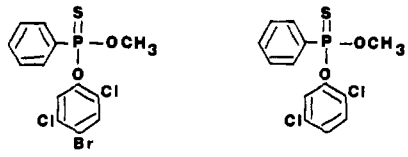
Early Pharmacokinetics of Leptophos and Desbromoleptophos following Intravenous Administration in the Hen

Elizabeth M. Cozzi,*[1] Alex J. Bermudez, and Larry G. Hansen

The pharmacokinetics, distribution into the neural tissue, and metabolism of leptophos and desbromoleptophos (DBL) were compared following intravenous administration of ^{14}C-labeled compound in the hen. Leptophos and DBL did not differ in their accumulation into the neural tissues; however, radioactivity accumulated in the sciatic nerve for both compounds in preference to other neural tissues. Radioactivity was excreted primarily into the urine for both compounds. Of the extractable radioactivity in the urine, most was present as methyl phenyl phosphonic acid, along with smaller amounts of methyl phenyl phosphonothioic acid. Distribution from the central compartment was at least triphasic for leptophos and DBL. The majority of radioactivity in the plasma 8 h after administration was protein-bound material that does not contribute to neuropathy.

Leptophos [*O*-(4-bromo-2,5-dichlorophenyl) *O*-methyl phenylphosphonothionate] is an organophosphorus compound capable of producing organophosphorus-induced delayed neuropathy (OPIDN) in several species. Leptophos and desbromoleptophos (DBL) both show strong inhibition of the proposed biochemical target of delayed neuropathy, neuropathy target esterase (NTE), in vitro and in vivo, but DBL is 8× more neurotoxic than leptophos by the oral route (Hansen, 1983).



LEPTOPHOS DBL

The first study in this series (Hansen et al., 1985) was designed to investigate any pharmacokinetic differences between leptophos and DBL in hens that might result in different amounts of the toxicants becoming available at the site of action. The pharmacokinetics of leptophos in the hen had previously been studied (Abou-Donia, 1977, 1979, 1980). Two important results from these experiments, namely that (1) leptophos was poorly metabolized in the hen following oral and topical administration and (2) leptophos had a biologic half-life of 15 days in the hen, served as guidelines for the design of the previous study. In this first experiment, blood levels and excretion of radiolabel were measured for 3–4 weeks following intravenous treatment with [*O-methyl*-^{14}C]leptophos or DBL. Leptophos and DBL were similar with respect to elimination into the excreta and biological half-lives for elimination of radioactivity from the central compartment. Possible differences between the two analogues were indicated in the very early distribution out of the central compartment. These could be significant, since it is proposed that events leading to neuropathy occur within 24–48 h after dosing with delayed neurotoxicants (Johnson, 1975).

The specific objectives of the present study were the following: (1) to study differences between leptophos and DBL in the early accumulation of radioactivity into the neural tissue, distribution from the central compartment, elimination, and metabolism; (2) to separate radioactivity in the plasma between parent and protein-bound entities; (3) to discover the route of excretion (urinary vs fecal) of leptophos and DBL through the use of colostomized hens.

Department of Veterinary Biosciences, University of Illinois, Urbana, Illinois 60120.

[1] Present address: Abbott Laboratories, North Chicago, IL 60064.

Table I. Mass Spectral Data for the Methylated Derivative of MPPA Synthesis Product

peak	rel intens (*m/e*)	proposed struct
227	3	$M^+ + C_3H_5$
215	11	$M^+ + C_2H_5$
187	100	$M^+ + H$
155	2	$M^+ - OCH_3$
109	3	$M^+ - C_6H_5$

MATERIALS AND METHODS

Care of Birds. Hyline White Leghorn pullets (strains W-36 and W-77) were obtained at 18–20 weeks of age from Hyline International (Watseka, IL). Pullets were identified by wing band numbers, aged, acclimated, exercised, and given food (Layer Ration, formulated by the Poultry Science Dept., University of Illinois) and water ad libitum.

Chemicals. [*O-methyl*-^{14}C]Leptophos (final SA = 0.1 mCi/mol, purity 95%) and [*O-methyl*-^{14}C]desbromoleptophos (final SA = 0.3 mCi/mol, purity 95%) were synthesized by R. L. Metcalf by methods described in Metcalf et al. (1983). Since both leptophos and DBL are neurotoxic compounds, all manipulations with concentrated materials were carried out under a fume hood with hand protection.

Potassium methyl phenyl phosphonothionate (90%) was a gift from Velsicol Chemical Corp. (Chicago, IL). Oxons of leptophos and DBL were produced by the method of Giang and Hall (1951). MPPA (methyl phenyl phosphonic acid) was synthesized in this laboratory by esterification of phenyl phosphonic dichloride (Sigma) with anhydrous methanol as described in Metcalf et al. (1983) and subsequent hydrolysis in 2 N NaOH. The identity of MPPA was established by mass spectrometry of a diazomethane-derivatized sample. (See Urine Extractions for description of derivatization and spectral conditions.) Table I shows the relative abundance of the molecular ion and prominent ion fragments for the derivatized sample. The pattern of breakdown was consistent with the structure of MPPA.

Colostomy Procedure. The surgical procedure for separation of urine and feces was essentially the same as in Dixon (1956).

Preparation/Administration of Dose. Intravenous doses were prepared as 2:1:4 ethanol–Emulphor (GAF, New York, NY)–water suspensions and delivered via a butterfly cannula to the jugular vein. No more than 0.93 mL of ethanol/kg of body weight was administered.

0021-8561/88/1436-0108$01.50/0

Atropine sulfate, when used, was administered as an aqueous solution via the cannula immediately following the organophosphorus compound. Although atropine did affect the total grams of feces excreted, a subsequent study showed that the percent radioactivity excreted in the feces was not affected by atropine administration.

Experiments. Two separate studies were conducted. The first was designed to compare the early pharmacokinetics of leptophos and DBL. The second study was conducted to identify the radioactive material present in the plasma following administration of radiolabeled DBL.

Experiment 1: Hens were colostomized 5–7 days prior to dosing, and their health was monitored until full recovery from surgery was assured. Hens were dosed in the jugular vein with 35 mg/kg (hen 366) or 30 mg/kg (hens 371 and 471) radiolabeled leptophos or 30 mg/kg radiolabeled DBL (hens 470, 476, and 469). To combat acute OP effects, 10 mg atropine sulfate was administered intravenously after the toxicant to each hen except hen 476, which received only 5 mg of atropine. Although atropine did affect the total amount of fecal elimination, studies in this laboratory indicated that it did not have a significant effect on the route of elimination of radioactivity. Blood samples (1.5 mL) were removed at 0.08-, 0.25-, 0.50-, 1.0-, 2.0-, 8.0-, and 24.0-h time points. Smaller blood samples were taken at 4.0 and 16.0 h. Urine and feces were collected separately throughout the study and frozen at −10 °C until analyzed. Birds were sacrificed at 24 h and brain, spinal cord, and sciatic nerve removed.

Experiment 2: Four uncolostomized hens of various ages were dosed with 30 mg/kg DBL. Blood samples (1.5 mL) were collected at 0.08–0.25, 0.5, 1, 2, 4, 8, 24, and 72 h.

Analytical Procedures. *Radiolabel Counting.* Whole-blood samples (0.05–0.20 g) were solubilized with NCS (Nuclear Chicago, Arlington Heights, IL) or Protosol (New England Nuclear, Boston, MA) solubilizers and decolorized with 3% benzoyl peroxide in toluene. Prior to sampling, tissues and excreta were prepared as follows: fecal samples were homogenized, adding water if necessary; urine was homogenized on a Polytron blender; tissues were minced. Duplicate 0.2-g samples were removed from each type and incubated overnight at 50 °C with a solubilizer. Samples were counted for 20 min or 10 000 counts on a Tri-Carb liquid scintillation counter. All counts were corrected for dilution, efficiency, quenching, and color.

Urine Extractions. Thawed urine samples (about 2.0 g) were extracted 4× with 30 mL of methylene chloride (Baker, analytical grade), acidified to pH 1, and reextracted. The organic layer was dried and concentrated to 2.0 mL. Samples (100 μL) were removed for liquid scintillation counting and the extracts dried to a residue and stored at −10 °C for later analysis. There was no change in the metabolite profile with storage in this manner for up to 8 months. The water layer from the extractions was filtered and sampled. Residue was collected, solubilized, and counted.

Thin-layer chromatography (TLC) followed by GC–MS was used to identify the radioactive species present. Samples (100 μL) were spotted onto 1000-μm PKF-525A silica gel plates (Whatman, Hillsboro, OR) and developed. Extracts were subjected to two TLC systems: System 1 [chloroform–benzene (1:1)] as in Holmstead et al. (1973) was used to characterize the percent parent vs oxon. System 2 [acetonitrile–water–ammonium chloride (8.9:0.9:0.2)] was used to identify acidic metabolites. Lanes developed in system 2 were divided into four zones and scraped. Although there appeared to be two major areas of radioactivity in samples in this system, which were reported to correspond to MPPA and MPPTA (methyl phehyl phosphonothioic acid), the activity in each spot traveled farther than the standard. Spiking standards with the extract indicated that the effect was not attributable to the matrix of the sample. The identity of the material in these spots was confirmed to be MPPA (zone 2) and MPPTA (zone 3) by GLC–MS of methylated derivatives as follows: Diazomethane solution was prepared fresh by adding 50 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich) to a 25-mL flask containing 5 mL of diethyl ether and 0.4 mL of 20% NaOH (Rivers et al., 1970). Combined urine extracts from several high-counting urine samples were subjected to preparative TLC in system 2. Bands of radioactivity were scraped, extracted from the gel with ethyl acetate and ethanol, and then concentrated. An excess of diazomethane (based on radioactivity in sample) was added. The samples and standards (300 μg by weight for standards or activity for samples) were dissolved in 0.1 mL of methanol, and 1 mL of diazomethane was added in 0.1 mL of cold ether. The reaction was allowed to proceed at room temperature for 2 h, and then the solvent was evaporated and the samples dissolved in an appropriate solvent for GC–MS (Chrzanowski and Jelinek, 1981). Because of the irritation and carcinogenic hazard of diazomethane, all manipulations were confined to a fume hood.

The GC–MS analyses were performed on a Extranuclear Simulscan (Pittsburg, PA) with a DB1701 (J & W Scientific, Folsom, CA) 30-m silica capillary column of 0.25-mm i.d. and 0.25-mm film thickness: flow rate, 50 cm/s of He; split ratio, 10:1; column program, isothermal at 85 °C, hold 1 min, program 85–280 °C at 39 °C/min. Chemical ionization mass spectrometry was employed with methane gas: source temperature, 160 °C; electron energy, 300 eV. The GC–MS spectra of the isolated urinary metabolites were identical with those of MPPA and MPPTA standards.

Plasma Extractions. Samples (0.6 g) were extracted three times with 10 mL of ether, precipitated with 1.0 M trichloroacetic acid, and extracted three times more with ether. Recovery of leptophos from spiked samples was good (>80%).

Samples (100 μL) were spotted on 1000-μm PKF-525A silica gel plates and developed in system 1 as described for the urine extractions. Plates were scraped into liquid scintillation vials into which cocktail was added and the resultant mixtures counted for radioactivity by liquid scintillation counting.

Pharmacokinetic Modeling. The decay in radioactivity in whole blood was at least biexponential in nature, and the number of terms in the model was limited by the number of data points. Data were fitted by the biexponential equation

$$C_p = Ae(-\alpha t) + Be(-\beta t)$$

where α is the rate constant for the initial elimination phase and β is the rate constant for the terminal elimination phase (Gibaldi and Perrier, 1982) using the SAAM-27 iterative fitting program (Berman and Weiss, 1978).

Statistical Analyses. Pharmacokinetic parameters from leptophos and DBL-treated hens were compared with use of *t*-tests ($p < 0.05$). Planned comparisons of radioactivity in neural tissues were performed by analysis of variance of tissue concentration using a randomized complete block (RCB) model and a 0.05 level of significance.

RESULTS

Tissue Distribution. The radioactivity in the tissues of the nervous system of hens is presented in Table II.

Table II. Concentration of Radioactivity in the Neural Tissues of Hens 24 h after an Intravenous Dose of [*O*-*methyl*-^{14}C]Leptophos (30–35 mg/kg) or DBL (30 mg/kg)

tissue	% dose/g wet wt tissue ($\times 10^3$)[a] leptophos	DBL
forebrain	9.8 ± 2.0	9.3 ± 0.8
midbrain	8.6 ± 1.2	6.8 ± 0.8
hindbrain	11.7 ± 2.8	9.4 ± 0.4
cervical spinal cord	8.1 ± 1.5	6.0 ± 0.5
thoracic spinal cord	8.0 ± 2.5	6.9 ± 1.5
sciatic nerve	15.8 ± 4.1	12.5 ± 1.2

[a] Mean ± SD (n = 3).

There was no difference in the accumulation of radioactivity into the individual neural tissues between leptophos and DBL-treated hens at 24 h. However, concentrations of radioactivity were significantly higher in the sciatic nerve than in other neural tissues 24 h after treatment with leptophos or DBL.

Elimination. The percent dose excreted in the urine and feces from colostomized hens is summarized in Table III. Some of the samples were not discretely separated and are designated as "mixed". This generally did not distort excretion patterns except for a 7-h sample from hen 366 which contained 7.29% of the dose (Table III). Most of the radioactivity was excreted in the urine for both leptophos and DBL-treated hens. In addition, leptophos and DBL were excreted at similar rates and to similar extents during the first 24 h.

Metabolism. Urine samples from treated hens were selected at approximately 4-, 8-, 16-, and 24-h time points for further characterization. Uniformity (time, size, radioactivity) could not be controlled, especially since these hens responded differently to acute cholinesterase inhibition as well as atropinization. In spite of the variation in timing and proportion of total urine extracted, the patterns of distribution vs time among CH_2Cl_2-extractable, water-soluble, and bound radioactivity were similar for the two treatment groups (Table IV). The proportion of organic-extractable radioactivity decreased dramatically between the first and second samples. It may be seen that significant amounts of radioactivity are present as water-soluble and unextractable entities.

The organic extracts were subjected to two TLC systems. Results from system 1 (data not given) indicated that none of the radioactivity in the urine was attributable to parent leptophos or DBL.

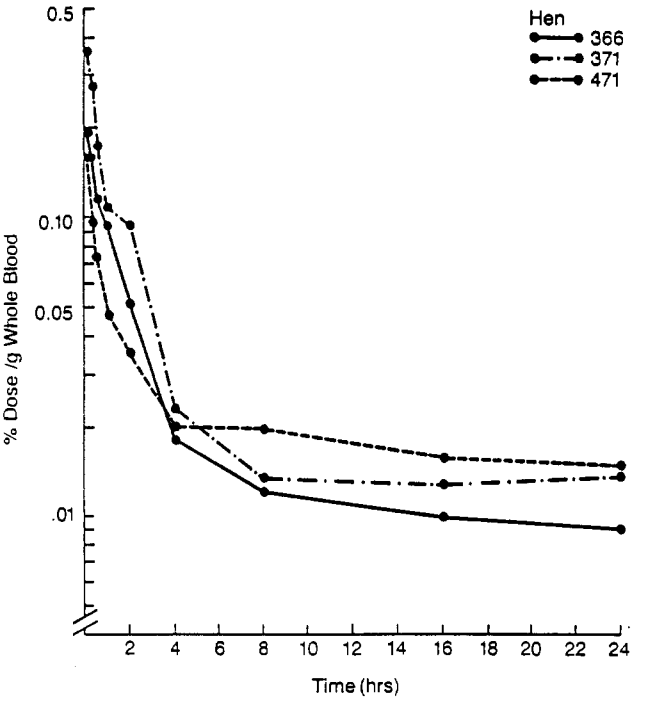


Figure 1. Percent dose radioactivity/gram of whole blood in hens for 24 h following a 30–35 mg/kg intravenous dose of [*O*-*methyl*-^{14}C]leptophos.

The TLC behavior of extracts in system 2 is summarized in Table V. Peaks of radioactivity were ascribed to MPPA and MPPTA. With data from Tables III–V, it can be seen that MPPA was the principal extractable metabolite of leptophos detected. Of the extractable radioactivity, 72–75% was attributable to MPPA and about 17% to MPPTA in both leptophos and DBL-treated hens. The route of metabolism did not appear to differ between the two compounds.

Distribution from the Central Compartment. Percent dose/gram whole blood data for leptophos and DBL following iv administration are shown in Figures 1 and 2. SAAM-generated fits to these data are given in Table VI. The true distribution phases of radioactivity, defined by more intense sampling, are much shorter than determined in the previous study (Hansen et al., 1985). These phases could not be defined in the oral and topical studies of Abou-Donia (1977, 1979, 1980). Distribution of radioactivity out of the central compartment can now be described

Table III. Cumulative Percent Dose of Radioactivity Excreted from Hens for 24 h following an Intravenous Dose of [*O*-*methyl*-^{14}C]Leptophos (30–35 mg/kg) or DBL (30 mg/kg)

Leptophos

	hen no. 366			hen no. 371			hen no. 471		
time, h	feces	urine	mixed	feces	urine	mixed	feces	urine	mixed
4.0	0.30	4.87			10.14		T[a]	6.00	
8.0	0.47	19.45	7.29	0.01	26.51		T	23.36	
16.0	1.27	19.45	7.29	0.21	31.14		0.84	34.29	0.41
24.0	1.81	34.25	7.29	0.65	38.07	0.12	1.34	38.57	0.50
total		43.35			38.84			40.41	

DBL

	hen no. 470			hen no. 476[b]			hen no. 469	
time, h	feces	urine	mixed	feces	urine	mixed	feces	urine
4.0	T	1.93		T	11.98	0.87	T	10.57
8.0	T	18.72		0.08	19.40	1.00	T	22.96
16.0	T	24.33		0.56	32.01	1.01	T	35.09
24.0	T	34.30	0.09	2.07	36.39	8.01	0.24	42.94
total		34.39			46.47			43.18

[a] T = trace (<0.005). [b] Given only 5 mg of atropine sulfate.

Table IV. Disposition of Radiolabel in Extracts from Urine of Colostomized Hens for 24 h following an Intravenous Dose of [*O-methyl*-^{14}C]Leptophos (30–35 mg/kg) or DBL (30 mg/kg)

hen	time, h	% radioactivity in fraction CH_2Cl_2	H_2O	residue	% rec
		Leptophos			
366	1.00	80.8	15.1	4.1	99.2
	7.00	55.8	16.4	27.8	102.0
	20.33	26.5	28.0	45.5	92.6
	24.50	28.4	30.5	41.1	80.0
371	2.66	74.9	18.9	6.2	100.1
	8.00	46.4	32.2	21.4	69.2
	16.50	9.5	43.3	47.2	85.6
	24.00	9.3	74.8	15.9	60.1
471	0.50	85.7	10.2	4.1	104.1
	7.08	23.3	42.0	34.7	75.6
	15.30	14.0	42.0	44.0	73.8
	22.30	12.6	37.4	49.9	14.0
		DBL			
470	0.58	62.3	36.4	1.3	89.0
	9.50	28.1	59.4	12.5	84.1
	18.83	37.9	52.8	9.3	75.0
	24.00	32.9	66.2	0.9	88.8
476	4.00	75.7	13.4	10.9	85.3
	8.33	24.6	53.3	22.1	63.5
	16.00	21.7	49.5	28.8	69.6
	24.00	8.7	88.6	2.7	42.9
469	2.42	86.0	6.9	7.1	77.3
	8.33	16.3	80.0	3.7	119.0
	16.00	37.0	43.0	20.0	62.6
	24.00	18.8	52.8	28.4	81.3

according to the equation $C_p = Ae(-\alpha t) + Be(-\beta t) + Ce(-\gamma t)$, where the third term probably represents the long, slow elimination phase. This phase is inadequately defined by 24-h sampling but was previously estimated to be 2–4 weeks. Early distribution of radioactivity out of the central compartment was statistically identical for leptophos and DBL.

Plasma Radioactivity. Table VII shows the distribution of radioactivity in the plasma into the various fractions following extraction. The behavior is illustrated in Figure 3 for hen 796. The radioactivity in the ether layer decreased with time, while that in the protein-bound fraction increased. Most of the radioactivity in plasma was protein bound after 8 h. The magnitude of the terminal slope for DBL elimination cannot be determined from these data, but it would appear that DBL reaches a slow elimination phase.

DISCUSSION

The present study was designed to elucidate pharmacokinetic and metabolic differences between leptophos and DBL that could account for the strikingly different neurotoxic potentials. Results indicate that there is no difference between the analogues with respect to distribution from the central compartment and into the presumed target tissue, route and extent of elimination, and pattern of biotransformation. Thus, differences between leptophos and DBL with respect to oral delayed neurotoxic potential are likely the result of differences in absorption from the gastrointestinal tract (Hansen et al., 1985).

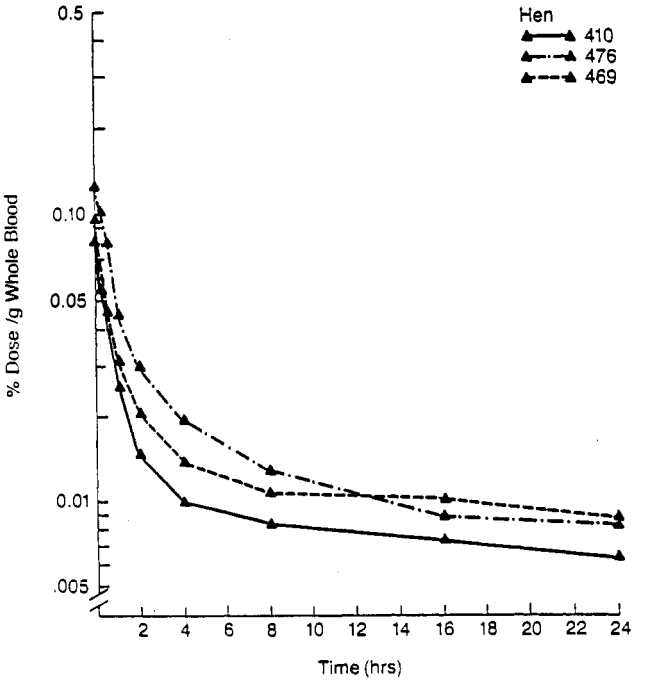


Figure 2. Percent dose radioactivity/gram of whole blood in hens for 24 h following a 30 mg/kg intravenous dose of [*O-methyl*-^{14}C]DBL.

Numerous studies (Lowndes et al., 1974; Howland et al., 1980; Caroldi et al., 1984) give evidence that the peripheral nerve is the site of action of delayed neurotoxicants. The present data provide limited evidence for sequestration of the delayed neurotoxicants into the target tissue and support previous findings (Hansen et al., 1985). Radioactivity did not localize into the sciatic nerve following oral or topical administration of leptophos in the hen (Abou-Donia, 1977, 1979, 1980); however, it did for EPN (*O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate) a delayed neurotoxicant of structure similar to leptophos following dermal administration in the cat (Abou-Donia et al., 1983).

This study demonstrates that leptophos and DBL are primarily excreted into the urine of hens as water-soluble and acidic metabolites. The urinary route of excretion for leptophos agrees with studies from the rat (Hassan et al., 1977; Whitacre et al., 1976) and mouse (Holmstead et al., 1973) and, in general, for the excretion of organophosphorus compounds. Although intuitively obvious with

Table V. Summary of TLC Separation of Extracts from Urine of Colostomized Hens for 24-h following an Intravenous Dose of [*O-methyl*-^{14}C]Leptophos (30–35 mg/kg) or DBL (30 mg/kg)

hen	% extractable radioactivity in zone 1	MPPA 2	MPPTA 3	4	% extr
		Leptophos			
366	7.6	76.8	8.7	5.5	100.0
371	1.9	74.9	19.4	2.8	79.6
471	1.2	72.4	24.5	1.9	25.8
mean ± SD	3.6 ± 3.5	74.7 ± 2.2	17.5 ± 8.1	3.4 ± 1.9	
		DBL			
470	3.7	57.4	29.0	7.9	34.8
476	2.0	85.4	7.2	4.0	34.3
469	1.2	73.8	14.9	10.4	36.3
mean ± SD	2.3 ± 1.3	72.2 ± 14.1	17.0 ± 11.1	7.4 ± 3.2	

Table VI. Pharmacokinetic Parameters from 24-h Study of Hens Receiving an Intravenous Dose of [*O-methyl*-^{14}C]Leptophos (30–35 mg/kg) or DBL (30 mg/kg)

	leptophos			DBL		
parameter	366[a]	371	471	470	476	469
α, h^{-1}	0.809	0.893	1.46	1.49	1.21	1.37
β, $h^{-1} \times 10^2$	1.66	1.38	1.96	3.79	3.98	2.16
A, % dose/g	0.181	0.293	0.132	0.066	0.111	0.0728
B, % dose/g	0.0132	0.0163	0.0228	0.010	0.0189	0.0141
mean ± SD						
α, h^{-1}		1.054 ± 0.354			1.328 ± 0.140	
β, $h^{-1} \times 10^2$		1.670 ± 0.290			3.31 ± 1.00	
A, % dose/g		0.203 ± 0.084			0.083 ± 0.024	
B, % dose/g		0.017 ± 0.005			0.015 ± 0.004	
$t_{1/2}(\alpha)$, h		0.657			0.510	
$t_{1/2}(\beta)$, h		41.58			20.94	

[a] Hen.

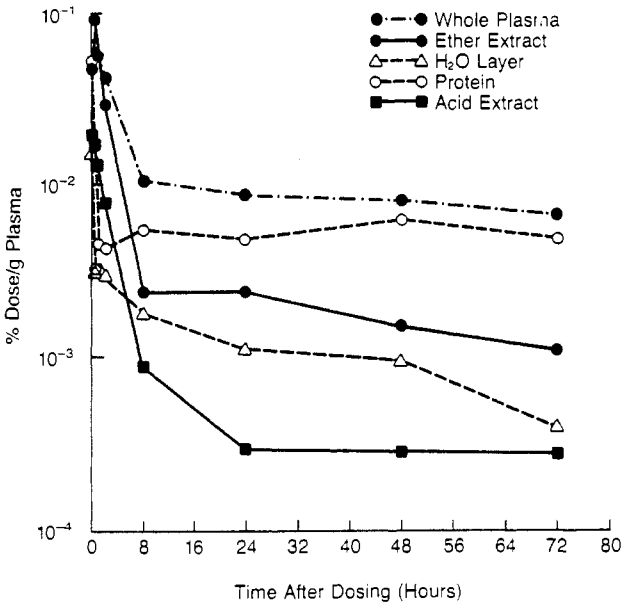


Figure 3. Disposition of radiolabel in plasma of hen 796 for 3 days following a 30 mg/kg intravenous dose of [*O-methyl*-^{14}C]-DBL.

respect to mammalian excretion of organophosphates, avian excretion patterns had not been well-defined since avian excreta are extruded mixed. The data also demonstrate that the distinct susceptibility of the hen to delayed neurotoxicity is not related to an inability to excrete and metabolize neurotoxic compounds.

Radioactivity in the urine was comprised of acid-extractable, water-soluble, and bound residues. The acid-extractable portion was comprised of MPPA and MPPTA. These probably resulted from hydrolysis of the oxons (Lasker et al., 1982) and oxidative or glutathione (GSH) dearylation of the thionates (Nomeir and Dauterman, 1979), respectively, by analogy to EPN. The presence of substantial amounts of the sampled radioactivity in the water layer gave some evidence to the GSH S-dealkylation route as significant for these compounds. The quantitative significance of this route in the hen has been shown for tetrachlorvinphos metabolism where 25% of the dose was excreted as desmethylated compound. Akhtar and Foster (1977) cited GSH demethylation as the predominant route of metabolism in the hen. The presence of significant amounts of nonextractable residues in hen excreta following op administration was also reported by Akhtar and Foster (1981) who found 18–35% of the radioactivity in the residue of hen excreta after treatment with labeled tetrachlorvinphos. Comparison of recovery from spiked

Table VII. Disposition of Radiolabel in Plasma of Hens for 3 Days following a 30 mg/kg Intravenous Dose of [*O-methyl*-^{14}C]Desbromoleptophos

		% dose				
hen	time, h	whole plasma	extract	H_2O	protein	acid
796	0.25	0.17460	0.14630	0.01500	0.05230	0.01920
	0.53	0.11840	0.09220	0.00320	0.00320	0.01700
	1.00	0.07750	0.05689	0.00330	0.00440	0.01250
	2.00	0.04456	0.02950	0.00299	0.00423	0.00750
	8.00	0.01024	0.00237	0.00177	0.00544	0.00088
	23.87	0.00850	0.00237	0.00110	0.00474	0.00029
	48.15	0.00788	0.00149	0.00093	0.00611	0.00028
	72.40	0.00643	0.00108	0.00038	0.00470	0.00027
714	0.10	0.06759	0.05434	0.00108	0.00289	0.00453
	0.50	0.03580	0.02602	0.00136	0.00308	0.00526
	1.00	0.02426	0.01657	0.00104	0.00277	0.00388
	2.00	0.01627	0.00997	0.00114	0.00264	0.00251
	8.00	0.00556	0.00123	0.00106	0.00310	0.00017
	24.17	0.00743	0.00062	0.00129	0.00658	0.00028
	48.50	0.00908	0.00088	0.00106	0.00698	0.00015
	69.00	0.00862	0.00131	0.00086	0.00622	0.00022
P-1	0.25	0.21180	0.18975	0.00254	0.00508	0.01480
	0.50	0.14185	0.11575	0.00355	0.00539	0.01700
	1.00	0.06879	0.05310	0.00289	0.00406	0.00887
	2.00	0.03460	0.02380	0.00294	0.00363	0.00422
	8.00	0.01526	0.00524	0.00237	0.00655	0.00111
	23.38	0.01136	0.00170	0.00132	0.00783	0.00051
	47.68	0.00827	0.00115	0.00104	0.00571	0.00036
	71.88	0.00659	0.00096	0.00062	0.00477	0.00024
P-2	0.08	0.00880	0.00775	0.00026	0.00045	0.00034
	0.50	0.01503	0.01139	0.00077	0.00123	0.00100
	1.00	0.01516	0.01284	0.00052	0.00064	0.00115
	2.00	0.01751	0.01385	0.00086	0.00110	0.00170
	8.00	0.01099	0.00577	0.00131	0.00311	0.00087
	23.92	0.01053	0.00158	0.00193	0.00622	0.00080
	48.25	0.01087	0.00100	0.00074	0.00829	0.00083
	68.92	0.00947	0.00093	0.00153	0.00666	0.00035

samples vs experimental samples in that study indicated that radioactivity in the residue resulted, at least in part, from metabolic incorporation.

The data from the second study with DBL serve to provide information about the identity of the radiolabel that appears in the blood following DBL administration. The majority of the radioalabel was present as protein-bound material 24 h after compound administration. This is not unexpected, since the irreversible binding of organophosphate pesticides to serum esterases is well documented. It may thus be seen that the 2–4-week half-life of radioactivity following leptophos administration previously noted was most likely due to slow turnover of protein-bound material and is not relevent to neuropathy.

ACKNOWLEDGMENT

We are grateful for the help of B. M. Francis and R. L. Metcalf for input into this report as well as the Environmental Protection Agency from providing support.

Registry No. DBL, 53490-78-1; MPPA, 10088-45-6; MPPTA, 42976-67-0; leptophos, 21609-90-5.

LITERATURE CITED

Abou-Donia, M. B. "Pharmacokinetics of a Neurotoxic Oral Dose of Leptophos in Hens". *Arch. Toxicol.* **1977**, *36*, 103–110.

Abou-Donia, M. B. "Pharmacokinetics and Metabolism of a Topically Applied Dose of O-4-Bromo-2,5-Dichlorophenyl O-Methyl Phosphorothioate in Hens". *Toxicol. Appl. Pharmacol.* **1979**, *51*, 311–328.

Abou-Donia, M. B. "Metabolism and Pharmacokinetics of a Single Oral Dose of O-4-Bromo-2,5-Dichlorophenyl O-Methyl Phenylphosphorothioate (Leptophos) in Hens". *Toxicol. Appl. Pharmacol.* **1980**, *55*, 131–145.

Abou-Donia, M. B.; Kinnes, C. G.; Abdo, K. M.; Bjornsson, T. D. "Physiological Disposition and Metabolism of O-Ethyl-O-4-Nitrophenyl Phenylphosphonothioate in Male Cats Following

a Single Dermal Administration". *Drug Metab. Dispos.* 1983, *11*(1), 31–36.
Akhtar, M. H.; Foster, T. S. "Metabolism of Tetrachlorvinphos by the Soluble Fraction (105000*g*) from Chicken Liver Homogenates". *J. Agric. Food Chem.* **1977**, *25*, 1017–1022.
Akhtar, M. H.; Foster, T. S. "Tetrachlorvinphos Metabolism in Laying Hens". *J. Agric. Food Chem.* **1981**, *29*, 766–771.
Berman, M.; Weiss, M. F. *Users Manuel for SAAM*; DHEW Publ. No. 78-180; National Institute for Arthritis and Metabolic Diseases: Bethesda, MD, 1978.
Caroldi, S.; Lotti, M.; Masutti, A. "Intra-arterial Injection of Diisopropylfluorophosphate or Phenylmethanesulphonyl Fluoride Produces Unilateral Neuropathy or Protection, Respectively, in Hens". *Biochem. Pharmacol.* **1984**, *33*(20), 3213–3217.
Chrzanowski, R. L.; Jelinek, A. G. "Metabolism of Phenyl Carbon-14-Labeled *O*-Ethyl *O*-(4-Nitrophenyl) Phenylphosphonothioate in the Rat and in Hens at Toxic and Subtoxic Dose Levels". *J. Agric. Food Chem.* **1981**, *29*, 580–587.
Dixon, J. M. "Investigation of Urinary Water Reabsorption in the Cloaca and Rectum of the Hen". *Poultry Sci.* **1956**, *37*(2), 410–414.
Giang, P. A.; Hall, S. A. "Enzymatic Determination of Organic Phosphorus Insecticides". *Anal. Chem.* **1951**, *23*, 1830–1834.
Gibaldi, M.; Perrier, D. *Pharmacokinetics*; Marcel Dekker: New York, 1982.
Hansen, L. G. "Biotransformation of Organophosphorus Compounds Relative to Delayed Neurotoxicity". *Neurotoxicology* **1983**, *4*, 72–86.
Hansen, L. G.; Cozzi, E. M.; Metcalf, R. L.; Hansen, T. K. "Effect of Disposition on the Relative Delayed Neurotoxicity Potency Among Leptophos Analogs". *Pestic. Biochem. Physiol.* **1985**, *24*, 136–148.
Hassan, A.; Abdel-Hamid, F. M.; Mohammed, S. I. "Metabolism of 14C-Leptophos in the Rat". *Arch. Environ. Contam. Toxicol.* **1977**, *6*, 447–454.
Holmstead, R. L; Fukuto, T. R.; March, R. B. "The Metabolism of O-(4-Bromo-2,5-Dichlorophenyl) O-Methyl Phenylphosphonothioate (Leptophos) in White Mice and on Cotton Plants". *Arch. Environ. Contam. Toxicol.* **1973**, *1*, 133–147.
Howland, R. D.; Lowndes, H. E.; Baker, T.; Richardson, R. J. "DFP Mononeuropathy: Evidence for a Periferal Site of Initiation". *Brain Res.* **1980**, *184*, 248–251.
Hutson, D. H.; Pickering, B. A.; Donniger, C. "Phosphoric Acid Triester-Glutathione Alkyltransferase. A Mechanism for the Detoxification of Dimethyl Phosphate". *Biochem. J.* **1972**, *127*, 285–293.
Johnson, M. K. "The Delayed Neuropathy Caused by Some Organophosphorus Esters: Mechanism and Challenge". In *CRC Crit. Rev. Toxicol.* **1975**, *3*, 289–316.
Lasker, J. M.; Graham, D. G.; Abou-Donia, M. B. "Differential Metabolism of O-Ethyl O-4-Nitrophenyl Phenylphosphonothioate by Rat and Chicken Hepatic Microsomes". *Biochem. Pharmacol.* **1982**, *31*(11), 1961–1967.
Lowndes, H. E.; Baker, T.; Riker, W. F., Jr. "Motor Nerve Dysfunction in Delayed DFP Neuropathy". *Eur. J. Pharmacol.* **1974**, *29*, 66–73.
Metcalf, R. L.; Francis, B. M.; Metcalf, R. A.; Hansen, L. G. "Acute and Delayed Neurotoxicity of Leptophos Analogs". *Pestic. Biochem. Physiol.* **1983**, *20*, 57–66.
Nomeir, A. A.; Dauterman, W. C. "*In Vitro* Metabolism of EPN and EPNO by Mouse Liver". *Pestic. Biochem. Physiol.* **1979**, *10*, 190–196.
Rivers, J. B.; Yanzer, W. L., Jr.; Klemmer, H. W. "Simultaneous Gas Chromatographic Determination of 2,4-D and Dicamba in Human Blood and Urine". *J. Chromatogr.* **1970**, *50*, 334–337.
Whitacre, D. M.; Badie, M.; Schwemmer, B. A.; Diaz, L. I. "Metabolism of 14C-leptophos and 14C-4-Bromo-2,5-Dichlorophenol in Rats: A Multiple Dosing Study". *Bull. Environ. Contam. Toxicol.* **1976**, *16*(6), 689–696.

Received for review December 1, 1986. Accepted August 19, 1987.

Occurrence of the Mycotoxin Cyclopiazonic Acid in Meat after Oral Administration to Chickens

William P. Norred,* James K. Porter, Joe W. Dorner, and Richard J. Cole

Four-week old chickens were administered 0.5, 5.0, or 10.0 mg of cyclopiazonic acid (CPA)/kg of body weight by crop intubation and were killed 3, 24, 48, or 96 h later. Breast and thigh muscle was removed from the carcasses, and the content of CPA in the meat was measured by a high-performance liquid chromatographic procedure. CPA was found in the meat from birds in each dose group, and the amount found was dependent on both dose given and the elapsed time after dosing. The maximum muscle content of CPA occurred 3 h after administration and ranged from 50 ppb in birds given 0.5 mg/kg to over 5000 ppb in some birds given 10.0 mg/kg. Birds given the highest dose of CPA eliminated CPA from meat at a slower rate than other treatment groups.

The fungal metabolite cyclopiazonic acid (CPA; Figure 1) is a toxic indole tetramic acid first isolated from *Penicillium cyclopium* Westling (Holzapfel, 1968). The toxicity of CPA to rats was demonstrated by Purchase (1971) and has since been shown by others (van Rensburg, 1984; Morrissey et al., 1985). Toxicity occurs in a number of other species, including pigs (Lomax and Cole, 1983; Lomax et al., 1984), chickens (Dorner et al., 1983), mice (Nishie et al., 1985a), dogs (Neuhring et al., 1985), and rabbits (Nishie et al., 1984). CPA is also suspected of causing symptoms of "kodua poisoning" in humans that consumed kodo millet seed in India (Rao and Husain, 1985). The discovery that *Aspergillus flavus* isolates are capable of producing CPA (Luk et al., 1977; Gallagher et al., 1978) has caused increased concern over the potentially harmful effects of this toxin, since *A. flavus*, one of the fungi responsible for the production of the highly toxic and carcinogenic aflatoxins, is a frequent contaminant of major agricultural commodities. Instances of natural occurrences

Toxicology and Biological Constituents Research Unit, Richard B. Russell Agricultural Research Center, USDA—ARS, Athens, Georgia 30604 (W.P.N, J.K.P.), and National Peanut Research Laboratory, USDA—ARS, Dawson, Georgia 31742 (J.W.D., R.J.C.).