Lee, P. W.; Allahyari, R.; Fukuto, T. R. *Pestic. Biochem. Physiol.* **1978**, *8*, 146.
March, R. B.; Metcalf, R. L. *Calif. Dept. Agric. Bull.* **1949**, *38*, 1.
Michalski, J.; Mikolajczyk, M. *Chem. Ind. (London)* **1964**, *16*, 661.
Michalski, J.; Mikolajczyk, M. *Tetrahedron* **1966**, *22*, 3055.
Mitsunobu, O. *Yuki Gosei Kagaku Kyokai Shi* **1970**, *28*, 206.
Ohkawa, H.; Mikami, N.; Miyamoto, J. *Agric. Biol. Chem.* **1977a**, *41*, 369.
Ohkawa, H.; Mikami, N.; Okuno, Y.; Miyamoto, J. *Bull. Environ. Contam. Toxicol.* **1977b**, *18*, 534.
Rohrbaugh, W. J.; Jacobson, R. A. *Inorg. Chem.* **1974**, *13*, 2345.
Wustner, D. A.; Desmarchelier, J.; Fukuto, T. R. *Life Sci.* **1972**, *11*, 583.

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Identification and Toxicological Evaluation of Impurities in Technical Malathion and Fenthion

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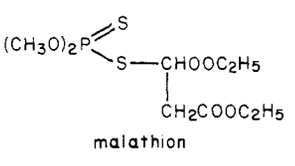
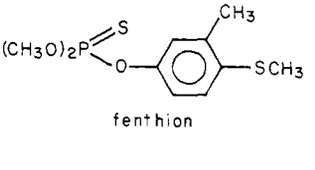
In a continuing study on the toxicological effects of impurities in technical malathion, three additional phosphorus-containing compounds were identified by GC–MS and, together with four previously identified compounds, tested in mice. All potentiated the toxicity of purified malathion, though to varying degrees. *O,S,S*-Trimethyl phosphorodithioate was the most powerful potentiator, while *O,O,O*-trimethyl phosphorothioate was the weakest. A direct relationship between inhibition of mouse serum and liver malathion carboxylesterase activities and malathion lethality in mice was observed. Irradiation of malathion on glass or silicic acid surfaces by ultraviolet light gave rapid breakdown to three major products. Two of these were identified as *O,O,O,O*-tetramethyl pyrophosphorodithioate and bis(dimethoxyphosphinothioyl) sulfide. GC–MS analysis of technical fenthion (96%) allowed structural assignment to four phosphorus and four nonphosphorus containing impurities. However, toxicological studies suggest that potentiation phenomena are not involved for this material.

Several organophosphorus pesticides including malathion [*O,O*-dimethyl *S*-(1,2-dicarboethoxy)ethyl phosphorodithioate] and phenthoate [*O,O*-dimethyl *S*-(α-(ethoxycarbonyl)benzyl) phosphorodithioate] have low mammalian toxicities and are regarded as "safe" for general use. However, combinations of some organophosphorus compounds (Frawley et al., 1957) or impurities present in the technical materials (Casida and Sanderson, 1963), arising either from synthesis or during storage, may lead to markedly different toxicities than would be expected from the toxicities of the individual components. The potentiation of malathion and phenthoate toxicity by several trimethyl phosphorothioate and phosphorodithioate esters has been investigated (Pelligrini and Santi, 1972) and more recently, several other impurities from technical grade malathion and acephate (*O,S*-dimethyl *N*-acetylphosphoramidothioate) and their effects on the mammalian toxicity of the purified insecticides were reported (Umetsu et al., 1977). Of the acephate impurities found, two had no effect on the inherent mammalian toxicity of acephate, one caused very slight potentiation, and a fourth significant antagonism. Storage of the technical material (40 °C, 6 months) had little effect on insecticidal activity but resulted in decreased mammalian toxicity. In contrast, malathion toxicity to mice increased under these storage conditions. Whereas malathion purified by multiple recrystallization had a rat oral LD_{50} of 12 500 mg/kg, contamination with as little as 0.05% of the *S*-methyl isomer of malathion or *O,S,S*-trimethyl phosphorodithioate led to LD_{50} values of 4400 and 3100 mg/kg, respectively. Several other compounds were identified and found to act as potentiators, though to a lesser degree than the two above. In consideration of the widespread use of malathion, and the potential hazard represented by either poor quality control or improper storage, a detailed knowledge of the impurities present and their effects on toxicity is of importance. The present study is concerned with the identification of additional malathion impurities and the assessment of their inherent toxicity and effectiveness as potentiators. Preliminary work also was carried out on another organophosphorus insecticide fenthion [*O,O*-dimethyl *O*-(3-methyl-4-methylthiophenyl) phosphorothioate].



malathion



fenthion

MATERIALS AND METHODS

General. Analytical and preparative thin-layer chromatography (TLC) utilized silica gel (60 PF, 254, EM Laboratories Inc.) layers of 0.25- and 1.0-mm thickness, respectively, on glass plates and solvent systems were as specified below. Chromatograms were visualized initially by ultraviolet (UV) examination and then with 2,6-dibromoquinone-4-chloroimide (DBQ) spray reagent (Menn et al., 1957). Silicic acid (Mallinckrodt CC-7) was used for column chromatography.

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Analytical gas chromatography was carried out with a Hewlett-Packard Model 402 high efficiency gas chromatograph fitted with a 6 ft × 2 mm i.d. glass U-tube column and an alkali (KCl) flame ionization detector (AFID). The column packing was prepared after the surface-modified support methodology of Aue et al. (1973) utilizing 6% EGSP-Z (Applied Science Laboratories, State College, PA) on HCl-extracted 80/100 mesh Chromosorb W, vacuum-coated and fluidized, conditioned for 24 h at 230 °C, and exhaustively extracted with chloroform. Gas flows for H, He, and air were 40, 36, and 320 mL/min, respectively. For malathion the column oven temperature was programmed from 70 °C for 4 min to 230 °C at 5 °C/min. For fenthion the temperature was programmed from 80 °C for 5 min to 180 °C at 5 °C/min, held for 30 min through emergence of peak 16 and increased to 220 °C at 5 °C/min. Preparative gas chromatography was carried out with the same equipment and conditions except for a 6 ft × 5 mm i.d. glass column and the effluent stream being split between a flame ionization detector (FID) and a collector.

Mass spectra (MS) of synthetic compounds were recorded by direct insertion probe in a Finnigan Model 1015 mass spectrometer interfaced with a data acquisition and reduction system (System Industries, System 150). An electron energy of 70 eV was used. Gas chromatography–mass spectroscopy (GS–MS) utilized the above instrument coupled to a Varian Aerograph Model 1400 gas chromatograph equipped with a column analogous to the analytical column described above. Helium was used as carrier gas (~10 mL/min). Samples were injected as acetone solutions (~1 mg/mL) with injection volumes of 0.5–3 μL, and an integration time of 10 s/scan with a 1-s delay between scans was generally used. Molecular ion (M^+) and fragment ion (m/e) intensities are quoted as percentages of the base peak.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian EM-390 instrument with tetramethylsilane as internal standard and lock signal. Chloroform-*d* was generally employed as solvent. Spectral data are quoted on the δ scale, in terms of chemical shift, assignment, multiplicity, and coupling constants. Abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet.

Chemicals. Technical fenthion (96%) was obtained through the Environmental Protection Agency from the Batelle Memorial Institute repository, Columbus, OH. Technical malathion (95%) was obtained from American Cyanamid Co., Princeton, NJ. *O,S,S*-Trimethyl phosphorodithioate, *O,O,S*-trimethyl phosphorothioate and bis(dimethoxyphosphinothioyl) sulfide were available from previous studies (Umetsu et al., 1977). *O,O*-Dimethyl phosphonothionate was prepared from dimethyl phosphite (Aldrich Chemical Co.) and phosphorus pentasulfide was prepared according to Yamasaki (1959).

O,O-Dimethyl phosphorodithioic acid was prepared from phosphorus pentasulfide and methanol by literature methods (Fletcher et al., 1950). The vacuum distilled product was further purified by salt formation, washing and recovery. NMR δ (neat liquid), 3.79 (–OCH_3, d, J = 15 Hz), 3.70 (–SH, s).

O,O,O-Trimethyl phosphorothioate was prepared from methanol and *O,O*-dimethyl phosphorochloridothioate. The product was vacuum distilled, then purified by column chromatography using an ether/hexane (1:2) solvent mixture.

Malaoxon was prepared by oxidation of malathion using bromine water (Wolfe et al., 1975), then purified by preparative TLC (ethyl acetate/benzene, 1:1): NMR δ (CCl_4) 4.20 and 4.08 (–OCH_2CH_3, overlapping q, J = 6 Hz), 3.8 and 3.79 (OCH_3, overlapping d, J = 12.5 Hz), 3.0 and 2.81 (–$CH_2CO_2C_2H_5$, ABq, each split into a d, J_{AB} = 13.5 Hz, J_A = 5 Hz, J_B = 4 Hz), 1.30 and 1.28 (–OCH_2CH_3, overlapping t, J = 6 Hz).

O,O,O,O-Tetramethyl pyrophosphorodithioate was synthesized by reaction of *O,O*-dimethyl phosphorochloridothioate with a water/pyridine mixture (Toy, 1951). Vacuum distillation yielded several fractions which by NMR and TLC analysis were found to contain the desired product together with varying amounts of the *O,O,O,S*-tetramethyl isomer. Purification was effected by preparative TLC (ethyl acetate/benzene, 1:1).

Purification of Technical Products. Malathion was purified by slow recrystallization of the technical material, as previously described (Umetsu et al., 1977). The mother liquors were concentrated, then fractionated as described below. Attempts to purify fenthion by crystallization at reduced temperatures were unsuccessful. Accordingly, the material was subjected to column chromatographic cleanup (hexane to ether gradient). NMR δ 7.18 (Ar–H, broad m), 3.79 (OCH_3, d, J = 14 Hz), 2.38 and 2.31 (–SCH_3 and $ArCH_3$, s).

Fractionation of Malathion and Fenthion. The impurity rich mother liquors from the malathion crystallization were column chromatographed (5 g applied to 140 g of silicic acid) using a hexane to ether gradient. Fractions (~18 mL) were automatically collected, then combined as dictated by analytical TLC (hexane/ether, 3:1 or 1:1). The combined fractions were analyzed by GC prior to GC–MS experiments. A similar approach was utilized for technical fenthion, and samples for GC–MS were also prepared by preparative GC.

Photolysis. Purified malathion was dissolved in acetone (40 mg/mL) and the solution spread on either a glass surface or analytical silicic acid TLC plate. The solvent was evaporated, then the plates were either placed in direct sunlight or irradiated with a medium pressure 140-W Hanovia mercury lamp set 10 in. above the surface. In the latter case, air was rapidly circulated over the plates to prevent ozone buildup, and the average reaction temperature was 38 °C. After the desired exposure time, products were recovered by rinsing the glass surfaces or silicic acid with acetone, and the mixtures were analyzed by GC.

Toxicological Evaluation. Mouse acute oral LD_{50} determinations utilized female Swiss white mice (25–30 g, Simonsen Laboratories, Gilroy, CA). Individual compounds were administered as corn oil solutions (0.10 mL/animal) following a 6-h fasting period. In potentiation studies, the "pretreatment" method using mice was adopted to obtain higher sensitivity in detecting potentiating activity. Purified malathion was administered in corn oil 4 h after treatment with the impurity. For fenthion LD_{50} data, female rats (Sprague-Dawley derived, ~250 g), from the Psychology Department colony, University of California, Riverside, were administered corn oil solutions (1.0 mL/200 g rat) following a 6-h fasting period. Mortalities were evaluated 48 h after treatment. Groups of 30 animals, divided into six different dose groups of five each, were used. Dose vs. percentage mortality was plotted on logarithm-probit paper, and the LD_{50} was obtained from an eye-fitted line.

Enzymology. Reagents for malathion carboxylesterase enzyme assay were obtained from Sigma Chemical Co., St. Louis, MO. Mouse serum and liver malathion carboxylesterase activities were assayed spectrophotometrically by coupling malathion hydrolysis to reduction of a tetrazolium dye at 37 °C (Talcott et al., 1979). The assay mixture in

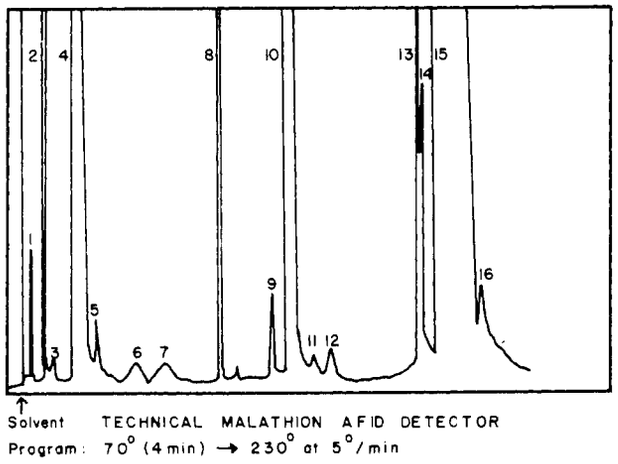

Figure 1. GC trace showing impurities present in technical malathion having shorter retention times than malathion: compound 1, $(CH_3O)_2P(S)H$; 2, $(CH_3O)_3P{=}S$; 3, unknown; 4, $(CH_3O)_2P(S)SCH_3$; 5, S_8; 6, $(CH_3O)_2P(O)SCH_3$; 7, $(CH_3S)_2P(O)OCH_3$; 8, $(CH_3O)_2P(S)OP(S)(OCH_3)_2$; 9, $(CH_3O)_2P(S)SP(O)(OCH_3)_2$; 10, $(CH_3O)_2P(S)SP(S)(OCH_3)_2$; 11, unknown; 12, unknown; 13 and 14, mixed carboethoxy and carbomethoxy malathion esters; 15, malathion; 16, malaoxon.

0.1 M Tris-HCl buffer (2.5 mL, pH 7.5) contained alcohol dehydrogenase (25 U/mL), NAD (1.8 mM), NAD-diaphorase (0.1 U/mL), *p*-iodonitrophenyltetrazolium violet (INT, 437 μg/mL), malathion (400 μM), and either liver homogenate (250 μg of protein/mL) or mouse serum (5 μL). To assay mouse serum carboxylesterase activity as a function of time, *O,S,S*-trimethyl phosphorodithioate (50 mg/kg) was administered orally to a group of eight mice. Blood samples were then drawn alternately from the orbital sinus (Riley, 1960) of four of the eight mice at selected intervals. The sera were assayed, values corresponding to each time point were averaged, and the results were expressed as percentages of control activity. To compare the effects of different impurities on esterase activity, groups of four mice were each dosed with the test compounds (50 mg/kg) and sacrificed 4 h later, and liver homogenates and sera samples were assayed for esterase activity (Talcott et al., 1979). Results were calculated in units of mg of malathion hydrolyzed min^{-1} (kg of body weight)$^{-1}$.

RESULTS

Phosphorus-Containing Impurities in Malathion. Separation of 14 impurities of shorter retention times than malathion, as shown in Figure 1, and 10 impurities of longer retention times was achieved by analytical temperature-programmed GC of technical malathion. For GC–MS analysis, however, impurities of longer retention times were unsuited since the ready fragmentation of the larger molecules precluded the observation of molecular ions and only malaoxon and the *S*-methyl isomer of malathion were assigned following GC comparisons with authentic compounds.

With the exception of the S_8 impurity (**5**), only phosphorus-containing impurities are indicated in Figure 1; the nonphosphorus compounds with shorter retention times than malathion have low sensitivities to the AFID. They were, however, readily observed in the gas chromatogram reconstructed from total ion current in the MS. *O,O*-Dimethyl phosphonothionate (**1**) and *O,O,O*-trimethyl phosphorothioate (**2**) showed molecular ions at *m/e* 126 (40%) and 156 (31%), respectively, with base peak at *m/e* 93. The latter fragment ion, assigned as $(CH_3O)_2P^+\cdot$, was characteristic of all dimethyl derivatized P=S compounds examined. Compounds **1** and **2**, which were also found in synthetic *O,O*-dimethyl phosphorodithioic acid, were confirmed by comparisons with authentic samples. *O,O,O,O*-Tetramethyl pyrophosphorodithioate (**8**) had an M^+ (*m/e*) 266 (8%) with fragment ions at 203 (7%), 125 (28%), 93 (100%), 79 (18%), and 63 (9%). Confirmation was also by comparison with an authentic sample. Compound **9** was tentatively assigned as the sulfide isomer of the pyrophosphorodithioate (**8**) on the basis of its MS fragmentation pattern [M^+, (*m/e*) 266 (2%), 235 (18%), 203 (28%), 142 (11%), 125 (100%), 109 (38%), 93 (72%), 79 (42%), 63 (30%), 47 (23%)] and its slightly shorter GC retention time than an authentic sample of the *S*-methyl isomer of **8**. *O,O*-Dimethyl phosphorodithioic acid proved too polar for GC analysis, and its structure was assigned after TLC comparisons with authentic material. None of these impurities constituted a significant percentage (<0.1%) of technical malathion.

Nonphosphorus Containing Impurities in Malathion. Structures were assigned to four nonphosphorus containing impurities on the basis of their mass-spectral fragmentation patterns. Diethyl fumarate almost coeluted with *O,O,S*-trimethyl phosphorodithioate and was characterized by fragment ions at *m/e* 127 (22%), 126 (16%), 99 (100%), 82 (8%), 71 (6%), and 55 (6%). Although there were some intensity differences, this pattern was otherwise identical with that for the authentic compound. In neither case was the molecular ion of *m/e* 172 found. Two related compounds, diethyl methylmercaptosuccinate and diethyl mercaptosuccinate were also found, with fragmentation patterns *m/e* 220 (M^+, 5%), 174 (41%), 146 (100%), 128 (30%), 105 (25%), 100 (40%), 75 (59%) and 55 (17%) and *m/e* 206 (M^+, 62%), 178 (77%), 159 (45%), 131 (100%), 103 (46%), 79 (61%), 73 (46%) and 55 (47%), respectively. The breakdown in each case is characteristic with initial loss of $C_2H_5O^-\cdot$ leading to the corresponding *O*-ethyl derivative of the anhydride radical cation, followed by loss of 28. The fourth compound was isolated by preparative TLC as a yellow crystalline solid. The MS showed a molecular ion of *m/e* 256 (10%), followed by a loss of 64, then consecutive losses of 32 to a base peak at *m/e* 64. This compound was assigned as S_8.

Malathion Potentiation. Table I lists the impurities tested for their effects on malathion toxicity in mice. The pyrophosphorodithioate (**8**) had the highest inherent toxicity (LD_{50}, 25 mg/kg), while malaoxon (**16**) and the dithiolate (**7**) showed moderate toxicity (215 and 400 mg/kg, respectively). The remaining compounds were less toxic but still more toxic than malathion itself. Our previous paper (Umetsu et al., 1977) showed that the malathion impurities caused less malathion potentiation in mice than in rats. This appears to be attributable to the lower rate of inhibition of carboxylesterase (which detoxifies malathion) by the impurities in mice than in rats. However, when mice were pretreated with potentiator and given sufficient time for inhibition of serum esterase to occur, then the potentiating effects on pure malathion were substantially greater than when the impurities and pure compounds were given together. By using this "pretreatment" method, it is possible to work with mice (less costly animal) to determine potentiating activity. Figure 2 shows that maximum inhibition of malathion mouse serum carboxylesterase activity following treatment with the dithiolate (**7**) was realized after 4 h. Therefore, for subsequent potentiation studies, malathion was administered to the animal 4 h after treatment with the impurity. The dithiolate (**7**) was found to be the best potentiator with a 10 mg/kg dose leading to a 3.5-fold enhancement of malathion toxicity, while the phosphoro-

Table I. Effect of Malathion Impurities on the Toxicity of Purified Malathion to Mice

impurity	LD_{50} of impurity, mg/kg	48-h oral LD_{50} of purified malathion (mg/kg) after treatment with dose of impurity, mg/kg[a]				
		0	5	10	50	100
2, $(CH_3O)_3P=S$	1150	6100			4400 (1.4)	3600 (1.7)
4, $(CH_3O)_2P(S)SCH_3$	1850	6100			2700 (2.3)[b]	2500 (2.4)
7, $(CH_3S)_2P(O)OCH_3$	400	6100		1750 (3.5)	600 (10.1)	
8, $(CH_3O)_2P(S)OP(S)(OCH_3)_2$	25	6100	3400 (1.8)	2100 (2.9)		
10, $(CH_3O)_2P(S)SP(S)(OCH_3)_2$	1500	4200[c]				3550[d]
16, $(CH_3O)_2P(O)SC(CH_2COOC_2H_5)HCOOC_2H_5$	215	6100		4100 (1.5)	2000 (3.1)	
17, $(CH_3O)_2P(S)SH$	1550	6100			2600 (2.3)	2150 (2.8)

[a] Mice were treated with each impurity 4 h before treatment with purified malathion. [b] Ratio: LD_{50} of purified malathion (6100 mg/kg) divided by the LD_{50} of purified malathion after pretreatment with impurity. [c] For a different batch of mice; a difference in sensitivity was indicated. [d] Purified malathion and impurity (5% in the purified malathion) were treated simultaneously.

Table II. Inhibition of Malathion Carboxylesterase Activities by Impurities from Technical Malathion[a]

impurity	dose, mg/kg	serum esterase, mg of MT[b] min^{-1} kg^{-1}		liver esterase, mg of MT[b] min^{-1} kg^{-1}		total esterase
control (no inhibitor)		30.2	(100)[c]	41.0	(100)[c]	71.2 (100)[c]
2, $(CH_3O)_3P=S$	50	27.3	(90)	34.4	(83)	61.7 (86)
	100	26.7	(88)	27.9	(68)	54.6 (77)
4, $(CH_3O)_2P(S)SCH_3$	50	21.5	(71)	26.4	(64)	47.9 (67)
7, $(CH_3S)_2P(O)OCH_3$	50	8.0	(26)	9.6	(23)	17.6 (25)
16, $(CH_3O)_2P(O)SC(CH_2COOEt)HCOOEt$	50	19.8	(64)	16.3	(40)	36.1 (50)
17, $(CH_3O)_2P(S)SH$	50	24.0	(79)	23.0	(56)	47.0 (66)

[a] Mice were pretreated with impurities at the given dose and 4 h later serum and liver malathion carboxylesterase activities were determined. Four animals were used at each dose level. [b] Malathion. [c] Percentage of control activity.

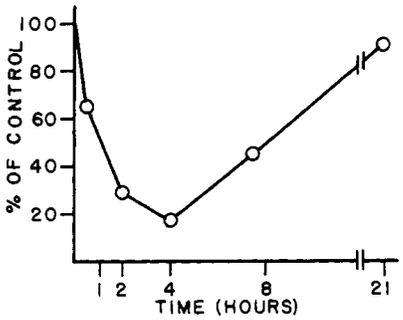

Figure 2. Variation of mouse serum malathion carboxylesterase activities with time after dosing orally with *O,S,S*-trimethyl phosphorodithioate (50 mg/kg). Eight mice received the test compound at time zero, and blood samples were obtained from four of the eight mice at various times after dosing. Residual malathion carboxylesterase activity in the serum was assayed. Results for each time point were averaged and expressed as percentage of activity measured in a control group.

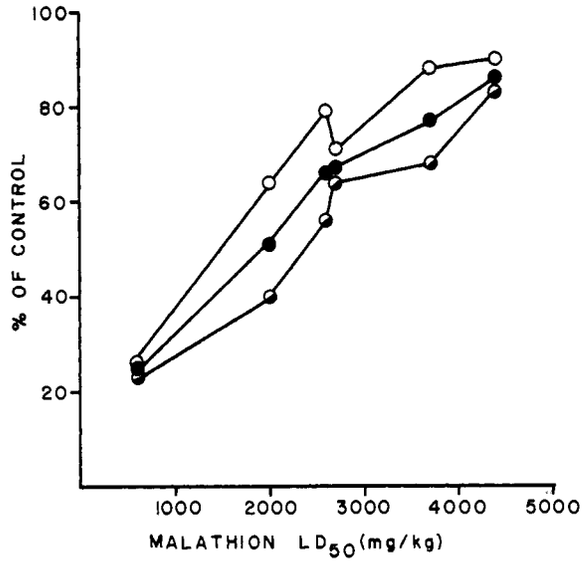

Figure 3. Relationship between malathion LD_{50} (mice) and malathion carboxylesterase activities. LD_{50} values for purified malathion after pretreatment of mice with impurities are from Table I; levels of inhibition of malathion carboxylesterase are from Table II. (○) Serum esterase, (◒) liver esterase, (●) total esterase (serum + liver).

thioate (**2**) was the weakest, with 100 mg/kg treatment resulting in a 1.7-fold increase (Table I). These results closely correlated with those for inhibition of mouse serum and liver carboxylesterase activities (Table II), and a direct relationship between malathion toxicity and enzyme inhibition by the impurities was found (Figure 3).

Photolysis of Malathion. Sunlight irradiation of recrystallized malathion, shown by GC to contain traces of *O,O,S*-trimethyl phosphorodithioate, malaoxon, and malathion mixed esters, on glass or silicic acid surfaces for periods up to 8 days led only to small changes in the relative intensities of the various components. However, rapid changes in composition did occur on irradiation with a medium pressure mercury lamp. After 1 h on the glass surface, GC analysis indicated the formation of three new phosphorus-containing compounds which eluted at 120, 141, and 157 °C. On the basis of comparison of retention times with those of authentic materials, the compounds eluting at 120 and 141 °C were assigned as *O,O,O,O*-tetramethyl pyrophosphorodithioate (**8**) and bis(dimethoxyphosphinothioyl) sulfide (**10**), respectively. Following irradiation for a further 3 h, all three of the new components increased at the expense of malathion, which was judged by relative intensities to have undergone an approximate 40% reduction. Malaoxon also showed a slight increase, and traces of two new compounds eluting at 163 and 164 °C appeared. (Under these GC conditions, malathion mixed esters, malathion, and malaoxon elute at 168, 170, and approximately 178 °C, respectively). A similar result occurred on the silicic acid surface; however, the compound eluting at 157 °C was formed in only minor

amounts and malaoxon formation was enhanced.

Fenthion Impurities. GC analysis of technical fenthion (96%) indicated 23 impurities, 9 of shorter and 14 of longer retention times than fenthion. Following GC–MS of impurity rich fractions obtained from either column chromatography or preparative GC, structures were assigned to eight compounds on the basis of their fragmentation patterns, with two, *O,O,S*-trimethyl phosphorodithioate and *O,O,O,O*-tetramethyl pyrophosphorodithioate, being confirmed by comparisons with authentic samples. Of the two other phosphorus-containing impurities, one showed a molecular ion of 232 (50%) with significant fragment ions at *m/e* 125 (49%), 109 (100%), 93 (49%), 79 (50%), 63 (35%), and 47 (28%). This pattern is characteristic of the breakdown of an $(-O)P(S)(OCH_3)_2$ moiety, as described above for the malathion impurities, and the additional substituent group must therefore be C_7H_7. In support of this assignment, fragment ions were found at *m/e* 91 (50%) and 77 (42%). Also an ion at *m/e* 107 (18%) was assigned as C_7H_7O. Since *m*-tolylphenol is a precursor to fenthion, the above impurity was formulated as *O,O*-dimethyl *O*-3-methylphenyl phosphorothioate. The fourth phosphorus-containing compound and the only compound of longer retention time than fenthion which was identified by GC–MS showed a molecular ion at *m/e* 278 (8%), thereby suggesting it to be an isomer of fenthion. The pattern attributable to the substituted phenyl moiety appeared unchanged but several differences in relative intensities of ions at *m/e* 110 (20%), 109 (20%), 79 (40%), 47 (38%), and 45 (30%) and the absence of an ion at *m/e* 93 suggested a phosphorothiolate rather than the phosphorothionate grouping. Similar differences were found on comparison of the MS of *O,O,O*-trimethyl phosphorothioate with *O,O,S*-trimethyl phosphorothioate. Accordingly, this impurity was assigned as *O,S*-dimethyl *O*-(3-methyl-4-methylthiophenyl) phosphorothioate.

Four other phosphorus-containing impurities with retention times longer than fenthion have also been tentatively identified as the sulfoxide and sulfone of fenthion and the sulfoxide and sulfone of fenoxon on the basis of GC comparisons with authentic samples.

Four nonphosphorus containing compounds were also assigned as follows: 3-methyl-4-methylthioanisole [M^+, *m/e* 168 (100%), 153 (88%), 138 (47%), 125 (15%), 121 (15%), 109 (50%), 107 (25%), 91 (25%), 77 (38%), and 45 (63%)], 3-methyl-4-methylthiophenol [M^+, *m/e*, 154 (92%), 139 (62%), 109 (23%), 95 (54%), 78 (23%), 77 (46%), 69 (23%), 65 (23%), 55 (31%), 51 (23%), and 45 (100%)], 5-methyl-2,4-di(methylthio)phenol [or the 2,6-di(methylthio) isomer] [M^+, *m/e*, 200 (60%), 212 (30%), 109 (40%), 107 (30%), 93 (30%), 91 (30%), 77 (40%), 51 (29%), 44 (70%), 40 (100%)], and 4-methyl-2,4-di(methylthio)anisole [or the 2,6-di(methylthio) isomer] [M^+, *m/e*, 214 (78%), 199 (33%), 184 (45%), 155 (33%), 109 (44%), 107 (33%), 77 (44%), 69 (56%), 55 (45%), 44 (78%), 40 (100%)].

Toxicological Evaluation of Technical Fenthion. Technical fenthion (96%) had a rat acute oral LD_{50} value of 1025 mg/kg compared with 1100 mg/kg for material purified by column chromatography. This minor difference most likely reflects the small amount of fenoxon present in the technical material. The poisoning symptoms shown by the rats were those typical of cholinesterase inhibition.

DISCUSSION

Malathion. The question of impurity mediated potentiation is particularly pertinent to malathion since the *S*-methyl isomeride of malathion has appeared as a likely contributor to an epidemic of malathion-induced poisoning in Pakistan (Baker et al., 1978). In consideration of the magnitude of the potentiation by *O,S,S*-trimethyl phosphorodithioate (Umetsu et al., 1977), this compound may also be implicated (Talcott et al., 1979). In the present study, several additional impurities have been identified. However, none have high potentiating activity.

The occurrence of *O,O*-dimethyl phosphonothionate (1) and *O,O,O*-trimethyl phosphorothioate (**2**) as impurities is expected during synthesis of *O,O*-dimethyl phosphorodithioic acid and they could be carried through along with the dithioic acid during subsequent steps. The presence of traces of the pyrophosphorodithioate suggests some radical-type side reaction since this compound occurs as a major photoalteration product. The use of GC–MS to determine impurities in technical products is generally a rapid and convenient method, although in the present situation application was found to be limited to the impurities with shorter retention times than malathion. Apart from the poorer GC resolution at higher elution temperatures, molecular ions could not be obtained for any of the larger molecules; malathion itself, using direct insertion probe, gave only a 2% M^+, and this was not enhanced on lowering the ionizing energy. Application of chemical ionization mass spectroscopy should overcome this problem.

As in the previous study (Umetsu et al., 1977) several nonphosphorus containing impurities were found, each of which could be anticipated from either the synthesis or the subsequent decomposition of malathion. Since these compounds were not of interest to the toxicological aspects of the present study, they were not pursued further.

Malathion Potentiation Studies. The organophosphorus impurities tested in mice varied widely in their inherent toxicities and effectiveness as potentiators of malathion toxicity (Table I). With the exception of the dithiolate (**7**) and malaoxon (**16**), the compounds generally were poor potentiators. Whether or not the pyrophosphorodithioate (**8**) is a malathion potentiator is unclear since it has such a high inherent toxicity. In contrast to rat studies, potentiation effects in mice are not readily apparent if the impurity and malathion are administered simultaneously. For example, simultaneous treatment of mice with malathion and 126 mg/kg of **4** led to a 1.27 enhancement of malathion toxicity (Umetsu et al., 1977) compared with a 50 mg/kg pretreatment, giving a 2.3-fold increase in the present study. Similarly, 10 mg/kg pretreatment with **7** gave a 3.5-fold enhancement compared with 22.3 mg/kg simultaneously administered, leading to a 1.42-fold increase previously. Comparisons of the present data with those from the previous study (Umetsu et al., 1977) clearly show rats to be the more sensitive species for the recognition of potentiation effects. Thus while the "pretreatment" method may further enhance the magnitude of the potentiation effect when evaluated in rats, it also demonstrates the feasibility of using the smaller animal.

Since the primary cause of potentiation appears to be carboxylesterase inhibition by the impurities (Talcott et al., 1979), the relationships between total serum and liver carboxylesterase activities and malathion lethality in mice following treatment with selected impurities were examined (Table II). The linear correlations observed (Figure 3) further supports the thesis that inhibition of carboxylesterase by the malathion impurities is the primary cause of malathion potentiation in mammals.

Malathion Photoalteration. As found by previous workers (Okada et al., 1962), sunlight induced decompo-

sition of malathion was slow, and accordingly GC analysis using a phosphorus specific detector indicated only minor changes in the relative intensities of the components initially present. In contrast, irradiation with ultraviolet light gave rapid breakdown. For example, in a 10^{-3} M solution of the 50% emulsifiable concentrate malathion had a half-life of 1.2 h (Okada et al., 1962). Other workers have also reported the degradation of malathion by ultraviolet light to less polar compounds, but no products were identified (Cook and Ottes, 1959; Mitchell, 1961). In the present study, the identification of *O,O,O,O*-tetramethyl pyrophosphorodithioate and bis(dimethoxyphosphinothioyl) sulfide as two of the three major phosphorus-containing photoproducts is of interest from both the mechanistic and toxicological points of view. Although the latter compound is relatively nontoxic (LD_{50}, rat: 1500 mg/kg), the pyrophosphorodithioate has high acute toxicity (LD_{50}, rat: 25 mg/kg). These compounds most likely arise from radical formation and recombination reactions. The GC characteristics of the third photoproduct, move prevalent from irradiation of malathion on a glass surface than on silicic acid, also suggest it to be of the pyrophosphate type. This compound was not found as an impurity in technical malathion. The investigation of the photochemical behavior of malathion, including solution photolyses, directed toward a refined definition of both the types of products formed and mechanisms involved is proceeding.

Fenthion. GC–MS techniques, as applied to impurity rich fractions from either column chromatography or preparative GC of technical fenthion (96%), were well suited to the determination of impurities with shorter retention times than fenthion. As with malathion, GC analysis of the impurities with longer retention times was not generally successful. In this case, however, this most likely reflects the poorer GC–MS resolution of these compounds rather than their ready fragmentation. Authentic standards of fenthion and its *S*-methyl isomer both gave significant molecular ions; 68 and 50%, respectively.

Fenthion is produced by the condensation of *O,O*-dimethyl phosphorochloridothioate and 3-methyl-4-(methylthio)phenol, the latter being synthesized from reaction of *m*-cresol with either Me_2SO or methyl disulfide and sulfuryl chloride (Eto, 1974). The nonphosphorus containing impurities then most likely arise during synthesis of the substituted phenol, and when possible isomers are indicated, the first-mentioned compound in each case is favored from steric considerations. The presence of *O,O,S*-trimethyl phosphorodithioate is feasible if the phosphorochloridothioate is produced from chlorination of *O,O*-dimethyl phosphorodithioic acid, which could then undergo methylation. The *S*-methyl isomer of fenthion represents the thiono–thiolo rearrangement reaction, and this may arise either during the condensation reaction, or on storage. The 3-methylphenyl analogue of fenthion would arise from condensation of 3-methylphenol with the phosphorochloridothioate and the occurrence of the pyrophosphate suggests a trace of moisture was present in the latter compound.

In an earlier fenthion study (Metcalf et al., 1963), the fenthion and fenoxon sulfoxides and sulfones were also found and their insecticidal activities were examined. All of the oxidation products showed toxicities similar to fenthion when applied topically to houseflies with the exception of fenthion sulfone which was significantly less toxic. In a study of the anticholinesterase activities and acute mammalian toxicities of these types of compounds (DuBois and Kinoshita, 1964), the fenoxon series and the *S*-methyl isomer of fenthion were found to be good cholinesterase inhibitors and markedly more toxic than fenthion. While the increased anticholinesterase activity was also recognized in the insect study (Metcalf et al., 1963), the minor changes in acute toxicity were rationalized in terms of reduced penetration.

In the present study, little difference in the mammalian toxicity was found between purified and technical fenthion. Since the presence of impurities did not suggest potentiation phenomena, individual compounds were not further evaluated.

LITERATURE CITED

Aue, W. A.; Hastings, C. R.; Kapila, S. *J. Chromatogr.* **1973**, *77*, 299.
Baker, E. L., Jr.; Zack, M.; Miles, J. W.; Alderman, L.; McWilson, W.; Dobbin, R. D.; Miller, S.; Teeters, W. R. *Lancet* **1978**, *1*, 31.
Casida, J. E.; Sanderson, D. M. *J. Agric. Food Chem.* **1963**, *11*, 91.
Cook, J. W.; Ottes, R. *J. Assoc. Off. Anal. Chem.* **1959**, *42*, 211.
DuBois, K. P.; Kinoshita, F. *Toxicol. Appl. Pharmacol.* **1964**, *6*, 86.
Eto, M., "Organophosphorus Pesticides: Organic and Biological Chemistry"; C.R.C. Press, Cleveland, OH, 1974.
Fletcher, J. H.; Hamilton, J. C.; Hechenbleicher, I.; Hoeberg, E. I.; Sertl, B. J.; Cassaday, J. T. *J. Am. Chem. Soc.* **1950**, *72*, 2461.
Frawley, J. P.; Fuyat, H. N.; Hagan, E. C.; Blake, J. R.; Fitzhugh, O. C. *J. Pharmacol. Exp. Ther.* **1957**, *121*, 96.
Menn, J. J.; Erwin, W. R.; Gordon, H. T. *J. Agric. Food Chem.* **1957**, *5*, 601.
Metcalf, R. L.; Fukuto, T. R.; Winton, M. Y. *Bull. WHO* **1963**, *29*, 219.
Mitchell, L. C. *J. Assoc. Off. Anal. Chem.* **1961**, *44*, 643.
Okada, K.; Nomura, K.; Yamamoto, T. *Nippon Noegi Kagaku Kaishi* **1962**, *32*, 117.
Pelligrini, G.; Santi, R. *J. Agric. Food Chem.* **1972**, *20*, 944.
Riley, V. *Proc. Soc. Exp. Biol. Med.* **1960**, *104*, 751.
Talcott, R. E.; Mallipudi, N. M.; Umetsu, N.; Fukuto, T. R. *Toxicol. Appl. Pharmacol.* **1979**, *49*, 107.
Toy, A. D. F. *J. Am. Chem. Soc.* **1951**, *73*, 4670.
Umetsu, N.; Grose, F. H.; Allahyari, R.; Abu-El-Haj, S.; Fukuto, T. R. *J. Agric. Food Chem.* **1977**, *25*, 946.
Wolfe, N. L.; Cox, R. H.; Gordon, J. A. *J. Agric. Food Chem.* **1975**, *23*, 1213.
Yamasaki, T. *Sci. Repts. Res. Inst., Tohoku Univ., Sera A* **1959**, *11*, 73; *Chem. Abstr.* **1960**, *54*, 24339.

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