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Ecotoxicology of Phenylphosphonothioates

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The phenylphosphonothioate insecticides EPN and leptophos, and several analogs, were evaluated with respect to their delayed neurotoxic effects in hens and their environmental behavior in a terrestrial-aquatic model ecosystem. Acute toxicity to insects was highly correlated with $\Sigma\sigma$ of the substituted phenyl group (regression coefficient $r = -0.91$) while acute toxicity to mammals was slightly less well correlated (regression coefficient $r = -0.71$), and neurotoxicity was poorly correlated with $\Sigma\sigma$ (regression coefficient $r = -0.35$)

Both EPN and leptophos were markedly more persistent and bioaccumulative in the model ecosystem than parathion. Desbromoleptophos, a contaminant and metabolite of leptophos, was seen to be a highly stable and persistent terminal residue of leptophos.

Introduction

The first reference to the chronic toxicity of phenylphosphonates was the evocation of delayed neurotoxicity in hens fed diets containing EPN [*O*-(4-nitrophenyl) *O*-ethyl phenylphosphonothioate] (*1*). EPN in 1956 was a minor pesticide, and since there was no indication of human danger, this early warning was ignored. Ten years later, as the phenylphosphonothioates began to complement the alkyl phosphates in replacing the extremely persistent organochlorine pesticides, the neurotoxicity of leptophos [*O*-(2,5-dichloro-4-bromophenyl) *O*-methyl phenylphosphonothioate] forced reconsideration of the real hazards of organophosphate-induced neurotoxicity (*2*). The present study compares the toxicity of nine phenylphosphonothioates in terms of the traditional parameters of their acetylcholinesterase inhibition in flies and their acute toxicity in rats, and also in terms of their neurotoxic activity in hens. In addition, the behavior of leptophos and EPN in an aquatic-terrestrial ecosystem (*3-5*) is assessed in order to evaluate environmental effects and food chain transfers.

In this manner, the toxicological profiles of leptophos and EPN have been characterized with respect to their acute toxicity in target and nontarget organisms, their environmental degradation and persistence, and their chronic toxicity. Comparisons with the other phenylphosphonothioates permit some generalizations about the relationship between delayed neurotoxicity and the classical organophosphorus ester toxicity due to acetylcholinesterase inhibition. Such comparisons in turn permit realistic evaluation of the hazards which must be set against the benefits of using a particular pesticide or class of pesticides.

Materials and Methods

Ecosystem

In the terrestrial-aquatic model ecosystem studies, 5 mg of the pure ^{14}C-labeled EPN, leptophos or desbromoleptophos, was applied from acetone solution to sorghum leaves in the terrestrial phase of the ecosystem, simulating application of these pesticides to crops. The contaminated sorghum leaves were eaten by salt marsh catepillars (*Estigmene acrea*), dispersing the ra-

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diolabeled products into the seven liters of standard reference water (*6*) containing plankton, alga (*Oedogonium cardiacum*), daphnia (*Daphnia magna*), and snails (*Physa* sp.). Subsequently mosquito larvae (*Culex pipiens*) and fish (*Gambusia affinis*) were added to complete the food-chain interactions. The entire ecosystem unit in a 10-gal aquarium was kept in a programmed environmental growth chamber for 12 hr daylight cycles of 5000 ft-candles light intensity and with constant temperature of 26 ± 1°C.

At the termination of the 33-day experimental period, the organisms and water samples were collected separately and weighed. The organisms were extracted with acetone, and the water extracted with diethyl ether. The total bioaccumulation and the nature of the degradation products was determined by liquid scintillation counting and thin layer chromatography. The environmental properties of the parent compound and major metabolites were expressed quantitatively as ecological magnification (EM = concentration of parent compound in the organism/concentration of parent compound in the water) and biodegradability index (BI = concentration of polar metabolites/concentration nonpolar metabolites).

The unextractable radioactivities (water—partitioning metabolites) were assayed by the Schöniger oxygen flask technique (*7*). Quenching was corrected by channels ratio methods (*8*). The radioassay technology, separation and identification were carried out as previously described (*5*).

Fly Toxicity

The insecticidal activity of the chemicals, due to the acute inhibition of acetylcholinesterase, was measured by using standard weight/volume solutions in acetone and applying microliter droplets to adult houseflies (*Musca domestica* NAIDM) as has been described previously (*9*).

Mammalian Toxicity

Data were taken from the World Health Organization's Programme for Testing New Insecticides, 1960–1977, and from the published literature (*10–12*). Because of the heterogeneous sources, these data are not as consistent as the LD_{50} for flies.

Neurotoxicity

Hens. White leghorn hens were obtained from Cornbelt Hatcheries, Forrest, Ill. Young hens weighed 1366 ± 200 g (SD) on arrival and were a minimum of 20 weeks old. Old hens from Cornbelt Hatcheries and from a red hybrid strain bred by the University of Illinois Poultry Farms were also used for comparison. Hens were banded on arrival, caged individually, and fed layer mash and water *ad libitum*.

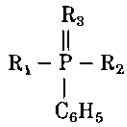
Chemicals. The organophosphorus esters discussed in the text are listed in Table 1 with their chemical structures and trade or generic names. The structural relationships among tested compounds and other phenylphosphonothioates are shown in Table 2. Technical grade chemicals were used for neurotoxicity tests and fly toxicity tests, but highly purified radiolabeled compounds were used for ecosystem studies.

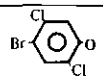
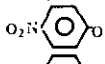
Treatment. Several dosing schedules were used, but compounds were always administered in gelatin capsules. Hens were not protected from

Table 1. Organophosphorus esters discussed in the text.

Number/Name	Chemical name
Leptophos	*O*-[2, 5-dichloro-4-bromophenyl] *O*-methyl phenylphosphonothioate
Desbromoleptophos	*O*-[2, 5-dichlorophenyl] *O*-methyl phenylphosphonothioate
Leptophos oxon	*O*-[2, 5-dichloro-4-bromophenyl] *O*-methyl phenylphosphonate
Ethoxyleptophos	*O*-[2, 5-dichloro-4-bromophenyl] *O*-ethyl phenylphosphonothioate
RF_1	*O*-[2, 6-dichlorophenyl] *O*-methyl phenylphosphonothioate
RF_2	*O*-[2, 5-dichlorophenyl] *O*-butyl phenylphosphonothioate
RF_3	*O*-[3-chlorophenyl] *O*-methyl phenylphosphonothioate
RF_4	*O*-[2, 5-dichlorophenyl] *O*-propyl phenylphosphonothioate
EPN	*O*-[4-nitrophenyl] *O*-ethyl phenylphosphonothioate
Cyanofenphos	*O*-[4-cyanophenyl] *O*-ethyl phenylphosphonothioate
#3	*O*-[2, 4-dichlorophenyl] *O*-methyl phenylphosphonothioate
#8	*O*-[3, 4-dichlorophenyl] *O*-methyl phenylphosphonothioate
#9	*O*-[3, 5-dichlorophenyl] *O*-methyl phenylphosphonothioate
#10	*O*-phenyl *O*-methyl phenylphosphonothioate
M_1	*O*-phenyl *O*-ethyl phenylphosphonothioate
OMS 160	*O*-[2, 4, 6-trichlorophenyl] *O*-ethyl phenylphosphonothioate
EPBP	*O*-[2, 4-dichlorophenyl] *O*-ethyl phenylphosphonothioate
Parathion	*O*-[4, nitrophenyl] *O*-ethyl *O*-ethyl phosphorothioate

Table 2. Structural relationship among phenylphosphonothioates.



R_2	R_3	$R_1 = OCH_3$	$R_1 = OC_2H_5$	$R_1 = OC_3H_7$	$R_1 = OC_4H_9$
	S O	Leptophos Leptophos-oxon	Ethoxyleptophos		
	S	Desbromoleptophos		RF4	RF2
	S		OMS 160		
	S	RF1			
	S	#3	EPBP		
	S	#8			
	S	#9			
	S	RF3			
	S		EPN		
	S		Cyanofenphos		
	S	#10	M1		

acute cholinergic effects with atropine; instead, doses were manipulated to fall below acutely lethal levels. After initial attempts at using single or multiple doses at maximum tolerated levels, two treatment schedules were adopted. Acute doses were begun at 50 mg/kg and chronic dosing was begun at 10 mg/kg/day and continued for 90 days. Treatment levels in subsequent hens were decreased until a nonlethal, non-neurotoxic dose was established and/or increased until a neurotoxic or lethal dose was established.

In several cases testing was limited by the small quantities of chemical available. Thus negative results do not necessarily demonstrate non-neurotoxicity but only inactivity at the levels tested.

Observation. Hens were weighed on arrival and as necessary to calibrate dosages. In chronic dosing studies, hens were weighed every second week. All hens were observed daily in their cages and evaluated at intervals for their ability to stand, walk and fly. Because of the variability in posture, gait and activity between hens and between observations for the same hen, only gross ataxia (+2), paraplegia (+3), paralysis (+4), and quadriplegia (+5) were used to define neurotoxicity. Increased nervousness or minor disturbances of gait (+1) were noted but not used to define neurotoxic chemicals or levels of treatment.

Results and Discussion

Rho-Sigma Evaluation

The acute toxicity of organophosphorus insecticides is well known to result from their irreversible inhibition of acetylcholinesterase. This occurs in phosphonothioates (P=S) following microsomal oxidation to the corresponding phosphonates (P=O) which then undergo a bimolecular reaction with a serine hydroxyl group in the enzyme to irreversibly inactivate it (*13*). The relative rates of inactivation of acetylcholinesterase by phosphonates are governed by the electrophilic properties of the phosphorus atom and these are a function of the electron-withdrawing properties of the groups attached to the phenoxy group as well as the electronic and steric properties of the

phosphonate group (*14, 15*). These effects can be quantified by the Hammett ρ-σ (rho-sigma) analysis, and by the Taft steric parameter E_s (*16*).

The concept of a neurotoxic esterase that is inactivated by interaction with organophosphorus ester neurotoxins (*17*) suggests that the reactivity, i.e., electrophilic character of the P atom, should relate quantitatively to the magnitude of neurotoxic effect in a manner similar to acute toxicity, i.e., phosphorylation of acetylcholinesterase. We have explored this possibility by plotting the summation of σ values for the aromatic substituents of the *O*-phenyl *O*-alkyl phenylphosphonothioates vs. acute topical LD_{50} to the female house fly *Musca domestica* as determined in this laboratory and versus the rat oral LD_{50} using values from the literature (*10-12*). The Taft σ^* values were used for the *ortho* substituents (*16*) after correcting them to H=O rather than CH_3=O. The general validity of the σ values was also confirmed by relating them to the K_a values for the ionization of the various phenols. The resulting plots (Fig. 1) leave no doubt of the validity of this concept of phosphorylation in regard to acute toxicity for the phosphonothioates. The correlation coefficient for σ vs. fly LD_{50} was $r = -0.91$ and for rat LD_{50} was $r = -0.71$. These indicated significance at $p < 0.001$ and $p < 0.05$, respectively, and also show the relative efficiency of the microsomal oxidation of P=S to P=O which does not appear to be significantly affected by the electronic and/or steric character of the remainder of the molecule.

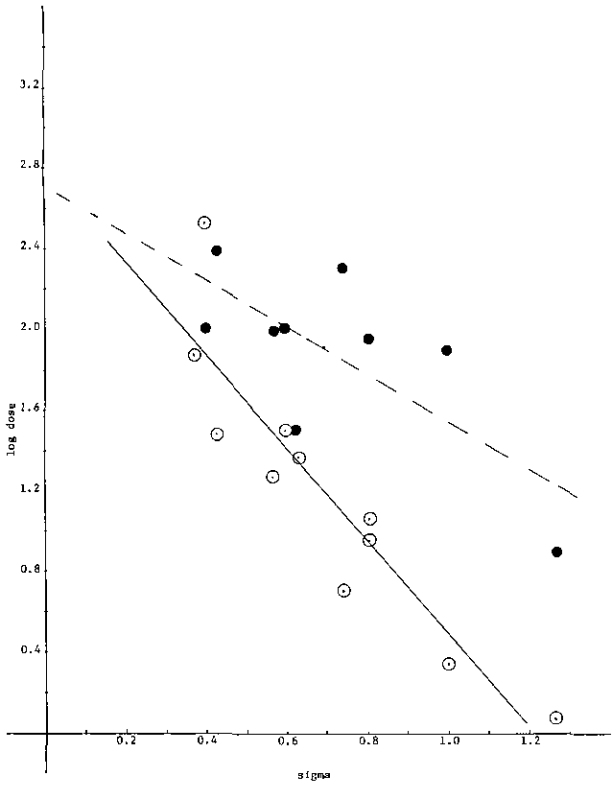


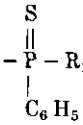
FIGURE 1. Relationship between $\Sigma\sigma$ and LD_{50} of substituted phenyl phenylphosphonothioates: (○) LD_{50}, flies; (●) LD_{50}, rats/mice; (—) regression line for fly LD_{50} vs. σ; (---) regression line for rat/mouse LD_{50} vs. σ.

In contrast is the corresponding plot of σ versus minimum neurotoxic dose (Table 3 and Fig. 2). The correlation coefficient was $r = -0.35$, significance level $p > 0.20$. Since these data include ethoxyphenyl-phosphonothioates (EPN, ethoxyleptophos) as well as methoxy phenylphosphonothioates, some heterogeneity would be expected. But within the class of methoxyphenylphosphonothioates, the neurotoxicity of desbromoleptophos ($\Sigma\sigma = 0.573$) is sixfold that of leptophos ($\Sigma\sigma = 0.805$). The effect of the *O*-alkyl chain can be seen in the absence of overt neurotoxicity when ethoxyleptophos is given at 1000 mg/kg (*18*). Other studies have shown that the nature of the group involved in the direct phosphorus-carbon bond is also critical; for example, the methylphosphonothioate analog of compound 160 [i.e., *O*-(2,4,6,-trichlorophenyl) *O*-ethyl methyl-phosphonothioate] is neurotoxic at a single oral dose of 20 mg/kg, while OMS 160 did not induce paralysis at 300 mg/kg (unpublished data).

The neurotoxic esterase theory of Johnson (*17, 19-21*) requires the neurotoxin to phosphorylate an esterase of unknown specificity within the nervous system. It is therefore necessary for a would-be neurotoxin to enter the nervous system in sufficient quantity, and to persist a sufficiently long time, to reach this esterase. A minimum lipophilicity and stability are required. Subsequently the neurotoxin must "fit" the enzyme sufficiently well to inhibit it, and finally the neurotoxin must possess the necessary electron-withdrawing/donating characteristics to phosphorylate the enzyme.

Thus it is not surprising that the correlation between neurotoxic activity and σ is not as pronounced as the correlation between σ and the acetylcholinesterase inhibition of the peripheral nervous system. It is even plausible that in some cases the requirements of persistence and lipophilicity counteract the electronegativity requirements sufficiently well that the observed results are an increase in neurotoxicity with decreasing σ values, as is seen for leptophos and its desbromo analog. Precisely which aspects of molecular structure relate to fit must await either a better understanding of the natural substrate of the

Table 3. Toxicity data and Σσ for phenylphosphonothioates.



Name	R_1	R_2	Acute Neurotoxic dose, mg/kg	Topical LD_{50} (flies), μg/g	Acute oral mammalian LD_{50}, mg/kg	σ
Leptophos	OCH_3	4-Br-2, 5-diCl	250	11.5	90	0.805
Desbromoleptophos		2, 5-diCl	38[a]	18.5	95	0.573
RF_1		2, 6-diCl	75	340	105[b]	0.400
RF_3		3-Cl	300-325[b]	75	>500[b]	0.373
#3[b]		2, 4-diCl	160-175	30	240	0.430
#9[b]		3, 5-diCl	175-225	5	200	0.746
#8[b]		3, 4-diCl	100-175	32	100	0.600
#10[b]		H	>500	>500	>500	0
EPN	OC_2H_5	4-NO_2	60[c]	1.2	7	1.267
Cyanofenphos		4-CN	—	2.2	79	1.000
EPBP		2, 4-diCl	1200[d]	—	—	0.430
Ethoxyleptophos		4-Br-2, 5-diCl	>1000[b]	9.0	—	0.805
M_1		H	—	>250,000	—	0
OMS 160		2,4,6-triCl	>300	23.4	32	0.630

[a] Data from Sanborn et al (*24*).
[b] Data from Hollingshaus et al. (*18*).
[c] Data from Witter and Gaines (*26*).
[d] Data from Abou-Donia (*27*).

neurotoxic esterase or of its function within the nervous system.

In addition to treating hens with single oral doses of phenylphosphonothioates, we tested the effects of low level chronic treatment with EPN, cyanofenphos, and leptophos, the three commercial phenylphosphonothioates available to us. All three were neurotoxic at 10 mg/kg/day (Table 4), but EPN was so acutely toxic that hens had to be treated at lower doses for the first week. Even so, EPN treated hens became ataxic 24 days after the start of treatment; ataxia progressed to irreversible paralysis (+4) and in one case to quadriplegia (+5). For leptophos, ataxia (+2) resulted from 90 days of 10 mg/kg/day. Cyanofenphos was intermediate, with ataxia (+2) after 34 days treatment at 10 mg/kg/day.

The precise ordering of neurotoxic potential at low levels of chronic dosing is unfortunately irrelevant, since it is already established that the least potent compound, leptophos, caused nerve damage in factory workers and in farm animals. The critical factor in these studies is that paralysis can occur in the absence of symptoms of acute poisoning (leptophos, cyanofenphos) or with sublethal doses which the animal could survive even without atropine (EPN). Inasmuch as human acute poisoning with organophosphorus esters is aggressively treated with atropine and acetylcholinesterase regenerators, the levels of ex-

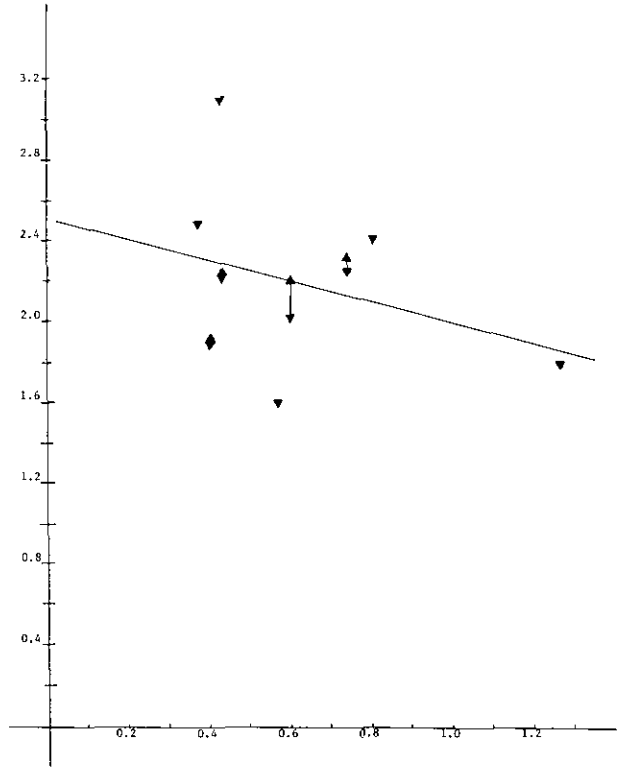
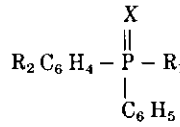


FIGURE 2. Relationship between σ and the minimum acute neurotoxic dose in the hens: (▼) single value reported as minimum neurotoxic dose; (↕) range of doses reported as minimum neurotoxic dose.

Table 4. Compounds tested for neurotoxicity.



Name	R_1	R_2	X	Minimum neurotoxic dose: Acute, mg/kg	Minimum neurotoxic dose: Chronic, mg/kg/day (days)	Maximum ineffective dose: Acute mg/kg	Maximum ineffective dose: Chronic, mg/kg/day (days)
Leptophos	OCH_3	2,5-dichloro-4-bromo	S	250	10 (92)	200	a
Leptophos-oxon		2,5-dichloro-4-bromo	O	87[b]	c	89[b]	c
Desbromoleptophos		2,5-dichloro	S	38[b]	c	40[b]	c
RF_1		2,6-dichloro	S	75	c	a	c
RF_3		3-chloro	S	—	c	120	c
EPN	OC_2H_5	4-nitro	S	d	10 (24)	31	2.5 (250)
Cyanofenphos		4-cyano	S	d	10 (34)	100	6
OMS 160		2,4,6-trichloro	S	—	—	300	40 (10)
RF_4	OC_3H_7	2,5-dichloro	S	—	c	138	c
RF_2	OC_4H_9	2,5-dichloro	S	—	c	120	c

[a] Lowest dose tested was neurotoxic.
[b] Data from Sanborn et al. (*24*).
[c] Chronic dosing not attempted because of small quantities available.
[d] Highest dose tested was acutely toxic and lower doses were not neurotoxic.

posure we used are conservative. The cumulative nature of the neurotoxic potential must be considered in determining the risk involved in manufacturing, formulating and using organophosphorus pesticides.

The aquatic-terrestrial model ecosystem provides a valid method for predicting the behavior of pollutants in the environment. It is particularly valuable, because over 150 chemicals have been compared under standardized conditions, allowing assessment of relative persistence, degradation and bioaccumulation. The design of the system corresponds to a farm pond surrounded by a watershed under cultivation which has been treated with 1 lb/acre (1.12 kg/hectare) of the test compound. Data for EPN, leptophos, and desbromoleptophos (Tables 5–7) can be compared with the data for parathion [*O*-(4-nitrophenyl *O*-ethyl phosphorothioate], which is generally regarded as a nonpersistent insecticide. Parathion in the model ecosystem was found in algae, snails, and fish as well as in water (Table 8). The highest levels were found in snails, which accumulated parathion 746-fold over the levels found in water.

Table 5. Distribution of ^{14}C-EPN and its metabolites in the terrestrial-aquatic model ecosystem.

Metabolite	R_f[a]	^{14}C-EPN equivalents, ppm: H_2O	Alga (*Oedogonium*)	Daphnia (*Daphnia*)	Snail (*Physa*)	Mosquito (*Culex*)	Fish (*Gambusia*)
Total extractable ^{14}C		0.0010	0.093	0.215	3.49	0.111	0.124
EPN	0.62	0.00023	0.017	0.0176	2.89	0.031	0.080
Unknown I	0.31	0.00009	—	—	—	—	—
EPN-NH_2	0.29	0.00003	0.0044	0.0505	0.069	—	0.013
EPN-oxon	0.22	0.00006	0.0025	0.0374	0.107	0.0025	0.0044
Unknown II	0.12	0.00012	—	—	—	—	—
Unknown III	0.08	0.00012	—	—	0.119	—	—
Phenylphosphonic acid	0.66[b]	0.00009	0.040	0.0205	0.219	—	0.027
O-Ethylphenylphosphonic acid	0.29[b]	0.00011	0.0085	0.0826	0.015	—	—
O-Ethylphenylphosphonothioic acid	0.11[b]	0.00006	—	—	0.020	—	—
Polar	0.00	0.00012	0.021	—	0.051	0.078	—
Unextractable ^{14}C		0.0076	0.228	0.135	0.794	0.393	0.100

[a] TLC with hexane:diethyl ether:ethyl acetate, 6:1:3 by volume unless otherwise noted.
[b] TLC with benzene:hexane:acetic acid, 4:4:3 by volume.

Table 6. Distribution of ^{14}C-leptophos and its metabolites in the terrestrial-aquatic model ecosystem.[a]

			^{14}C-leptophos equivalents, ppm[b]			
	R_f[c]	H_2O	Alga (*Oedogonium*)	Snail (*Physa*)	Mosquito (*Culex*)	Fish (*Gambusia*)
Total extractable ^{14}C		0.0095	0.262	6.38	0.655	0.565
Unknown I	0.84	—	0.0059	—	—	—
Leptophos	0.76	0.00023	0.164	4.80	0.313	0.220
Desbromoleptophos	0.76	0.00001	0.015	0.112	0.061	0.039
Unknown II	0.52	—	0.016	—	—	—
Unknown III	0.44	0.0091	—	0.367	0.066	0.258
S-CH_3 leptophos	0.25	—	0.0080	—	—	—
Unknown IV	0.22	—	0.027	—	—	—
Leptophos-oxon	0.18	0.00003	0.0060	0.088	0.035	0.0014
Unknown V	0.16	0.00001	0.0091	0.029	0.0038	—
Unknown VI	0.14	0.0062	—	—	—	—
Unknown VII	0.11	0.00002	0.0037	—	—	—
Unknown VIII	0.007	—	0.0021	0.447	—	—
Unknown IX	0.03	—	0.0037	0.032	—	—
Polar	0.0	0.00008	0.021	0.501	0.176	0.047
Unextractable ^{14}C		0.0103	1.037	1.78	0.937	1.036

[a] Purity of the treated compound was 97.75%.
[b] All daphnia were killed.
[c] Solvent system for TLC was benzene:chloroform:acetic acid, 50:50:1.

Some bioaccumulation was seen in algae and fish as well, but relatively little. The Biodegradability Index (BI) for snails was greater than one, demonstrating the metabolism of parathion to polar, water soluble products. The presence of aminoparathion, *p*-nitrophenol and *p*-aminophenol in most components of the ecosystem is in accord with the well documented degradation pattern of parathion (*5*, *22*).

The behavior of EPN in the model ecosystem is similar to that of parathion, but EPN is clearly more persistent. The values for bioaccumulation or ecological magnification (EM) of EPN in snail and fish were 12,561 and 346 vs. 747 and 87 for parathion (Tables 5, 8, and 9). Moreover, the EPN metabolites were relatively less polar than those of parathion, so that biodegradability index values (BI) of EPN for snail and fish were 0.15 and 0 vs. 1.23 and 0.24 for parathion. Leptophos in turn, was more bioaccumulative than EPN (Table 9) with EM values of snail 21,232 and fish 972; and was correspondingly more recalcitrant to biodegradation with BI values of snail 0.85 and fish 0.090. These values from the present study using ^{14}C-phenyl ring-labeled leptophos are in excellent agreement with EM values of 48,358 in snail and 1,443 in fish previously obtained with $^{14}CH_3O$-labeled leptophos (*23*).

Desbromoleptophos, a persistent metabolite of leptophos with enhanced neurotoxic properties (*24*) was found in all components of the model ecosystem treated with leptophos, at levels ranging from 2% of leptophos concentration in snails, 9% in alga, 19.5% in fish, and 19.6% in mosquito

Table 7. Distribution of ^{14}C-desbromoleptophos and its metabolites in the terrestrial-aquatic model ecosystem.[a]

		^{14}C-desbromoleptophos equivalents, ppm[b]			
	R_f[c]	H_2O	Alga (*Oedogonium*)	Snail (*Physa*)	Fish (*Gambusia*)
Total extractable ^{14}C		0.0024	2.44	53.9	5.52
Unknown I	0.98	—	0.119	0.012	—
Desbromoleptophos	0.76	0.0015	2.109	52.6	5.35
Unknown II	0.47	0.00058	0.056	0.957	—
Unknown III	0.38	—	0.053	—	—
Unknown IV	0.21	0.00007	—	—	—
Unknown V	0.16	0.00009	—	—	—
Polar	0.0	0.00008	0.107	0.345	0.162
Unextractable ^{14}C		0.0085	4.01	10.3	1.94

[a] Purity of the treated compound is 96.10%.
[b] All daphnia and mosquitos were killed.
[c] Solvent system for TLC is hexane:ether:ethyl acetate, 6:1:3.

Table 8. Distribution of ^{14}C-parathion and its metabolities in the terrestrial-aquatic model ecosystem.

		^{14}C-Parathion equivalents, ppm					
	R_f[a]	H_2O	Alga (*Oedogonium*)	Daphnia (*Daphnia*)	Snail (*Physa*)	Mosquito (*Culex*)	Fish (*Gambusia*)
Total extractable ^{14}C		0.0038	0.128	0.177	1.44	0.180	0.122
Unknown I	0.85	—	—	—	0.015	0.125	0.00093
Parathion	0.63	0.00036	0.035	—	0.272	—	0.032
Unknown II	0.47	—	0.0091	—	0.066	—	—
p-Nitrophenol	0.35	0.0022	0.023	0.029	0.165	0.0036	0.0320
Parathion-NH_2	0.23	0.00010	0.0073	—	0.045	0.0022	0.0091
Para-oxon	0.16	0.00017	0.0061	—	0.059	0.030	0.0071
p-Aminophenol	0.08	0.00034	0.0084	0.067	0.029	—	0.017
Polar	0.0	0.00061	0.039	0.082	0.792	0.020	0.024
Unextractable ^{14}C		0.013	0.837	0.216	2.11	0.869	0.275

[a] TLC with hexane:diethyl ether:ethyl acetate, 6:1:3 (v/v).

(Table 6). Desbromoleptophos is clearly a persistent bioaccumulative degradative metabolite with EM values of 11,187 in snail and 3,888 in fish. When desbromoleptophos itself was evaluated in the model ecosystem (Tables 7 and 9), the EM values were snail 34,164 and fish 3,477. The BI values were snail 0.006 and fish 0.030. Thus desbromoleptophos appears to represent a highly stable and persistent terminal residue of leptophos metabolism; its greater persistence in other systems has also been established (*24*). The five-fold greater neurotoxicity of desbromoleptophos, its role as a photodecomposition product of leptophos, and its substantially greater persistence and decreased biodegradability all argue for its suggested role as the active neurotoxin in agricultural poisonings by leptophos (*24*). The environmental persistence of this highly active delayed neurotoxin emphasizes the importance of evaluation of environmental residues of potentially neurotoxic compounds for neurotoxic action.

Summary

The evaluation of phenylphosphonothioates in terms of their acute, chronic, and environmental hazards illustrates the complexity of risk determination in pesticide use. The use of σ-coefficients to establish relative acetylcholinesterase inhibition within a class of closely related organophosphorus esters is an eminently reliable predictor of insecticidal activity, and a fairly reliable indicator of mammalian toxicity. To what extent the heterogeneity of the data used for assessment of mammalian acetylcholinesterase inhibition is responsible for the lower correlation between σ and LD_{50} in rodents and to what extent the complexities of oral administration (e.g., digestion, transport, hepatic metabolism) affect the lower correlation is uncertain.

Delayed neurotoxicity, in sharp contrast to acute toxicity, is not well correlated with σ. The marked effect of the ethoxy-group in reducing neurotoxic potential was far more significant than alterations in σ among *O*-methyl phenylphosphonothioates. Since a similar benign effect of ethoxy moieties—or malignant effect of methoxy moieties—is not seen in methyl phosphonothioates (unpublished data), the role of the P-C bond is also of considerable importance.

The ecosystem data demonstrate the variability of persistence of organophosphorus pesticides and suggest that their degradability is relative. Recent studies on the persistence of para-oxon on citrus foliage (*25*) illustrate the absence of absolutes in real world residue studies, but under the standardized conditions of the terrestrial aquatic model ecosystem, both EPN and leptophos are clearly more persistent than parathion. Their potential for inducing permanent paralysis after

Table 9. Ecological magnification (EM) and biodegradability index (BI)

	Ecological Magnification		Biodegradability Index	
	Snail	Fish	Snail	Fish
Parathion	747	87.4	1.22	0.247
EPN	12,561	346	0.010	—
Leptophos	21,232	973	0.085	0.090
Desbromoleptophos	34,092	3469	0.005	0.030

low level chronic exposure is therefore an extremely significant datum in the evaluation of their hazards to man and animals.

In a more general fashion, the multifaceted evaluation of closely related phenylphosphonothioates illustrates the inadequacy of a single determinant of toxicity. Acute toxicity, chronic toxicity, and knowledge of environmental behavior are required to assess the safety of environmental chemicals.

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