Potentiation of Toxicity of Organophosphorus Compounds Containing Carboxylic Ester Functions toward Warm-Blooded Animals by Some Organophosphorus Impurities

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Evidence is presented of the potentiating effects exhibited by some organophosphorus impurities, which are normally present in technical organophosphorus pesticides, on the toxicity of the active ingredients to warm-blooded animals. These impurities were quantitatively determined by thin-layer chromatographic and gas chromatographic methods, and identified by mass spectral techniques. The varying and significant potentiating effects of oral toxicity to the rat were experimentally demonstrated for phenthoate, malathion, and *O,O*-dimethyl *S*-α-isopropoxycarbonylbenzyl phosphorodithioate (experimental product). Tests performed on phenthoate demonstrated that the organophosphorus impurities in no way affected the toxicity of this product to heterothermic animals (fish, insects, and mites). A simple chemical method of detoxification was also devised and developed, based on the addition of acetyl bromide to the above-mentioned technical products. Regarding phenthoate, experimental evidence indicates that, compared with the technical product, the detoxified product is less toxic not only to the rat but also to other warm-blooded animals.

For several years it has been known that numerous organophosphorus compounds potentiate the toxicity of malathion to warm-blooded animals and resistant insects through a mechanism of inhibition of the esterases responsible for the detoxification of malathion by hydrolysis at the level of ester bonds of succinic acid moiety (Casida, 1961; Casida *et al.*, 1963; Plapp *et al.*, 1963; Plapp and Tong, 1966). Moreover, various authors have ascertained that simultaneous administration of pairs of organophosphorus esters to warm-blooded animals causes a potentiated toxicity.

These studies have been thoroughly summarized by Dubois (1961). The research being considered was undertaken on the grounds of observations which revealed that, while the rat oral toxicity of various samples of technical phenthoate (PHE) (*O,O*-dimethyl *S*-α-ethoxycarbonylbenzyl phosphorodithioate) decreased as their content of active ingredient increased, due to the improvements in the synthesis process, the toxicity of such samples to phythophagous insects and mites did not undergo any significant change.

Starting from this peculiar finding, it was reasonable to assume that impurities were present in technical PHE and could differently affect the active ingredient toxicity to homothermic and heterothermic animals.

The investigation was extended to include chemically similar compounds, that is, compounds resulting from the *O,O*-dimethyldithiophosphoric acid and containing carboxylic ester functions, *i.e.*, M 1703 (experimental product = *O,O*-dimethyl *S*-α-isopropoxycarbonylbenzyl phosphorodithioate) and malathion {*S*-[1,2-bis(ethoxycarbonyl)ethyl] *O,O*-dimethylphosphorodithioate}.

MATERIALS AND METHODS

Most compounds under study were synthesized according to methods described previously: PHE (Montecatini, 1957, 1967); M 1703 and $(MeO)_2P(S)CH(C_6H_5)COOH$ coded APH (Montecatini Edison, 1967); $(MeO)_2P(S)(SMe)$ coded TES (Norman *et al.*, 1952); $(MeO)_2P(O)(SMe)$ coded OTE (Hilgetag *et al.*, 1960); $(MeO)_2P(O)SCH(C_6H_5)COOC_2H_5$ coded OPH (Bayer Farbenfabriken, 1955).

$(MeS)_2P(O)(OMe)$ coded ITE is obtained by allowing 2 mol of sodium methyl mercaptide to react with 1 mol of methylphosphorodichloridate, holding at 5° for 20 min and at 50° for 30 min. The solvent is eliminated from the organic phase, dried with Na_2SO_4, and the residue is distilled at a reduced pressure. ITE distils at 75° to 74° ($Kp_{0.2}$). $(MeO)(MeS)P(O)SCH(C_6H_5)COOC_2H_5$ coded IPH is obtained by isomerizing PHE with $SnCl_4$, as described by Hilgetag *et al.* (1965), in order to obtain several isomers of thiophosphoric esters. Malathion was taken from commercial samples either bought in Italy or directly supplied by American Cyanamid Co.

Toxicological Evaluations. HOMOTHERMIC ANIMALS: ALBINO RAT (WISTAR STRAIN), ALBINO MOUSE, GUINEA PIG, ALBINO RABBIT, HARE, PHEASANT, QUAIL, CHICKEN (LEGHORN STRAIN). Toxicity tests on homothermic animals were carried out by administering the test products in undiluted form to these animals (half males and half females) by means of a gastric probe. The animals were fasted for 6 hr before treatment and 2 hr afterward, and then kept under observation for 7 days (rat, mouse, guinea pig, rabbit) or 10 days (hare, pheasant, quail, chicken). For any toxicity test, the following animals were used: 80 albino rats, 40 albino mice, 54 guinea pigs, 30 albino rabbits, 24 hares, 26 pheasants, 88 quail, and 54 chickens. The LD_{50} was determined by the Litchfield and Wilcoxon method (1949).

HETEROTHERMIC ANIMALS: INSECTS, MITES, AND FISH. Toxicity evaluations were carried out on adults of *Musca domestica* L. by topical application of the test compound dissolved in acetone; on third- and fourth-instar larvae of *Culex pipiens* L. using the technique described by Tomasucci and Michieli (1968); on *Macrosiphum solani* Kittel and *Tetranychus urticae* Koch, by spraying the product, suitably formulated and dispersed in water, on adult females infesting potato plants and the underside of bean leaves. In all cases mortality was recorded 24 hr after treatment. The tests on fish were carried out in glass containers (38 × 28 × 20 cm), each holding 16 l. of water and 10 fish. Water aeration was ensured by capillary tubes connected to a compressed air supply system.

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Table I. Tlc Behavior of PHE and Its Impurities

Compounds	Code names	R_f values in the different solvent systems[a] A	B	C
Phenthoate	PHE	0.50	0.98	0.83
$(MeO)(MeS)P(O)SCH(C_6H_5)COOC_2H_5$	IPH	0.14	0.68	0.78
$(MeO)_2P(O)SCH(C_6H_5)COOC_2H_5$	OPH	0.11	0.58	0.81
$(MeO)_2P(S)CH(C_6H_5)COOH$	APH	0.00	0.00[b]	0.52
$(MeO)_2P(S)(SMe)$	TES	0.64	0.98	0.82
$(MeS)_2P(O)(OMe)$	ITE	0.13	0.48	0.76
$(MeO)_2P(O)(SMe)$	OTE	0.08	0.32	0.75

[a] A = 90:10 *n*-hexane–acetone, two migrations; B = 60:40 *n*-hexane–ethyl acetate, five migrations; C = *n*-butanol saturated with ammonia N, one migration. Front height 14 cm. [b] Streaking.

The containers were illuminated by overhead fluorescent tubes Philips TLF 55 (light intensity on water surface about 300 foot-candles) with a 9-hr daily photoperiod. Known amounts of an acetone solution in which the test product had been dissolved were added to the water in the containers. After 10 days, with water held at a temperature of 24 to 26°, the LC_0 (a concentration producing no mortality among the test animals) and the LC_{100} (producing 100% mortality) were determined.

Purification of PHE, Malathion, and M 1703. Samples of PHE at a high purity level were obtained by four-fold crystallization of the technical product which was dissolved in a 5:2 *n*-hexane–ethanol mixture and cooled down to −9°; then an active ingredient crystal was added. PHE is a colorless crystalline solid with melting point at 17.5°.

M 1703 and malathion were purified by chromatography on a glass column (inside diameter 2.5 cm, height 50 cm), packed with 40 g of silica gel G (Merck), eluting the former with 85:15 *n*-hexane–ethyl acetate and the latter with 90:10 *n*-hexane–acetone. M 1703 and malathion were eluted, respectively, from 85 to 120 ml and 120 to 200 ml.

Active Ingredient Determination in PHE, Malathion, and M 1703 Technical Grades. The active ingredient was determined in PHE and M 1703 technical grades by using a method described by Bazzi *et al.* (1965), whereas the same determination in malathion was made according to a procedure described in the Official Methods of Analysis (Association of Official Agricultural Chemists, 1965).

Thin-Layer Chromatographic Determinations (tlc) of Organophosphorus Impurities. To separate PHE from its impurities, glass plates (20 × 20 cm) coated with a silica gel G (Merck) layer, 0.3 mm thick, were used and three different solvent systems were applied (Table I). For the identification of the impurities located on the silica gel layer, use was made of two reagents, *i.e.*, palladium chloride (Bazzi *et al.*, 1965) and 2,6-bromoquinonchloroimide (DQC) (Menn *et al.*, 1957).

With $PdCl_2$, PHE, APH, and TES produced yellow spots, whereas those resulting from IPH, OTE, and ITE were yellow-brown on a whitish background. With DQC, red-brown spots were produced by PHE, APH, and TES, and yellow-brown spots were produced by IPH, OTE, and ITE. A first attempt at the identification of organophosphorus impurities was made by cochromatography, whereas a visual determination technique was used for their quantitative evaluation, comparing the intensity of their color with that of a related standard.

Table II gives the lowest doses required to conduct an adequate quantitative evaluation of organophosphorus impurities, as well as the optimal operating conditions. For the separation, identification, and determination of TES, ITE, and OTE in malathion and M 1703, the same operating conditions as indicated in Table II were reproduced.

Table II. Chemical Amounts and Operating Conditions Required for the tlc Determination of Some Organophosphorus Impurities in PHE

Compounds	Highest PHE dose to be applied in spot form, mg	Lowest amounts of detectable impurities, μg	Reagents	Mixture used[a]
IPH	15	0.5	DQC	B
OPH	15	0.5	DQC	B
APH	2	0.5	$PdCl_2$	C
TES	2	0.3	$PdCl_2$	A
ITE	15	1	$PdCl_2$	B
OTE	15	1	$PdCl_2$	B

[a] See Table I.

Gas Chromatographic Determinations (glc) of Organophosphorus Impurities. The organophosphorus impurities were separated from PHE by preparative chromatography on glass plates (20 × 40 cm) coated with a 1-mm layer of silica gel G (Merck). 0.5 g of technical PHE was applied to each plate in a uniform thin streak 34 cm long, 3 cm from both sides of the plate, using a 90:10 *n*-hexane–acetone solvent system (three migrations, front displacement 14 cm). After the third migration, the silica gel G between 0 and 6 cm and that between 10 and 14 cm was removed from plates, transferred to two flasks containing 100 ml of acetone, stirred for 2 hr, and finally separated from the acetonic solution by filtration.

The phosphorated substances in the acetonic solution were determined by injection of 1 to 2 μl of solution into a Hewlett-Packard gas chromatograph equipped with columns (outside diameter 6 mm; inside diameter 4 mm) and with a potassium chloride thermionic detector maintained at a temperature of 400°. Further data with regard to operating conditions are given in Table III.

An hypothesis was also advanced on the nature of the impurities by comparing their retention times with those of the related compounds obtained by synthesis. The quantitative evaluation of such impurities was conducted utilizing the external standard system. For compound IPH a correction was necessary before comparing the areas, taking due account of the recovery experimentally verified (66%), and multiplying by 1.52 the peak area produced by the sample under test.

The above methods can also be applied for the identification and determination of TES, ITE, and OTE in malathion, as well as of ITE and OTE in M 1703.

Identification of Organophosphorus Impurities in Technical PHE by Mass Spectrometry. The mass spectrometric analysis followed a work designed to obtain individual impurities or solutions containing the highest possible concentrations of impurity mixtures, starting from technical PHE synthesized

Table III. Operating Conditions Used for glc Determination of Some Organophosphorus Impurities in PHE

Impurities	Length of column, m	Type of packing	Temperature, °C		Carrier gas flow rate, ml N_2/min	Retention time, sec	
			Column	Injector			
OTE and ITE	1.2 (Teflon)	NPGS 5% Chromosorb W AW DMCS 60–80 mesh	170	220	50	90 (OTE)	135 (ITE)
OPH and IPH	1.8 (glass)	OV-1 3% Chromosorb W AW DMCS HP 60–80 mesh	200	250	60	150 (OPH)	240 (IPH)
TES	1.8 (glass)	Apiezon L 2% + Epon 1001 0.2% Gas Chrom Q 80–100 mesh	120	240–250	50	90	

according to Montecatini (1957). In order to develop a first concentrate of impurities, technical PHE was subjected to crystallization as previously described in the section "Purification of PHE, Malathion and M 1703."

After separation of crystalline PHE by filtration, the solvent was removed from the filtrate at 40° by means of a vacuum rotary evaporator, and the residue was used to obtain TES, OTE, IPH, and OPH.

TES AND OTE. By subjecting the residue to vacuum distillation of 0.7 mm, a colorless liquid containing 64% TES and 0.95% OTE distilled at 41 to 43°. TES was separated from OTE by preparative chromatography, using the technique outlined in the section "Thin-Layer Chromatographic Determination of Organophosphorus Impurities," and applying 1 g of distilled substance to a plate. After the third migration, the silica gel comprised between 1 and 4 cm from the starting line (containing OTE), and that between 9 and 12 cm (containing TES) was removed from the plate and eluted with acetone. TES was isolated from the eluate by complete solvent evaporation at 30° in a vacuum rotary evaporator. The solvent was removed from the other eluate by evaporation, as indicated above, to a volume of 1 ml. A solution at 2.7% OTE was thus obtained. IPH and OPH were separated from the residue by preparative chromatography, according to the same technique previously illustrated for TES and OTE separation, removing and eluting with acetone the silica gel, which comprised between 1.5 and 5 cm from the starting line. A solution containing 2% IPH and 0.5% OPH was thus obtained.

The mass spectrometer used for the identification of OTE, OPH, and IPH was a Hitachi Perkin-Elmer, Model RMU/GE, coupled through a Watson–Biemann type of molecular separator with a gas chromatographic column (outside diameter 6 mm; inside diameter 3 mm). Under such conditions, with compounds of considerable molecular weight (OPH and IPH), variations were noted in the ion relative abundance only. These, however, were not such as to preclude the identification.

The identification of TES was made by analyzing the product directly in the mass spectrometer. All mass spectra were recorded at 70 eV electron energy, with 1800 V ion accelerating voltage. The filament emission current was 80 μA. The operating conditions for the gas chromatographic separation of the peaks of the unknown impurities are given in Table IV.

Detoxification Process. This method is based substantially on the treatment of the technical product to be detoxified, or solutions containing it, with bromides, iodides of aliphatic organic acids (*e.g.*, acetyl bromide), the mass being stirred for varying periods of time according to temperature, and finally removing the excess acyl halide and other volatile substances under vacuum or by water vapor countercurrent (Montecatini Edison, 1969).

Detoxification of PHE, malathion, and M 1703 was carried out by adding 2% acetyl bromide to a dichloromethane solution containing 38% (w/w) technical product, and by heating to 80°, under stirring, PHE and M 1703 for 2 hr, and malathion for 30 min only. The solution was then distilled by a water vapor countercurrent, using a glass column (inside diameter 3 cm; height 55 cm) filled to 43 cm with glass rings (outside diameter 0.5 cm, inside diameter 0.35 cm; height 0.6 cm) and fed from the bottom with water vapor and from the top with organic solution, ajdusting the speed so as to obtain about 800 g of condensation water and 20 g of technical product per hour.

Under these conditions the solvent and the other volatile substances were conveyed by the vapor current and could be collected after cooling, while the detoxified compound passed

Table IV. Operating Conditions for glc Separation of the Peaks of the Unknown Impurities for Mass Spectrometric Determination

Impurities	Length of column, m	Type of packing	Temperature, °C			Carrier gas flow rate, ml He/min	Retention time, sec	
			Column	Injector	Molecular separator			
OTE	1.5	NPGS 5% Chromosorb W AW DMCS 60–80 mesh	150	210	180	60	115	
OPH and IPH	1.15	OV-1 3% Gas Chrom Q 60–80 mesh	180	250	200	60	80 (OPH)	120 (IPH)

Table V. Data Relating to Active Ingredient Content, Rat Oral Toxicity, and Toxicity to Insects and Mites of the Different PHE, Malathion, and M 1703 Technical Samples, Compared with Samples of the Relevant Purified Products

Compounds	Active ingredient, %	Rat oral LD_{50}, mg[a]/kg	Toxicity referred to purified compound = 100			
			M. domestica	*C. pipiens* (larvae)	*M. solani*	*T. urticae*
PHE technical 1	61.2	77.7	62.2	60	65	68.3
PHE technical 2	78.7	118	88.2	88.6	83.3	91.3
PHE technical 3	90.5	242.5	93.9	90.5	88.3	100.9
PHE purified	98.5	4728	100	100	100	100
Malathion technical	92.2	1580	116	81.9	99	
Malathion purified	98.2	8000	100	100	100	
M 1703 technical	83.2	205	86.5	106.9	86.4	94.4
M 1703 purified	98	2750	100	100	100	100

[a] As active ingredient.

down through the glass rings and flowed out with water from the column bottom. It was then separated from the water by decantation and reduced to dryness at 40° under vacuum (1 mm).

RESULTS AND DISCUSSION

The Table V data indicate that an increase in the active ingredient content of PHE, malathion, and M 1703 is associated with a marked decrease in their oral toxicity to the rat, whereas their toxicity to insects and mites, where referred to the active ingredient content and within the limits of analytical accuracy, remains in most cases practically unchanged. It was also verified that, when highly purified, such products exhibited a very low degree of oral toxicity to the rat. At least as far as the rat oral toxicity is concerned, it is reasonable to presume from the above that various toxicity levels exhibited by the different samples of the three technical products under study could be ascribed to a different concentration of toxicologically important impurities.

By tlc and glc analysis, various organophosphorus substances were detected in PHE, malathion, and M 1703 technical products. Six of these substances were identified (Table VI). Their chemical structure was assumed initially through a comparison of the R_f values and retention times of the substances extracted from the technical products with those of the related standards obtained by synthesis.

The mass spectra of four of these substances, *i.e.*, OTE, OPH, IPH, and TES (Figures 1, 2, 3, and 4) separated from technical PHE, are well in agreement with their formula. The final confirmation was obtained by making a comparison with the mass spectra of the synthesized compounds. On the other hand, the identification of the substances contained in malathion and M 1703 by means of less specific techniques (cochromatography and gas chromatography) was conducted on a few compounds only (TES, OTE, ITE). However, bearing in mind that such techniques, though not highly specific, made it possible to forecast the structure, afterward confirmed by mass spectrometry, of the four impurities contained in PHE, and considering that results comparable to those of PHE were obtained by subjecting the relevant technical products to the detoxification process outlined above, it is most likely that the structure of the impurities in question corresponds to that expected.

In view of the content of the impurities in the technical products and of their toxicity to the rat (Table VI), it can be excluded that such substances act additively, while it can be reasonably assumed that their greater toxicity to warm-

Table VI. Impurities Detected in PHE, Malathion, and M 1703, Their Percentage in the Technical Products Examined, and Rat Oral LD_{50}

Impurities	Percent impurity content				Rat oral LD_{50} of impurities, mg/kg
	PHE[a]		Malathion,	M 1703,	
	tlc	glc	tlc	tlc	
APH	1				4500
OPH	0.03	0.024			63
IPH	0.4	0.32			250
TES	2.25	2.01	1	0.1	450
OTE	0.015	0.019	0.1	0.2	47
ITE	0.0066	0.0053	0.02	0.03	96

[a] Technical product at 90.5% active ingredient. In addition to those set forth above, there are other impurities, mostly nonphosphorated, which have no toxicological significance.

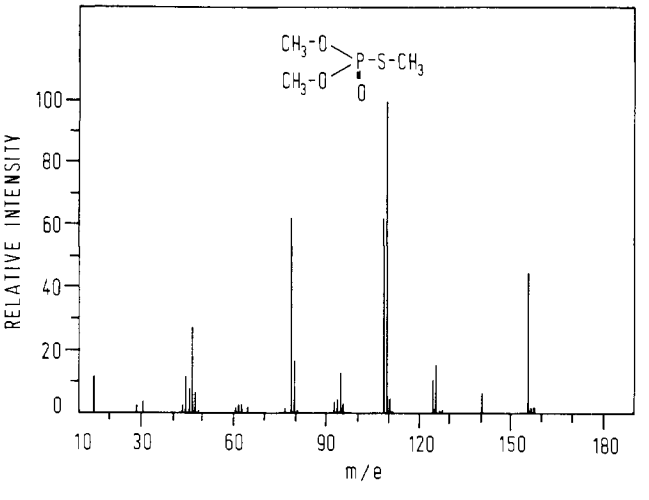

Figure 1. Mass spectrum of supposed OTE

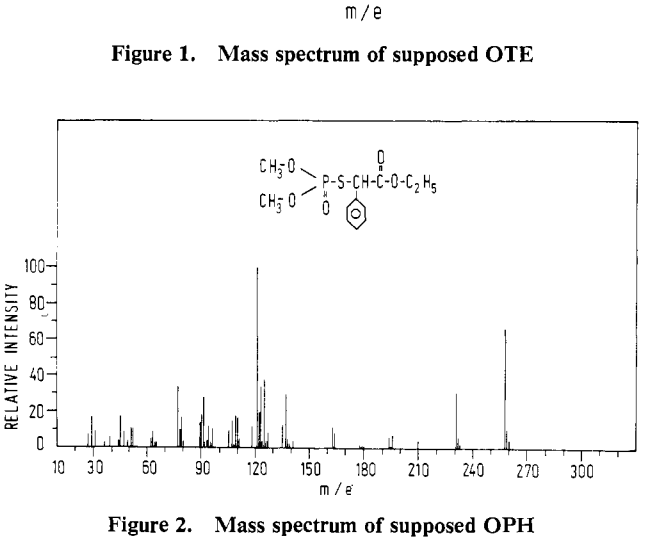

Figure 2. Mass spectrum of supposed OPH

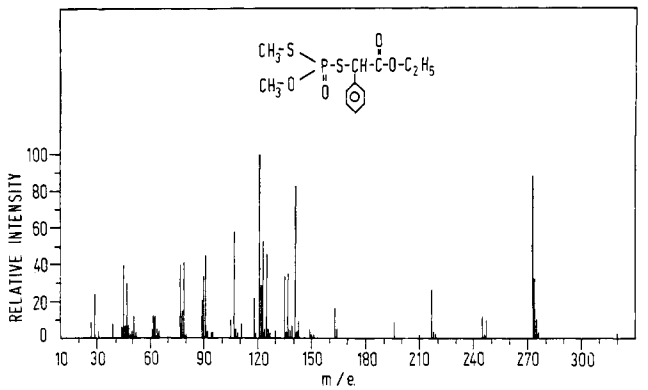

Figure 3. Mass spectrum of supposed IPH

blooded animals was most probably the result of a potentiated action.

Table VII reports the data from potentiation tests carried out with the different organophosphorus impurities, whereas Table VIII reproduces the percentage of impurities required to reduce the rat oral LD_{50} of the different experimental samples to a half. This value was worked out by graphic interpolation from potentiation curves (Figure 5).

These data demonstrate that two compounds, ITE and OTE, proved highly potentiating, though to a different extent, in respect of PHE, malathion, and M 1703.

In all cases considered, the potentiating action of ITE was found to be greater than that of OTE.

Moreover, potentiation by TES was adequate on PHE and M 1703, but poor on malathion. OPH and IPH, too, caused potentiation (IPH > OPH) on PHE, whereas APH did not produce any such effect.

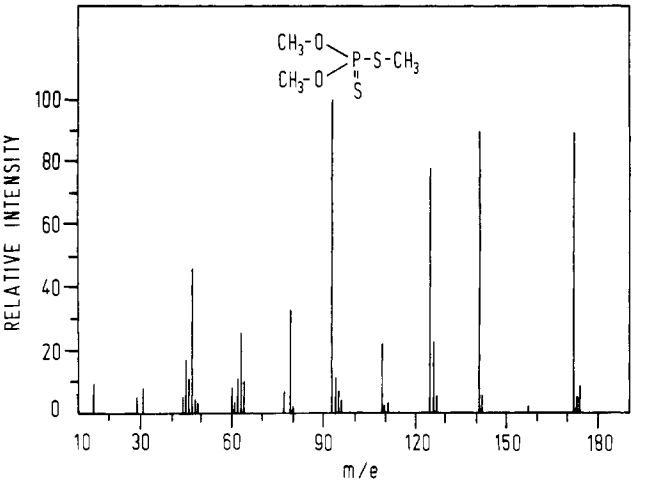

Figure 4. Mass spectrum of supposed TES

Although there is no experimental evidence, it can be presumed that the mechanism of potentiation of the compounds under study can be referred to that proposed by various authors (Casida, 1961; Plapp *et al.*, 1963; Plapp and Tong, 1966) to illustrate the potentiation of malathion toxicity by other organophosphorus compounds, *e.g.*, the inhibitory action of such potentiating compounds on the esterases responsible for the hydrolytic decomposition of malathion at the level of ester bonds of the succinic acid moiety. The experience so far acquired, even though incomplete, has led to an outstanding problem being resolved, namely, the detoxification of *O,O*-dimethyl phosphorodithioates having a carboxyalkylic function and belonging to the group of pesticides of extensive argicultural, public health, and household usage.

Table VII. Potentiating Action of Some Organophosphates on PHE, Malathion, and M 1703

Compounds	% Compound added to			Rat oral LD_{50}, mg/kg		
	PHE	Malathion	M 1703	PHE	Malathion	M 1703
OPH[a]	0			3100		
	0.5			2120		
	1			1300		
IPH	0			3100		
	0.1			2200		
	0.25			1370		
	0.5			730		
	1			500		
APH	0			3100		
	2			3200		
TES	0	0	0	3100	8000	2480
	0.1	3	0.05	2450	5500	1590
	0.2	3.5	0.15	1750	4000	1100
	0.4	4	1	1170	3000	400
	0.8			965		
	1.6			650		
	3.2			430		
ITE	0	0	0	3100	8000	2480
	0.0025	0.02	0.00025	1700	5200	2000
	0.005	0.035	0.001	900	4450	1240
	0.01	0.1	0.0025	600	2920	800
	0.1	0.2	0.005	360	2100	397
	1	0.5	0.05	145	1240	165
		1			605	
OTE	0	0	0	3100	8000	2480
	0.0025	0.1	0.01	1920	3900	1240
	0.005	0.2	0.025	1580	2720	780
	0.01	0.5	0.05	1240	2150	560
	0.1		0.2	620		303
	1			312		

[a] The potentiating action of OPH on PHE was also demonstrated by applying the Dubois method (1961). After simultaneous oral administration to rats of half the LD_{50} level of the two compounds (OPH 31.5 mg/kg and PHE 1550 mg/kg), 100% mortality was recorded among the test animals, instead of 50% as expected if no potentiating effects had developed.

Table VIII. % Organophosphorus Impurities Required for Reducing the Rat Oral LD_{50} to Half That of the Examined Compounds

Compounds	% impurities in PHE	Malathion	M 1703
ITE	0.003	0.045	0.001
OTE	0.0055	0.18	0.01
TES	0.25	3.5	0.12
IPH	0.2		
OPH	0.75		

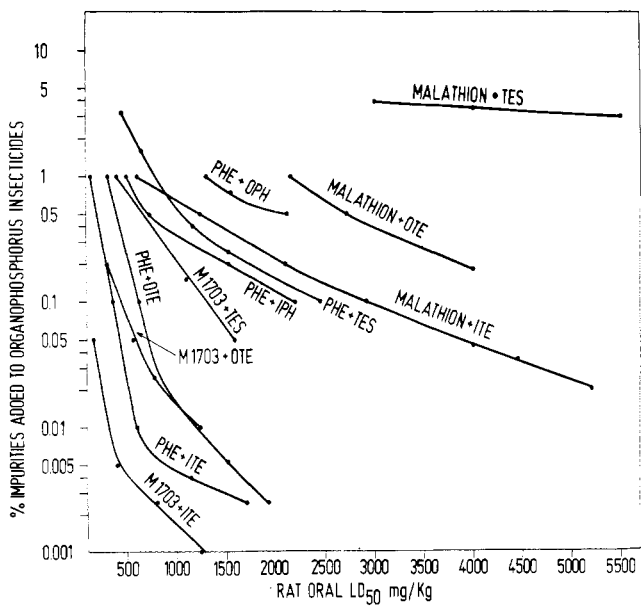

Figure 5. Potentiation of PHE, malathion, and M 1703 toxicity to warm-blooded animals by same phosphorus compounds

The detoxification method is based on the fact that bromides and/or iodides of carboxylic acids, *e.g.*, acetyl bromide, are capable of reacting electively with the potentiating organophosphorus impurities having a =P(=O)— bond, converting them into toxicologically inert compounds, and leaving the =P(=S)— compounds, which also include the active ingredients, almost unchanged.

The TES not attacked by such acyl halides must be eliminated by a vapor countercurrent or, during synthesis, by a special technique (Montecatini Edison, 1967).

Table IX gives the toxicological and chemical data showing the correlation between the content of potentiating compounds and the toxicity of PHE, malathion, and M 1703, prior to and after the process of detoxification.

It can be observed that a significant improvement of the toxicological properties of the technical products is obtained by this process and that the decrease in toxicity is associated with a reduced concentration of organophosphorus impurities responsible for potentiation.

The effects of the detoxification process are evident not only on the rat but also on other homothermic animals, whereas no appreciable influence is observed on heterothermic animals (Tables X and XI).

In the light of the results obtained in the present research, it is considered that a revision of the studies conducted by

Table X. Acute Toxicity of Two Technical PHE's to Various Warm-Blooded Animals

Test animals	Rat oral LD_{50}, mg/kg: Technical PHE, 90.5% active ingredient	Technical PHE detoxified with acetyl bromide, 93.5% active ingredient
Rat	280	3100
Mouse	360	2620
Guinea pig	377	2130
Rabbit	210	650
Hare[a]	72	180
Pheasant	218	3300
Quail[b]	300	1500
Chicken	255	2800

[a] Males only. [b] Undetermined sex.

Table XI. Toxicity of PHE, Both Technical[a] and Detoxified with Acetyl Bromide, to Insects, Mites, and Fish

Test animals	Technical PHE, 90.5% active ingredient		Detoxified technical PHE, 93.5% active ingredient	
Insects and mites	LD_{50}		LD_{50}	
M. domestica (μg fly)	0.14		0.14	
C. pipiens (ppm)[b]	9.9		9.9	
M. solani (ppm)[b]	20		19	
T. urticae (ppm)[b]	3		3	
Fish	LC_0, ppm	LC_{100}, ppm	LC_0, ppm	LC_{100}, ppm
Carassius auratus Val.	1.5	4.5	1.3	7
Cyprinus carpio L.	1.2	7	1	3
Alburnus albidus Costa	0.62	2.5	0.5	2.5
Cobitis taenia L.	0.75	2.25	1.25	3.5
Gambusia affinus B. and G.	0.05	0.3	0.035	0.15
Lebistes reticulates Pet.	0.62	1.5	0.25	1.75

[a] Synthesized according to Montecatini (1957). [b] PHE concentration in the liquid used to spray *M. solani* and *T. urticae* or to immerse *C. pipiens* larvae.

Table IX. Chemical and Toxicological Data Relating to PHE, Malathion, and M 1703 Prior to and Following Detoxification

Compounds	Active ingredient, % Prior to	After	Rat oral LD_{50}, mg/kg Prior to	After	% organophosphorus impurities determined by glc: IPH Prior to	IPH After	OPH Prior to	OPH After	TES Prior to	TES After	OTE Prior to	OTE After	ITE Prior to	ITE After
PHE[a]	90.5	93.5	268	1500	0.32	0.042	0.024	0.008	2.01	0.003	0.019	0.003	0.0053	<0.002
PHE[b]	92	92	800	3200	0.057	<0.002	0.008	<0.002	0.005	0.0013	0.012	<0.001	<0.002	<0.002
Malathion	92.2	93.4	1580	8000					1	0.003	0.061	0.005	0.014	<0.002
M 1703[b]	83.2	83.8	205	1760					0.1[c]	0.05[c]	0.058	0.013	0.0032	<0.002

[a] Synthesized according to Montecatini (1957). [b] Synthesized according to Montecatini Edison (1967). [c] Determined by tlc.

various authors, and reviewed by Dubois (1961), is needed for the purpose of verifying whether potentiation resulting from the administration of pairs of organophosphorus insecticides to warm-blooded animals was at least in part attributable to impurities of the type under study and present in the products used.

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Photodecomposition of 3′,4′-Dichloropropionanilide (Propanil)

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The action of sunlight on dilute aqueous solutions of 3′,4′-dichloropropionanilide (propanil) provided a large number of photodecomposition products separable by gas chromatography. 3′-Hydroxy-4′-chloropropionanilide, 3′-chloro-4′-hydroxypropionanilide, 3′,4′-dihydroxypropionanilide, 3′-chloropropionanilide, 4′-chloropropionanilide, propionanilide, 3,4-dichloroaniline, 3-chloroaniline, propionic acid, propionamide, 3,3′,4,4′-tetrachloroazobenzene, and a humic acid were identified. The tetrachloroazobenzene also resulted from the photolysis of a dilute aqueous solution of 3,4-dichloroaniline. The principal pathways of propanil photodecomposition were: replacement of chlorine substituents by hydroxyl groups; formation of propionamide; replacement of chlorine substituents by hydrogen; and hydrolysis of the amide. Propanil and its benzenoid photolysis products were not appreciably volatile, and only propanil was phytotoxic.

The anilide herbicide 3′,4′-dichloropropionanilide (propanil, I) is widely used for the selective control of broadleaf weeds in cultivated rice and as a postemergence herbicide in tomatoes. In 1968, a controversy developed over the use of propanil in the vicinity of prune orchards in several Northern California counties after some of the trees began to exhibit yellowing of the leaves allegedly due to incidental exposure to the herbicide following its use in nearby rice fields.

The purpose of the present investigation was to identify the products of propanil photodecomposition in aqueous solution, to propose a possible mechanism for their formation, and to determine whether or not the photodecomposition of propanil was related to prune tree damage.

EXPERIMENTAL

Synthesis of Photoproducts. 3′,4′-Dichloropropionanilide was received as a technical product, mp 85–89°C (Technical Stam, Rohm & Haas Co.). It was recrystallized twice from cyclohexane (charcoal) to a mp of 91–92°C [lit. 91–92°C (Good, 1961)].

3′-CHLOROPROPIONANILIDE (II). Propionyl chloride (0.92 g, 0.010 mol) was slowly added to a chilled suspension of 3-chloroaniline (Eastman Organic Chemicals) (1.28 g, 0.010 mol) in 6 ml of 10% sodium hydroxide solution to give 3′-chloropropionanilide (0.92 g, 50%) which was recrystallized twice from 3:1 water–ethanol; mp 85.5–86°C [lit. 88–89°C (Good, 1961)].

Anal. Calcd for $C_9H_{10}ClNO$: C, 58.85; H, 5.49; N, 7.65. Found: C, 59.00; H, 5.30; N, 7.70.

Infrared (ir) spectrum 3236 (NH) and 1667 (CO) cm^{-1}. Nuclear magnetic resonance (nmr) spectrum δ 8.80 (NH). Mass spectrum *m/e* 183 (parent), 127 (base, $ClPhNH_2$).

4′-CHLOROPROPIONANILIDE (III). The procedure was identical to that for 3′-chloropropionanilide except that 4-chloroaniline (Eastman Organic Chemicals) was used to give 4′-chloropropionanilide (0.84 g, 46%) which was recrystallized twice from benzene; mp 137.5–139°C [lit. 137°C (Good, 1961)].

Anal. Calcd for $C_9H_{10}ClNO$: C, 58.85; H, 5.49; N, 7.65. Found: C, 58.96; H, 5.39; N, 7.44.

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