interval between administration of 2.5 mg. of EPN per kg. and 25 mg. of malathion per kg. to rats it was found that symptoms of organophosphate poisoning (lachrymation, fasciculation, salivation) were evident with 0.5- to 2-hour intervals but not thereafter, nor were there symptoms in control rats which received either malathion or EPN alone.

Cholinesterase Inhibition. Table II shows that with an EPN-malathion interval of 2 hours, EPN potentiated the effect of malathion against brain cholinesterase 0.5 hour after the malathion; at later times no potentiation of inhibition was observed.

Tissue Metabolites. The levels of certain groups of metabolites in the whole rat and in rat blood were studied. The chloroform-extractable fraction containing malathion plus malaoxon was further analyzed in the case of blood, but not with the whole rat because this fraction contained too much fat for successful chromatography.

Table III shows that EPN raised the proportion of chloroform-extractables in the whole rat and in blood about threefold. However, malaoxon was detected in blood in the control animals but not in those treated with EPN. The total amount of metabolites in blood was about 3.3 p.p.m. (average of all rats).

Urinary Excretion. The rate of excretion of malathion metabolites was studied for various EPN-malathion intervals. Figure 1 shows that the curve for rats with malathion only does not differ greatly from that in which the rats were pretreated 2 hours prior with EPN. Full data were also obtained for other intervals, with substantially similar results. Thus for EPN-malathion inter-

Table IV. Identity of Malathion Metabolites in Rat Urine

	Compound	Control	EPN-Malathion Interval, Hours 0.5	2	3
1.	Dimethyl phosphate	6	6	5	8
2.	Malathion monoacid	12	36	26	14
3.	Malathion diacid	48	14	31	14
4.	Dimethyl phosphorothioate	10	16	13	21
5.	Unknown A	3	2	2	4
6.	Unknown B	0	4	1	1
7.	Desmethyl malathion	11	8	9	14
8.	Dimethyl phosphorodithioate	4	8	6	8
9.	Total products of carboxyester cleavage	59	50	57	28
10.	Total products of phosphorothiolester cleavage	24	37	33	51
11.	Total unknowns	3	5	3	5

Results expressed as percentages of total radioactivity recovered from column, including that which did not form peaks (8 to 14% of total). "Products of carboxyester cleavage" = 2 + 3. "Products of phosphorothiolester cleavage" = 1 + 4 + 7 + 8. Urine collected between 0 and 48 hours.

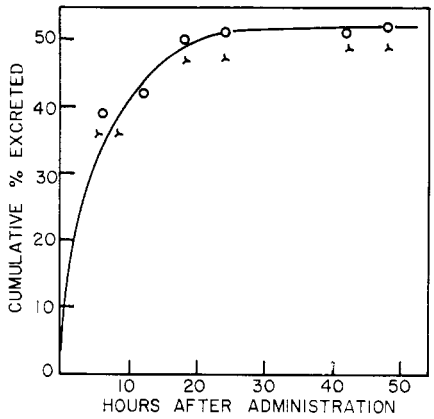

Figure 1. Urinary excretion of malathion metabolites by the rat

O Average for 6 rats administered malathion intraperitoneally at 25 mg. per kg.
Average for 4 rats administered EPN + malathion intraperitoneally at 2.5 and 25 mg. per kg., respectively. EPN administered 2 hours prior to malathion

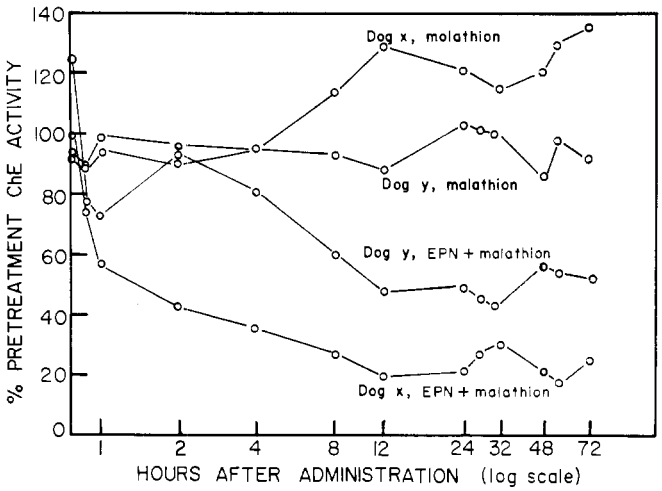

Figure 2. Effect of malathion and EPN plus malathion on dog erythrocyte cholinesterase in vivo

Time in hours after malathion administration
EPN at 2.5 mg. per kg. administered intraperitoneally 3 hours prior to malathion. In both cases malathion administered intraperitoneally at 25 mg. per kg.

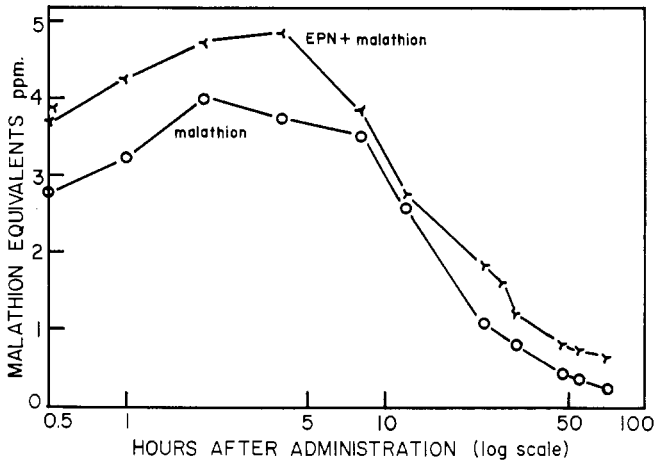

Figure 3. Total malathion equivalents in whole blood of dog X

For conditions see Figure 2

vals of 0.5, 1, 2, 3, 4, and 5 hours, the average cumulative per cent excretion 5 to 6 hours after the malathion was administered was 39, 36, 41, 41, 38, and 41 compared with the control value for malathion alone of 39%.

Ion exchange columns were used to identify and measure the proportion of urinary metabolites for various EPN-malathion intervals of from 0.5 to 7 hours. A part of these data is shown in Table IV, for urine accumulated from 0 to 48 hours. The most important observation was that the malathion diacid content was greatly reduced by EPN, while the various products from phosphorothiolate cleavage were correspondingly increased. Most of this increase was due to *O,O*-dimethyl phosphorothioate and dimethyl phosphate. This shift from products of carboxyesterase hydrolysis increased with increasing EPN-malathion interval up to 3 hours. After this time a steady return to normal was found. Thus in the urine accumulated from 0 to 12 hours (not shown in the table) the percentage as products of carboxyesterase hydrolysis increased, the figures for EPN-malathion intervals of 3, 4, 5, 6, and 7 hours being 26, 37, 40, 46, and 46%; the percentage as products of thiolophosphate hydrolysis decreased, the values being 59, 39, 42, 34, and 29.

The Dog. From the results of the rat studies a dose of 2.5 mg. of EPN per kg. and 25 mg. of malathion per kg. and EPN-malathion interval of 3 hours were selected for the dog. Two adult female mongrel dogs weighing 21 and 18 kg. were used (dogs X and Y). Dog X was treated first with malathion alone and then in a subsequent experiment with EPN plus malathion, while dog Y was treated with EPN plus malathion and later with malathion alone. This experimental design reduced the biological variation inherent in metabolism studies. The time interval between experiments was approximately 3 weeks, at which time no further radioactivity was being excreted from the first experiment. In both cases malathion alone gave no symptoms, but the combination caused central nervous system depression, and a reduction of heart rate—e.g., for dog X from 80 per minute to 62 at 15 minutes, recovering thereafter to 72 per minute at 75 minutes.

Cholinesterase Inhibition. Figure 2 shows that malathion alone had no inhibitory effect upon erythrocyte cholinesterase in vivo, while the EPN plus malathion gave pronounced inhibition.

Blood Metabolites. Figure 3 shows for one dog that EPN caused only a small increase in the total blood radioactivity; in the other dog a similar picture was seen, except that at 0.5 hour the malathion equivalents were raised more by EPN and after 2 hours the curves for the second dog were almost identical with and without EPN.

Table V. Identity of Malathion Metabolites in Dog Urine

Collection Interval	0–2 Hr.		4–8 Hr.		8–12 Hr.		12–24 Hr.		32–48 Hr.		56–72 Hr.	
	Control	EPN	Control	EPN	Control	EPN	Control	EPN	Control	EPN	Control	EPN
Dimethyl phosphate	1	8	1	8	1	7	1	9	1	5	0	2
Malathion monoacid	56	23	43	14	39	14	28	8	33	12	40	15
Malathion diacid	11	2	18	5	22	4	35	3	20	6	17	8
Dimethyl phosphorothioate	5	27	7	27	6	25	9	40	10	38	9	36
Unknown A	0	5	2	5	0	4	1	4	0	0	0	0
Unknown B	0	9	0	8	0	11	2	9	0	0	0	2
Desmethyl malathion	19	19	22	17	22	21	22	15	22	16	20	19
Dimethyl phosphorodithioate	5	4	5	4	6	5	10	4	10	5	8	6
Total products of carboxyester cleavage	67	25	61	19	61	18	63	11	53	18	57	23
Total products of phosphorothiolester cleavage	30	58	35	56	35	58	42	68	43	64	37	63
Total unknowns	0	14	2	13	0	15	3	13	0	0	0	2

Calculations as in Table IV.
Results average of two dogs.
Results expressed as percentages of total radioactivity recovered from column.

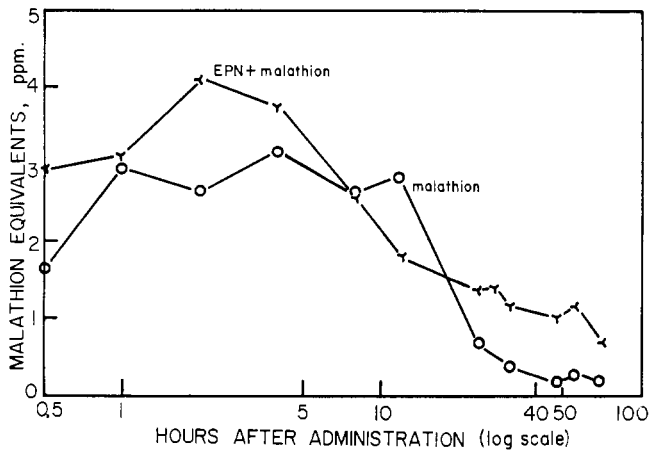

Figure 4. Total chloroform-inextractables in blood of dog X

For conditions see Figure 2

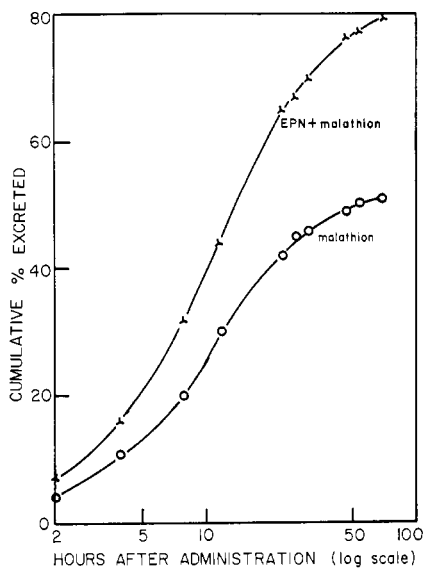

Figure 5. Urinary excretion of malathion metabolites by dog X

For conditions see Figure 2

Figure 4 shows that the chloroform-inextractables were very similar with and without EPN for dog X. In dog Y a comparable picture was found. The levels of chloroform-extractable metabolites were also studied, but were erratic and EPN had apparently opposite effects in the two dogs, raising the level in one, lowering it in the other. The levels varied between 0 and 2.2 p.p.m. expressed as malathion.

URINARY EXCRETION. Contrary to expectation, EPN increased the over-all urinary excretion of malathion metabolites. Figure 5 shows results for dog X. With dog Y the excretion of malathion metabolites was the same with or without EPN up to 10 hours after administration, at which time a difference occurred which yielded final excretion figures after 72 hours of 51% without EPN and 65% with EPN.

The nature of the urinary metabolites was studied for six collection periods between 0 and 72 hours (this procedure, which was more precise than for the rat, was made possible by catheterization and by the larger volumes of urine involved). The results for the two dogs were remarkably similar, seldom differing by more than 5%. Table V shows the averages for the two dogs. For any given treatment, the variation of metabolites with time was fairly small: In the controls, the main change was an increase in malathion diacid at the expense of malathion monoacid; with EPN treatment, the dimethyl phosphorothioate content increased with time. The difference between the control and EPN-treated animals was consistent at all times, in that products of carboxyesterase hydrolysis were severely reduced from about 60 to about 20%; malathion monoacid and malathion diacid were involved about equally in this reduction. There was a marked increase in products of phosphorothiolester cleavage caused by EPN, due almost entirely to extra dimethyl phosphorothioate.

Discussion

Of the anticipated results, the only unequivocal effect in both animals was a marked shift induced by EPN in the nature of the urinary metabolites, reflecting a change from the usual detoxification site, the carboxyester group, to the phosphorothiolate group.

A reversal of expectation was the finding that urinary excretion of malathion products was hardly affected by EPN in the rat, and was substantially increased by EPN in the dog. Because in the whole rat and in rat blood the amount of chloroform-inextractables—i.e., ionic compounds derived from carboxyester and phosphorothiolester cleavage, which should be readily excreted—was greatly reduced by EPN (Table III), it must follow that in the rat the products of phosphorothiolester cleavage are more readily excreted than the products of carboxyester cleavage. A similar conclusion is reached for the dog, in which the chloroform-inextractables of blood were little increased by EPN, yet their urinary output was considerably increased. The phenomenon may be connected with the fact that the carboxyacids are weaker acids than the phosphates, or

possibly with the larger molecular size of the carboxyacids—e.g., malathion monoacid, mol. wt. 302, compared with dimethyl phosphorothioate, mol. wt. 142.

It had been hoped to study fecal excretion in the dog, but the animals had to be fasted and were also disturbed by the restriction of the metabolism cage; thus dog X did not defecate during the whole of the control period.

In the rat, the fraction of chloroform-extractables (malathion plus malaoxon) was greatly increased in blood and in the whole animal; in the blood the malaoxon level was reduced. These observations parallel those of Seume and O'Brien for rat tissues in vitro. In the dog, the chloroform-extractable picture in blood was obscure: No conclusions could be drawn. The levels of total radioactivity of chloroform-inextractable metabolites in the blood were raised somewhat at first, but these effects had disappeared 10 hours after treatment.

These results do not permit a final verdict on the ultimate mechanism of potentiation. It appears certain that in vivo, as in vitro, carboxyesterase(s) is (are) inhibited by EPN. That this inhibition gives rise to an increase in malathion level has been shown only in the rat (for blood and whole animal). The rat results confirm the hypothesis of Seume and O'Brien (*9*) that there is an actual reduction of the concentration of malaoxon at the hypothetical target site and not an increased level, unless measurements on blood and the whole body are a very inaccurate reflection of events at the target site. If this is so, the effectiveness of EPN-potentiated malathion must be caused by an increased persistence of malaoxon at the target site, rather than an increased concentration. A final conclusion can be based only upon a more precise study of potentiation in vivo.

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INSECTICIDE DECOMPOSITION

Clay-Catalyzed Decomposition of Insecticides

F. M. FOWKES, H. A. BENESI, L. B. RYLAND, W. M. SAWYER, and K. D. DETLING

Emeryville Research Center, Shell Development Co., Emeryville, Calif.

E. S. LOEFFLER,[1] F. B. FOLCKEMER,[1] and M. R. JOHNSON[1]

Formulation Laboratory, Shell Chemical Corp., Denver, Colo.

Y. P. SUN[2]

Agricultural Research Division, Shell Development Co., Denver, Colo.

LARGE QUANTITIES OF INSECTICIDES are formulated in the form of dusts or wettable powders in which the toxicant is mixed with a finely ground, natural clay which dilutes the insecticide to concentrations suitable for agricultural use, and imparts the desirable properties of flowability and dispersibility to the dust. Various mineral clays (attapulgite, kaolinite, montmorillonite, etc.) are used as supposedly chemically inert diluents. Prior to these studies, it had been recognized that clay-diluted chlorinated insecticides occasionally decompose, sometimes becoming sufficiently hot to ignite spontaneously. Investigations (*3*) showed that the decomposition was promoted by the acid sites of the clay and that appropriate neutralization could reduce decomposition to negligible rates. Since the introduction and widespread use of clay-deactivators (*3, 5*) this problem of decomposition has virtually disappeared.

Early studies of the decomposition of dieldrin in solution showed that the reaction is catalyzed by mineral acids or even wet ferric chloride. Active clays were examined with indicator dyes and found to be surprisingly strong acids, especially when dry. Acidic diluents are shown to catalyze the decomposition of DDT, aldrin, toxaphene, chlordan, dieldrin, heptachlor, Aramite, and endrin. Although these compounds have the carbon-chlorine bond in common, the decomposition reactions do not appear to involve only this bond. With the epoxidized insecticides (dieldrin and endrin), the epoxy group disappears and the formation of carbonyl groups is indicated by infrared studies. With endrin, the main product of decomposition has been identified as a ketone of unchanged molecular weight. A wide variety of diluents has been investigated but most of the work has been done with Velvex (a kaolinite clay) or Attaclay (an attapulgite clay).

Two experimental innovations were used extensively: indicator dyes to

[1] Present address, Union Technical Service Laboratory, Shell Chemical Corp., Union, N. J.

[2] Present address, Agricultural Research Division, Shell Development Co., Modesto, Calif.