Metabolism of ^{14}C-Leptophos and ^{14}C-4-Bromo-2, 5-dichlorophenol in Rats: A Multiple Dosing Study

D. M. Whitacre, M. Badie, B. A. Schwemmer, and L. I. Diaz

Research Department
Velsicol Chemical Corporation
341 E. Ohio Street
Chicago, Ill. 60611

Leptophos [O-(4-bromo-2,5-dichlorophenyl)-O-methyl phenylthionophosphonate] is a phosphonate insecticide which is used internationally to control several pests on cotton, certain vegetable crops, and tobacco. The insecticide has been used under temporary permit in the U.S. for control of pests on citrus, certain fruits, and several vegetable crops.

The environmental fate of phosphonate insecticides has not been well defined (MENN, 1971). Only one paper has been reported on the fate of leptophos when administered to small mammals (HOLMSTEAD et al., 1973). They reported that a single oral dose of ^{14}C-leptophos given to mice was rapidly excreted in the urine, largely as polar metabolites. In a different study, ^{14}C-leptophos residues inhaled by rats in cigarette smoke were almost totally eliminated (>90%) after 4 days (ATALLAH et al., 1975).

The purpose of this study was to determine the fate of ^{14}C-leptophos and its major metabolite, 4-bromo-2,5-dichlorophenol (BDCP), after multiple administration to rats.

MATERIALS AND METHODS

Chemicals

Radiochemicals used in this study were synthesized by New England Nuclear Corporation, Boston, MA, and were >98% radiochemically pure by thin layer chromatography (TLC). These were ^{14}C-phenyl-labeled leptophos [O-(4-bromo-2,5-dichlorophenyl)-O-methyl phenyl (^{14}C) thionophosphonate], specific activity of 6.23mCi/mM; ^{14}C-phenoxy-labeled leptophos [O-(4-bromo-2,5-dichloro-

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 6 © 1976 by Springer-Verlag New York Inc.

phenyl (^{14}C)-O-methyl phenylthionophosphonate", specific activity of 7.35mCi/mM; and ^{14}C-BDCP "4-bromo-2,5-dichlorophenol(^{14}C)", specific activity of 11.51mCi/mM.

Analytical reference chemicals, including leptophos, leptophos oxon "O-(4-bromo-2,5-dichlorophenyl)-O-methyl phenyl phosphonate", BDCP, O-methyl phenylphosphonic acid (MPPA), O-methyl phenylphonothioic acid (MTPPA) and phenylphosphonic acid (PPA), were >97% pure and were either purchased or synthesized.

Treatment of Rats

Adult Holtzman rats, weighing between 300-400g, were used in these studies.

The desired dose in 0.30 ml of ethanol/water (1:1) was administered to lightly ether-anesthetized rats by oral intubation. Each rat received a net of about 2.0, 1.5 and 0.5μCi of the ^{14}C-phenoxy leptophos, ^{14}C-phenyl leptophos and ^{14}C-BDCP, respectively, per day. The daily dosage of each label was about 0.30 (♂), 0.50 (♀); 0.23 (♂), 0.30 (♀); and 0.035 mg/kg for ^{14}C-phenoxy and ^{14}C-phenyl leptophos and ^{14}C-BDCP, respectively.

Duplicate males and females were dosed with the labeled forms of leptophos and one female was dosed with ^{14}C-BDCP daily for five days. The rats were then placed on a withdrawal diet for an additional six days. Each animal was individually housed in a metabolism chamber which could collect urine and feces separately. Purina Rat Chow and water were provided ad libitum to animals throughout the experiment. At the end of the experiment, animals were sacrificed and organs excised for radioanalysis.

Excreta

Urine - Urine was collected frequently during each 24-hour period and was held at 5°C prior to radioanalysis. Daily collections were individually radioassayed for total ^{14}C by direct counting.

For the leptophos studies, urine from days 3 and 4 or 4 and 5 were combined for analysis. For the ^{14}C-BDCP study, urine from the first five collection days

was combined for analysis.

In all cases, urine was filtered and the pH adjusted to <3.0 or <1.0 with concentrated HCl for BDCP and leptophos-dosed rats, respectively.

Acidified urine from the ^{14}C-BDCP and ^{14}C-phenoxy leptophos-dosed rats was extracted directly with a 2X volume of ethyl acetate. The ^{14}C-phenyl leptophos urine was extracted with a 4X volume of acetonitrile: ethyl ether (1:1) after saturation with NaCl. Extracts were counted directly to determine distribution of radiocarbon between the organic and aqueous layers.

The urine extracts were reduced to the appropriate volume by flash evaporation and were subjected to TLC analysis.

Feces - Feces were collected frequently during each 24-hour period and were held at 5°C prior to analysis.

Feces composited from each 24-hour period were lyophilized to dryness, weighed, and ground into a powder with a C.R.C. micromill. Subsamples were then subjected to combustion analysis.

Tissues

Excised organs and tissues from the ^{14}C-phenyl leptophos-dosed rats were lyophilized and ground into a powder as described for feces. The skinned carcass, including muscle, bone, connective and adipose tissue, was homogenized in water using a Waring blender. Aliquots of the homogenate were then subjected to combustion analysis.

Radioassay

About 1-ml aliquots of urine or other liquids were counted directly, while 100-200-mg aliquots of dried, powdered tissues and feces were combusted followed by radioassay of trapped $^{14}CO_2$.

All samples were counted using a Nuclear Chicago Isocap 300 Liquid Scintillation Spectrophotometer,

while solids were combusted using a Packard 305 Biological Materials Oxidizer.

Spiked recovery of solids subjected to combustion indicated that the efficiency of the combustion technique employed was greater than 90%.

Solvents were counted using Permafluor® (Packard Instruments), while aqueous samples were counted in Aquasol® (New England Nuclear). Counting efficiency varied from 65 to 98%, depending on types of samples under assay. All counts were corrected for quench, using either sample channels ratio or external standardization techniques.

Thin Layer Chromatography

Precoated silica gel G-F254 thin layer chromatographic plates, 0.25mm thick (Brinkmann Instruments), were developed in one dimension with acetonitrile: water:ammonium hydroxide (80:17:3) for analysis of leptophos urinary extracts, or in hexane:ethyl ether: ethanol:acetic acid (70:30:1:1) for BDCP urinary extracts.

After development, reference standard spots were visualized under UV light, while radiocarbon spots were located by radioautography after exposure to Kodak no-screen X-ray film for from 1 to 7 days. Individual spots isolated using radioautography were quantitated by direct counting of scraped silica gel.

RESULTS AND CONCLUSIONS

Excretion

The cumulative excretion of ^{14}C in urine and feces at different stages of the study is presented in Table I. After 5 days of continuous leptophos treatment, about 80-95% of the cumulative dose had been eliminated in excreta, divided about 80:20 between urine and feces, respectively. Recovery of radiocarbon from the ^{14}C-phenoxy label was slightly better than the ^{14}C-phenyl label leptophos. After the leptophos source was removed, radiocarbon continued to be eliminated for the duration of the collection period, although less than

TABLE I.

Elimination of radiocarbon from rats administered five daily doses of ^{14}C-leptophos or ^{14}C-4-bromo-2,5-dichlorophenol (BDCP)

Days after 1st dose		Cumulative % of dose after treatment with:								
		Leptophos-phenyl-^{14}C			Leptophos-phenoxy-^{14}C			BDCP-ring-^{14}C		
		Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
1	Male	51.66	10.60	62.26	66.36	3.83	70.19	---	---	---
	Female	34.96	--*	34.96	60.26	--*	60.26	53.08	--*	53.08
3	Male	70.28	12.78	83.06	73.99	12.00	85.99	---	---	---
	Female	39.93	11.29	51.22	76.19	11.65	87.84	48.07	15.56	63.63
5	Male	71.48	20.36	91.84	86.04	12.66	98.70	---	---	---
	Female	60.80	17.74	78.54	76.96	--*	88.61	48.14	12.77	60.91
7 1/	Male	75.85	22.80	98.65	92.92	13.19	106.11	---	---	---
	Female	62.85	19.41	82.26	79.46	19.47	98.93	53.47	16.77	70.24
9	Male	78.48	23.70	102.18	93.60	13.33	106.93	---	---	---
	Female	67.35	19.67	87.02	80.30	19.56	99.86	54.33	16.95	71.28
11	Male	80.09	24.28	104.37	94.03	14.01	108.04	---	---	---
	Female	69.60	19.99	89.59	80.67	19.61	100.28	55.08	17.11	72.19

*No feces collected.

1/Animals were on an insecticide-free diet after the 5th day.

1% was eliminated in the last 24-48 hours excreta was collected. At the end of the experiment, the cumulative excretion of radiocarbon from leptophos-dosed rats averaged about 100%.

After 5 days of BDCP administration to a female rat, 61% of the cumulative dose had been excreted, about 80:20 urine to feces. The cumulative excretion of ^{14}C-BDCP or equivalents at the end of the experiment was 72%, much lower than the leptophos excretion. A possible explanation lies with the fact that the BDCP is relatively volatile.

It is perhaps worth mentioning that in initial studies with leptophos not reported here, problems in quantitatively recovering administered radiocarbon were encountered. The source of the problem was traced to the use of toluene as a preservative in urine collection cups. It was shown that the ^{14}C-leptophos and metabolites partitioned into the toluene in significant quantities and then co-evaporated with the solvent. The problem was rectified by eliminating the use of toluene and collecting excreta frequently during each collection period.

The data from this study demonstrate clearly that continuous exposure of rats to leptophos in no way limits the capacity of these animals to excrete the substance efficiently and quickly. Elimination is essentially complete within 72 hours of receiving a single dose or the last of a series of doses. The excretory pattern for leptophos presented here for rats is very similar to the pattern shown for mice by HOLMSTEAD et al. (1973).

Tissues

Table II presents the results of tissue radioassays. The recovery of radiocarbon through the drying and powdering process was checked and found to be good.

The highest residues were found in liver, but even this constituted <0.25% of the cumulative administered dose, six days following the last of five daily doses. No attempt was made to characterize the low levels of radiocarbon found in tissues.

TABLE II.

Radiocarbon in Tissues of Rats 6 Days after the Last of 5 Daily Doses of ^{14}C-Phenyl Leptophos

Tissue*	% of Administered Dose	(ppm)
Liver	0.234	0.166
Blood	0.024	0.010
Lung	0.017	0.049
Skin	0.111	0.020
Digestive Tract	0.059	0.020
Carcass**	0.20	0.009

*Kidney, heart, testes and brain each had less than 0.01%.

**Remains including muscle, bone, connective and adipose tissue.

Nature of Urinary Metabolites

Leptophos - More than 97% of urinary radiocarbon was organosoluble for both labeled forms.

Table III shows the types and quantities of metabolites excreted in urine. Leptophos was quantitatively hydrolyzed and the products excreted in urine. There were no detectable residues of leptophos in urine from the phenoxy or phenyl-labeled studies. The phenoxy radiocarbon was excreted in urine as BDCP almost quantitatively (>98%). The phenyl radiocarbon was excreted in urine as MPPA (~90%), PPA (~10%), and MTPPA (~1%) and an unknown. The major rat urinary metabolite (MPPA) is different from the major mouse urinary metabolite (MTPPA) as elucidated by HOLMSTEAD et al. (1973), and probably represents intraspecies differences in metabolism of the compound.

BDCP - More than 98% of the urinary radiocarbon was organoextractable and >98% of this was unchanged BDCP. The BDCP is known to be excreted in urine as a salt and in the free form, but in these studies we did not seek to identify the ratio between the two.

TABLE III.

Nature of ^{14}C-Leptophos Metabolites in Urine[1/] of Rats Orally Administered these Substances Daily for Five Days.

	(% of cumulative dose)			
	Phenoxy-^{14}C-leptophos		Phenyl-^{14}C-leptophos	
Metabolite	Female	Male	Female	Male
Leptophos	ND	ND	ND	ND
Unknown	ND	ND	0.23	0.32
BDCP	98.5	98.5	--	--
MPPA	--	--	89.30	89.40
MTPPA	--	--	0.52	1.10
PPA	--	--	9.20	9.95
Origin	1.5	1.5	--	--

ND - Nondetectable (<0.01% of administered activity)

1/ - Urine composites collected on days 3 and 4 or 4 and 5 of the study.

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