Interfering ECD-sensitive peaks at short t_R, from side reactions of PFB-Br, can be removed on a short silica gel column since they elute with 5% toluene in hexane and the PFB-pyrethroid derivatives can be subsequently eluted with toluene (modified from Kovač and Anderle, 1978).

Pentafluorobenzylation provides a rapid and convenient method for introducing a highly ECD sensitive substituent into α-cyanophenoxybenzyl pyrethroids. These PFB derivatives may be useful in confirming the identity of residues analyzed by other methods and, with a suitable cleanup procedure, in enhancing the sensitivity of residue analysis.

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Resolution, Absolute Configuration, and Acute and Delayed Neurotoxicity of the Chiral Isomers of O-Aryl O-Methyl Phenylphosphonothioate Analogues Related to Leptophos

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The chiral isomers of O-methyl phenylphosphonothioic acid, O-(4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonothioate (leptophos), and O-(2,5-dichlorophenyl) O-methyl phenylphosphonothioate (desbromoleptophos) were prepared and their toxicological properties were examined. The absolute configurations of the enantiomers of leptophos and desbromoleptophos were assigned by relating them to the configurations of the corresponding O-methyl phenylphosphonothioic acids. The absolute configuration of the (−)-α-methylbenzylammonium salt of (−)-O-methyl phenylphosphonothioic acid was established by X-ray diffraction analysis. Optical purity was assessed by chiral pseudo-contact lanthanide shift reagents and hydrolysis of the esters. The $(R)_p(+)$ isomers of leptophos and desbromoleptophos were more acutely toxic to the housefly and white mouse, while the $(S)_p(-)$ isomers were more delayed neurotoxic when administered intraperitoneally to the hen.

It is well known that chirality at the phosphorus atom of an organophosphorus ester often has a significant effect on the biological activity of the ester. The difference in toxicity of the enantiomers of chiral organophosphorus poisons has been attributed to differences in their ability to inhibit acetylcholinesterase and other esterases and to differences in their rates of metabolism in animals (Lee et al., 1978).

Recently, the effect of phosphorus chirality on the delayed neurotoxicity of the enantiomers of EPN [O-ethyl O-(p-nitrophenyl) phenylphosphonothioate] and the corresponding oxons was described (Ohkawa et al., 1977b; Abou-Donia et al., 1978). The dextrorotatory isomer was several-fold more acutely toxic to hens, houseflies, and the rice stem borer, while the levorotatory isomer was more delayed neurotoxic to hens. Such differences in the toxicological properties of the chiral isomers suggest that the active site associated with delayed neurotoxicity is stereochemically different from the site associated with acute toxicity, i.e., acetylcholinesterase. It has been hypothesized that organophosphorus induced delayed neurotoxicity is attributable to inhibition of an enzyme identified as neurotoxic esterase (Johnson, 1975).

O-(4-Bromo-2,5-dichlorophenyl) O-methyl phenylphosphonothioate (leptophos) and all the corresponding mono- and dichlorophenyl analogues also have been shown to cause delayed neurotoxicity (Hollingshaus et al., 1979). This paper is concerned with the resolution and determination of the absolute configuration of the chiral isomers of leptophos and desbromoleptophos and the toxicological properties of these compounds.

MATERIALS AND METHODS

General. Optical rotations were determined with a

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Table I. Physical Constants and Values of Specific Rotation in Degrees Arc for the Compounds of General Structure

	R	abs config	mp or bp (mm), °C	n^{23}D	sp $[\alpha]^{24}$D
1	OH	racemic	100 (0.02)	1.5772	
1a		$(S)_P$			−21.0 (neat)
1b		$(R)_P$			21.72 (neat)
2	O⁻H₃N⁺CH(CH₃)C₆H₅	racemic	142–144		
2a		$(S)_P$	142–144		−17.68 (c 14.5)^a
2b		$(R)_P$	142–144		17.83 (c 10.75)^a
3		racemic	71–72		
3a		$(S)_P$	88–90		−46.26 (c 10.0)^b
3b		$(R)_P$	88–90		46.13 (c 10.6)^b
4		racemic	46–47		
4a		$(S)_P$	46–47		−23.03 (c 2.08)^b
4b		$(R)_P$	46–47		22.72 (c 2.24)^b

^{a} Specific rotation was measured in methanol. ^{b} Specific rotation was measured in benzene.

Perkin-Elmer Model 241 polarimeter or with a Rudolph Model 80 high-precision polarimeter at the sodium D line (589 nm). Proton magnetic resonance spectra were recorded on a Varian Model EM-390 spectrometer in chloroform-d, using tetramethylsilane as the internal standard. Infrared spectra were obtained with a Beckman Model 4240 spectrophotometer.

Thin-layer chromatography (TLC) was carried out on silica gel plates (Silica Gel 60 PF-254) with phosphor indicator, and SilicAR CC-7 Special was used for column chromatography. All melting and boiling points are uncorrected.

Synthesis and Resolution. O-Methyl phenylphosphonothioic acid (1) was prepared from phenylphosphonothioic dichloride (Aldrich Chemical Co.) according to Mitsunobu (1970). The O-methyl phenylphosphonothioic acid was purified as the dicyclohexylamine salt by recrystallization from 2-propanol, mp 155–156 °C. Racemic 1 was treated with (+)- or (−)-α-methylbenzylamine (Aldrich Chemical Co.), and the diastereomeric salts, i.e., (−)-phenylphosphonothioic acid–(−)-α-methylbenzylamine salt (2a) and (+)-phenylphosphonothioic acid–(+)-α-methylbenzylamine salt (2b), were separated by repeated recrystallization from ethyl acetate–hexanes (9:1) or ethyl acetate, as described by Allahyari et al. (1977). X-ray diffraction analysis was conducted on a highly purified sample of 2a, as described in a later section. Physical constants and values of optical rotation for the enantiomeric acids and α-methylbenzylammonium salts are presented in Table I.

Racemic and optically active O-methyl phenylphosphonochloridothioate was prepared from racemic and enantiomeric 1 and phosphorus pentachloride in carbon tetrachloride at −5 °C according to Michalski and Mikolajczyk (1964). Because of its tendency to racemize upon heating, the chloridothioate was not isolated nor purified but was reacted with the potassium salt of 4-bromo-2,5-dichlorophenol or 2,5-dichlorophenol after removal of the phosphorus oxychloride. In the initial preparation of resolved leptophos, distillation of the chloridothioate under reduced pressure gave a final product of substantially lower

optical activity. Resolved leptophos (3a, 3b) and desbromoleptophos (4a, 4b) were purified by column chromatography using hexanes–benzene (9:1) as the solvent and recrystallization from pentane–benzene (7:1). Physical constants and values of optical rotation for the final esters are presented in Table I.

Optical Purity. Assessment of the optical purity of 1a (α^{23}D −21.0° neat) was accomplished by converting it to O,S-dimethyl phenylphosphonothioate (5a) and by NMR analysis of the ester with the aid of a pseudo-contact shift reagent (Koizumi et al., 1977). The acid, 1a, obtained from 2a, was converted to the sodium salt in methanolic sodium methoxide and treated with a slight excess of dimethyl sulfate, giving 5a, purified by means of a microdistillation still, 0.01 torr and bath temperature of 100 °C. The shift reagent, tris[3-(trifluoromethylhydroxymethylene)-d-camphorato]europium (III) (Aldrich Chemical Co.), was added to 5a in the molar ratio 2:3.5.

The optical purity of (−)-leptophos (3a) and (−)-desbromoleptophos (4a) was determined in a similar manner. A 350-mg sample of 3a ($[\alpha]^{24}$D −46.26) was converted to the corresponding oxon [6a, O-4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonate] by reaction with m-chloroperoxybenzoic acid in dichloromethane as previously described (Wustner et al., 1972; Allahyari et al., 1977). Increments of the europium shift reagent were added to a sample of 6a in deuteriochloroform, using Me₄Si as the internal standard. A similar sample of 4a ($[\alpha]^{24}$D −23.03) was converted to its corresponding oxon [7a, O-(2,5-dichlorophenyl) O-methyl phenylphosphonate] and analyzed for optical purity by the same procedure. In addition, a sample of 4b ($[\alpha]^{24}$D +22.72) was hydrolyzed with 2 equiv of sodium hydroxide in aqueous dioxane and the rotation of the resultant acid was compared with the rotation of the original acid 1a.

Determination of Absolute Configuration. Colorless crystals of 2a, mp 142–144 °C, $[\alpha]^{23}$D −17.68°, were obtained after four recrystallizations from ethyl acetate–hexanes. The following crystal data were obtained (from single crystal diffractometer, Mo Kα, λ = 0.70954 Å): PSO₂NC₁₅H₂₀, fw 309.4, orthorhombic, space group $P_{2_12_12_1}$,

Table II. Fractional Atomic Coordinates ($\times 10^4$)^a for $(S)_P$-(-)-α-Methylbenzylammonium O-Methyl Phenylphosphonothioate (2a)

atom	x	y	z	atom	x	y	z
S	3069 (1)	1627 (1)	4805 (3)	H1	5237	1009	9838
P	3740 (1)	1290 (1)	2302 (2)	H2	6703	1296	234
O1	3576 (2)	458 (2)	1553 (9)	H3	7200	1907	3329
O2	3560 (2)	1813 (3)	183 (7)	H4	6252	2243	6112
N	1675 (2)	151 (2)	243 (1)	H5	4780	1968	5744
C1	4864 (3)	1465 (3)	275 (1)	H6	55	138	9463
C2	5445 (3)	1265 (4)	112 (1)	H7	8593	9874	9929
C3	6311 (4)	1433 (4)	135 (1)	H8	7882	283	2999
C4	6600 (4)	1796 (4)	317 (1)	H9	8608	958	5681
C5	6042 (4)	1992 (3)	484 (1)	H10	83	1217	5345
C6	5171 (3)	1831 (3)	462 (1)	H11	3159	2874	1214
C7	3633 (5)	2657 (4)	26 (1)	H12	4196	2805	919
C8	1139 (3)	896 (3)	212 (1)	H13	3580	2881	8778
C9	1360 (4)	1264 (4)	993 (1)	H14	1046	1780	9755
C10	205 (3)	701 (3)	236 (1)	H15	1995	1373	9871
C11	9757 (3)	305 (3)	74 (1)	H16	1202	893	8733
C12	8894 (4)	148 (4)	102 (1)	H17	2264	283	2285
C13	8475 (4)	390 (4)	283 (1)	H18	1571	9938	3833
C14	8899 (5)	788 (4)	441 (1)	H19	1523	9771	1349
C15	9775 (4)	943 (4)	423 (1)	H20	1323	1315	3415

^{a} Only non-hydrogen parameters were varied.

$a = 15.611$ (7), $b = 16.787$ (9), $c = 6.136$ (1) Å, $V = 1608$ (1) Å^3, $D_c = 1.28$ g/cm^3 for $Z = 4$, $\mu = 2.35$ cm^{-1}.

Intensity data were collected at room temperature on an automated four-circle diffractometer designed and built in the Ames Laboratory, Iowa State University (Rohrbaugh and Jacobson, 1974). The diffractometer is equipped with a scintillation counter and is interfaced to a PDP-15 minicomputer which operates in a time-sharing mode. Graphite-monochromated Mo Kα radiation was used for data collection. All data (6739 reflections) within a 2θ sphere of 50° were measured in the hkl, $\bar{h}kl$, $hk\bar{l}$, and $\bar{h}k\bar{l}$ octants using a ω-scan data collection technique.

The intensity data were corrected for Lorentz–polarization effects. The σ_F's were calculated from the σ_I's by the finite difference method (Lawton and Jacobson, 1968). Equivalent data were averaged and 1561 reflections with $F_o > 3\sigma_F$ were retained for structural refinement.

The phosphorus and sulfur positions were determined by conventional Patterson techniques, and the remaining non-hydrogen atoms were located by successive structure factor and electron-density map calculations (Lapp and Jacobson, 1979; Hubbard et al., 1971). These positions were then refined by a block-diagonal least-squares procedure. Hydrogen atoms were then included; aromatic hydrogen positions were calculated at 0.95 Å from the respective carbons, and the methyl and amino hydrogens were then located on an electron-density difference map. All non-hydrogen atoms were refined anisotropically; hydrogen parameters were not refined. After two cycles of full-matrix refinement the conventional residual index, R, converged to 0.069.

Acute Toxicity. Insecticidal activity was determined using S_{NAIDM} strain, as described by March and Metcalf (1949). Treated flies were held at 15 °C and the mortality was estimated after 24 h. Mammalian toxicity was determined with 25–35-g female Swiss white mice obtained from Simonson Laboratories, Gilroy, CA. Solutions of the toxicants in corn oil were administered orally at 0.10 mL/mouse to animals fasted for 6 h before treatment. LD$_{50}$ values were based on 24-h mortality, using 5–11 mice/dose and at least five different doses/compound.

Delayed Neurotoxicity. Delayed neurotoxic activity was determined using adult white leghorn hens, 1.3–1.8 kg in weight and 24 months in age. Toxicants were administered intraperitoneally in Me$_2$SO, with both untreated
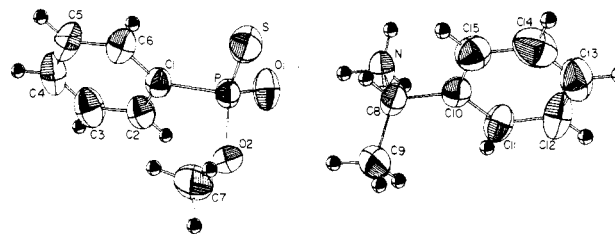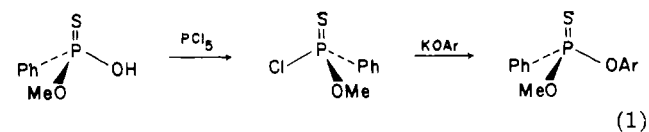

Figure 1. Stereoscopic view of $(S)_P$-(-)-α-methylbenzylammonium O-methyl phenylphosphonothioate.

and Me$_2$SO-treated controls. Leptophos isomers were tested using two–five hens/dose at doses from 50 to 150 mg/kg. Desbromoleptophos isomers were tested using three–ten hens/dose at doses from 1 to 50 mg/kg. Hens receiving toxicant were given 10 mg/kg of atropine sulfate in 0.9% saline (intramuscularly) 15 min before treatment. Hens were observed three times each week for symptoms of ataxia over a 4-week period. The severity of ataxia was graded as described by Davies and Holland (1972).

RESULTS AND DISCUSSION

Determination of Absolute Configuration. The synthesis of the chiral isomers of leptophos and desbromoleptophos was carried out according to eq 1 for one of

$$\underset{MeO}{\overset{S}{\underset{Ph}{\overset{\parallel}{P}}}}\!\!-OH \quad \xrightarrow{PCl_5} \quad \underset{OMe}{\overset{S}{\underset{Cl}{\overset{\parallel}{P}}}}\!\!-Ph \quad \xrightarrow{KOAr} \quad \underset{MeO}{\overset{S}{\underset{Ph}{\overset{\parallel}{P}}}}\!\!-OAr$$

(1)

the isomers. The procedure is essentially the same as described earlier for the synthesis of the chiral isomers of fonofos (Allahyari et al., 1977).

The absolute configuration of (-)-O-methyl phenylphosphonothioic acid (1a) was established to be $(S)_p$ by X-ray diffraction analysis of the (-)-α-methylbenzylammonium salt of the acid (2a). A computer drawing of 2a is shown in Figure 1. Fractional atomic coordinates are given in Table II and values for interatomic distances and interatomic angles are in Table III.

Owing to a pseudosymmetry problem, the determination of the absolute configuration of (-)-leptophos (3a) has not been successful. However, assignment of configuration of the leptophos and desbromoleptophos enantiomers may

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Table III. Values for Interatomic Distances and Angles for $(S)_P$-(-)-α-Methylbenzylammonium O-Methyl Phenylphosphonothioate (2a)

Interatomic Distances[a]					
P-S	1.943 (2)	C3-C4	1.35 (1)	C10-C11	1.384 (9)
P-O1	1.492 (4)	C4-C5	1.39 (1)	C11-C12	1.382 (9)
P-O2	1.594 (5)	C5-C6	1.393 (9)	C12-C13	1.35 (1)
P-C1	1.801 (6)	C6-C1	1.39 (1)	C13-C14	1.35 (1)
O2-C7	1.422 (8)	C8-C9	1.52 (1)	C14-C15	1.39 (1)
C1-C2	1.393 (9)	C8-N	1.516 (7)	C15-C10	1.40 (1)
C2-C3	1.387 (9)	C8-C10	1.502 (8)		

Interatomic Angles[b]			
S-P-O1	115.1 (2)	C4-C5-C6	119.7 (7)
S-P-O2	112.9 (2)	C5-C6-C1	120.2 (7)
S-P-C1	110.8 (3)	C9-C8-N	108.7 (6)
O1-P-O2	103.6 (3)	C9-C8-C10	113.3 (6)
O1-P-C1	111.6 (2)	N-C8-C10	110.1 (4)
O2-P-C1	102.0 (3)	C8-C10-C11	121.7 (6)
P-O2-C7	120.3 (5)	C8-C10-C20	119.0 (6)
P-C1-C2	118.9 (5)	C11-C10-C15	119.2 (6)
P-C1-C6	122.5 (5)	C10-C11-C12	119.8 (7)
C2-C1-C6	118.5 (5)	C11-C12-C13	121.0 (7)
C1-C2-C3	120.9 (7)	C12-C13-C14	120.1 (7)
C2-C3-C4	120.0 (7)	C13-C14-C15	121.0 (8)
C3-C4-C5	120.7 (6)	C14-C15-C10	118.9 (7)

[a] Angstroms. [b] Degrees.

be made on the basis of the known stereochemistry of reactions related to those described in eq 1. Previous work in this laboratory (Allahyari et al., 1977) showed that conversion of resolved O-ethyl ethylphosphonothioic acid to O-ethyl ethylphosphonochloridothioate by the action of phosphorus pentachloride occurred with inversion of the phosphorus center. Reaction of the chloridothioate with sodium benzenethiolate to give fonofos (O-ethyl S-phenyl ethylphosphonodithioate) also occurred with inversion. Others have shown that displacement reactions of these types take place with inversion of configuration (Michalski and Mikolajczyk, 1966). Assuming that inversion takes place in each step of the two-step reaction from 1a to either 3b or 4b, the absolute configuration of these products is $(R)_p$, as indicated in eq 1. Conversely, 3a and 4a are $(S)_p$.

Additional support for the assignment of absolute configuration was obtained from examination of the hydrolysis of both leptophos and desbromoleptophos to O-methyl phenylphosphonothioic acid. (-)-O-Methyl phenylphosphonothioic acid, 1a [α^{24}_D -21.0 (neat)] was converted to 4b ([α]$^{24}_D$ +22.72). Hydrolysis of this material with 2 equiv of sodium hydroxide in aqueous dioxane and subsequent acidification returned (+)-O-methyl phenylphosphonothioic acid [α^{24}_D +8.0 (neat)]. Allahyari et al. (1977) have previously shown that hydroxide displacement reactions on the phosphorus atom occur with inversion of configuration. The acid obtained from this hydrolysis should therefore have a sign of rotation equal but opposite to that of 1a. The rotation observed suggests that 4b was only 69% optically pure. This was substantiated through a pseudo-contact lanthanide shift reagent analysis described below.

Another sample of (-)-O-methyl phenylphosphonothioic acid [α^{24}_D -14.43 (neat)] was converted to (+)-leptophos ([α]$^{24}_D$ +22.08, c 3.5 benzene) in the usual manner. Hydrolysis of this material returned (+)-O-methyl phenylphosphonothioic acid ([α]$^{24}_D$ +10.66, c 4.3 methanol). This study was carried out with one of the earlier resolved products, and even though the sample used was also optically impure, the results indicate that displacement reactions on the phosphorus atom occur with inversion of configuration. It should be added that (+)-O-methyl phenylphosphonothioic acid was dextrorotatory both neat and in methanol solution.

Table IV. Acute Toxicity of Racemic, $(R)_P$(+), and $(S)_P$(-) Isomers of Leptophos and Desbromoleptophos to Houseflies and Mice[a]

	LD$_{50}$	
compound	housefly (topical), μg/g	mouse (oral), mg/kg
desbromoleptophos		
($\pm$)	11.4[a]	54.9[a]
$(R)_P$(+)	10.4[a]	51.3[a]
$(S)_P$(-)	17.3[b]	63.7[a]
leptophos		
($\pm$)	14.1[a]	71.9[a]
$(R)_P$(+)	5.7[b]	64.4[a]
$(S)_P$(-)	22.2[c]	75.0[a]

[a] Values with common superscript are not significantly different, $P = 0.05$.

Assessment of Optical Purity. The NMR spectrum of racemic O,S-dimethyl phenylphosphonothioate showed doublets for OCH$_3$ and SCH$_3$ protons at δ 3.8 and 2.1 (J = 14 Hz), respectively. Addition of the pseudo-contact shift reagent tris[3-(trifluoromethylhydroxymethylene)-d-camphorato]europium(III) to the racemic material resulted in two doublets (separated by 2.4 Hz) for the SCH$_3$ protons. Treatment of the sodium salt of (-)-O-methyl phenylphosphonothioic acid (1a, α^{24}_D -21.0°) with dimethyl sulfate resulted in (-)-O,S-dimethyl phenylphosphonothioate (5a). Addition of the shift reagent to this material resulted in no separation of the SCH$_3$ doublet, indicating that the minus rotating ester was optically pure. Since the configuration at the phosphorus center was not affected in the conversion of 1a to 5a, the starting acid 1a also was optically pure.

Several chiral shift reagents were tried to determine the optical purity of the enantiomers of leptophos. However, addition of shift reagents to racemic leptophos did not effect splitting of the OCH$_3$ absorptions into two separate signals. The shift reagents also had no effect on the ^{31}P signal. In contrast to leptophos, addition of tris[3-(trifluoromethylhydroxymethylene)-d-camphorato]europium-(III) to leptophos oxon resulted in a 24-Hz splitting of the OCH$_3$ signal into two doublets. A sample of $(S)_p$-leptophos (3a, [α]$^{24}_D$ -46.26) was converted to the corresponding (-)-leptophos oxon (6a) by reaction with m-chloroperoxybenzoic acid. Addition of the shift reagent to 6a resulted in two OCH$_3$ doublets centered at δ 3.8, separated by 24 Hz. These corresponded to 98% 6a and 2% 6b [(+)-leptophos oxon], indicating that 3a was at least 98% optically pure. Similarly, a sample of $(S)_p$-desbromoleptophos (4a, [α]$^{24}_D$ -23.03) was converted to its corresponding oxon (7a). Addition of the shift reagent to 7a resulted in two OCH$_3$ doublets centered at δ 4.2, separated by 2.4 Hz. These corresponded to 72% 7a and 28% 7b [(+)-desbromoleptophos oxon], indicating that 4a was only 72% optically pure. The conversion of fonofos to its oxon has been shown to occur with retention of configuration (Allahyari et al., 1977).

Toxicity. The $(R)_P$(+) isomers of leptophos and desbromoleptophos were more toxic to houseflies and mice than were the $(S)_P$(-) isomers, although differences in toxicities were not large (Table IV). The largest difference in toxicity was observed for leptophos against houseflies, where the $(R)_P$(+) enantiomer was about fourfold more active than the $(S)_P$(-) enantiomer. In all other cases, the difference was substantially less than twofold; however, greater differences would be expected from optically pure desbromoleptophos. Overall, the acute toxicological data were similar to those reported for the (+) and (-) isomers

Table V. Delayed Neurotoxic Effect of Racemic (±)-, (R)p(+)-, and (S)p(-)-Leptophos in Atropinized Hens

| dose, mg/kg	(±)				(R)p(+)				(S)p(-)			
	no. treated	acute toxicity[a]	delayed neuro-toxicity	deg of ataxia[b]	no. treated	acute toxicity	delayed neuro-toxicity	deg of ataxia	no. treated	acute toxicity	delayed neuro-toxicity	deg of ataxia
50	5	0	0									
100	5	0	3	4-6	2	0	0		2	0	2	8
150	5	0	5	8								

[a] Death occurring within 72 h of treatment.   [b] 2 = mild, 4 = moderate, 6 = severe, 8 = total paralysis and death as described by Davies and Holland (1972).

Table VI. Delayed Neurotoxic Effect of Racemic (±)-, (R)p(+)-, and (S)p(-)-Desbromoleptophos in Atropinized Hens

dose, mg/kg	(±)				(R)p(+)				(S)p(-)			
	no. treated	acute toxicity[a]	delayed neuro-toxicity	deg of ataxia[b]	no. treated	acute toxicity	delayed neuro-toxicity	deg of ataxia	no. treated	acute toxicity	delayed neuro-toxicity	deg of ataxia
1	5	0	0		5	0	0		5	0	0	
5	3	0	0		5	1	0		5	0	1	2
10	10	0	1	2	8	0	1	2	8	0	2	2
20	7	0	4	4	3	0	2	2	3	0	2	4
30	5	0	4	4	3	0	3	4	3	0	3	6
40	3	0	3	6	3	1	2	4	3	0	3	6
50	3	0	3	6	3	0	3	6	3	0	3	8

[a] Death occurring within 72 h of treatment.   [b] 2 = mild, 4 = moderate, 6 = severe, 8 = total paralysis and death as described by Davies and Holland (1972).

of EPN [ethyl O-(p-nitrophenyl) phenylphosphonothioate] (Ohkawa et al., 1977b), cyanofenfos (Ohkawa et al., 1977a), and for the (R)p(+) and (S)p(-) isomers of fonofos (Lee et al., 1978). In all these cases, the (+)-rotating isomer showed higher activity. It should be pointed out, however, that the relative positions of various groups attached to the phosphorus atom in (R)p(+)-leptophos [or (R)p(+)-desbromoleptophos] and (R)p(+)-fonofos are different, i.e., the phenyl and methoxy moieties in (R)p-leptophos and the ethyl and ethoxy moieties in fonofos occupy opposite corners of a tetrahedron, as depicted by structures a and b.

a   b
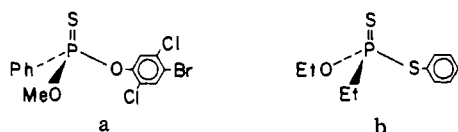
Sufficient quantities of completely resolved leptophos were not available for a comprehensive evaluation of its delayed neurotoxic activity. In a preliminary study, however, the (S)p(-) enantiomer (3a) produced severe paralysis in hens 19 days following treatment with 100 mg/kg, while the (R)p(+) enantiomer (3b) at the same time produced no symptoms of ataxia through 28 days posttreatment (Table V). Although quantitative conclusions cannot be made from the data, the results are in agreement with Ohkawa et al. (1977b) who reported that the (-) enantiomer of EPN produced ataxia in hens, while the (+) enantiomer produced no symptoms at any of the doses tested. Abou-Donia et al. (1978) similarly reported (-) enantiomers of both EPN and EPN oxon were more potent delayed neurotoxic agents than the respective (+) enantiomers.

A more extensive study with 72% optically pure desbromoleptophos is summarized in Table VI. There was little difference in the percentage of hens developing symptoms of ataxia at any given dose but the severity of ataxia was considerably greater for hens given the (S)p(-) enantiomer (4a) than for those given the (R)p(+) enantiomer (4b). In addition, hens treated with 4a developed symptoms of ataxia 11-14 days following treatment, while hens treated with 4b required 11-18 days to develop ataxia. The delay period was 11-14 days for all hens given racemic

mixture, 4a, or 4b at doses of 30, 40, and 50 mg/kg. At doses of 5, 10, and 20 mg/kg, however, hens given 4a developed ataxia 2-4 days sooner than hens given 4b or racemic compound. Such results would be expected from a partially resolved compound and are in agreement with the findings of Ohkawa et al. (1977b) and Abou-Donia et al. (1978).

It is apparent that both the (S)p(-) isomers of leptophos and desbromoleptophos and the (-) isomers of EPN and EPN oxon are more delayed neurotoxic, while the (R)p(+) isomers of leptophos and desbromoleptophos and the (+) isomers of EPN and EPN oxon are more acutely toxic. These findings add further support to the hypothesis that the biochemical lesion responsible for delayed neurotoxicity is separate from the lesion responsible for acute toxicity. Furthermore, the active site associated with organophosphorus induced delayed neurotoxicity must be stereochemically different from the acetylcholinesterase site associated with acute toxicity. To better understand the stereochemistry of organophosphorus induced delayed neurotoxicity, the stereospecificity of neurotoxic esterase as well as the metabolism of chiral phosphonates in both susceptible and nonsusceptible species must be determined. Investigations in these areas are currently in progress.

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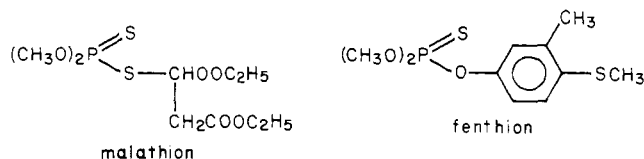
Identification and Toxicological Evaluation of Impurities in Technical Malathion and Fenthion

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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

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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*-di-

methyl *O*-(3-methyl-4-methylthiophenyl) phosphorothioate].

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.