The Retention, Distribution, Excretion, and Metabolism of Dibutyl Phthalate-7-^{14}C in the Rat

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The retention, distribution, excretion, and metabolism of dibutyl phthalate-7-^{14}C have been investigated in the rat. Of a single oral dose 80–90% is metabolized and excreted in the urine within 48 hr. Phthalic acid, monobutyl phthalate, mono(3-hydroxybutyl) phthalate, and mono(4-hydroxybutyl) phthalate have been identified as metabolites in the urine. Rats fed for 12 weeks on a diet containing dibutyl phthalate at 1 g/kg of feed did not accumulate either dibutyl phthalate or monobutyl phthalate in tissues or organs.

Phthalate esters have become ubiquitous contaminants of the environment and their occurrence, analysis, toxicology, and health hazards have been extensively reviewed (Autian, 1973; Bloom, 1972). Dibutyl phthalate (DBP) has been reported in air samples (Thomas, 1973), inland waters (Hites, 1973), and fish (Mayer et al., 1972; Williams, 1973). It has a low oral toxicity (Lefaux, 1968) but has been reported to have some toxic properties (Singh et al., 1972). Chambon et al. (1972) have reported that rats orally dosed with DBP excrete monobutyl phthalate (BP) and phthalic acid in the urine but accounted for only 2–3% of the ingested dose. Stalling et al. (1973) have shown that DBP is metabolized, by a microsomal preparation from channel catfish liver, to BP and at least three other unidentified metabolites. Albro and Moore (1974) have investigated the metabolism of several phthalate diesters and have identified, by infrared and mass spectrometry, six metabolites in the urine of rats dosed with DBP. They were able to account for only 28% of the ingested dose and the present study has, therefore, been carried out using DBP-7-^{14}C in order to quantitate the retention, distribution, excretion, and metabolism in the rat.

EXPERIMENTAL SECTION

Reagents. General reagents and precautions against phthalate contamination have been previously reported (Williams, 1973).

NMR Spectroscopy. A Varian analytical NMR spectrometer, Model A60A, coupled to a Varian time averaging computer, Model C-1024, was used to record the NMR spectra.

Thin-Layer Chromatography. Pre-coated TLC sheets of silica gel F-254 (0.25 mm, Merck) were used with the following solvent systems: (A) 10% ethyl ether in petroleum ether; (B) chloroform–methanol–acetic acid (147:7:2, v/v/v); (C) 2% ethyl ether in hexane.

Gas–Liquid Chromatography. A Varian Aerograph Model 2100 gas–liquid chromatograph equipped with flame ionization detectors was used with nitrogen (40 ml/min) as the carrier gas. U-Shaped glass columns were used: (A) 6 ft × 3.5 mm i.d. packed with 5% OV-210; (B) 6 ft × 3.5 mm i.d. packed with 5% OV-101, both on 100–120 mesh Chromosorb W (HP); (C) 6 ft × 2 mm i.d. packed with 10% SP-216-PS (Supelco Inc.) on 100–120 mesh supelcoport. Operating temperatures were: injection port, 220°; detector block, 270°; column temperature, 165° for dimethyl phthalate and methylbutyl phthalate, 192° for all other metabolite derivatives.

GLC–Mass Spectrometry. A Varian MAT Model 311A high-resolution mass spectrometer coupled with a Varian-Aerograph Model 1400 gas chromatograph was used. The 6 ft × 0.25 in. glass column was packed with 5% OV-101 on 100–120 mesh Chromosorb W (HP) and the carrier gas was helium at 40 ml/min. The operating conditions were as follows: injection port, 240°; detector block, 270°; the column temperature was as indicated.

Sample Preparation for GLC Analysis. Combined tissues or organs were homogenized with ether (5 vol) and then with 0.5 *N* sodium hydroxide (5 vol). Ether and base extracts were shaken together and the ether layer was analyzed for DBP and the base layer for BP.

DBP. Dried ether extract was concentrated to ca. 25 ml under a stream of nitrogen at room temperature, acetonitrile was added (10 ml), and then the remaining ether blown off. Acetonitrile was extracted with petroleum ether (15 ml) saturated with acetonitrile and then petroleum ether was back extracted with acetonitrile (3 × 10 ml), the acetonitrile extracts were combined, an equal volume of water added, and the aqueous acetonitrile extracted with petroleum ether (3 × 20 ml). The petroleum ether extracts were combined, washed with water, dried (Na_2SO_4), and concentrated under a stream of nitrogen at room temperature to ca. 15 ml.

BP. The base extract was acidified (pH 1) and extracted with ether (2 vol). The ether extracts were concentrated to dryness and the residue was methylated with diazomethane in ether–5% methanol. Acetonitrile (20 ml) was added and the ether was blown off under nitrogen at room temperature. Water was added (20 ml), the solution was extracted with petroleum ether (3 × 20 ml), washed with water, dried (Na_2SO_4), and the petroleum ether solution was concentrated to ca. 15 ml.

A small column (0.5 g) of activated silica gel was washed with ether (5 ml) followed by petroleum ether (5 ml). The 15-ml solution of the DBP or BP extract was added to the column and eluted with petroleum ether (5 ml) followed by 15% ether in petroleum ether (10 ml). The 15% ether eluate was collected, concentrated to 0.5 ml using a micro-Snyder column, and analyzed by GLC or GLC–MS. The overall recovery for DBP was 92% and that for BP was 44%.

Radioactivity Measurements. Quantitative measurements of radioactivity were made with a Packard Tri-Carb Model 3320 liquid scintillation spectrometer. Activity in aqueous solutions was measured by addition of 1-ml aliquots to 15 ml of Aquasol (New England Nuclear) and in organic solvents by addition of 1-ml aliquots to 15 ml of toluene containing 2,5-diphenyloxazole (0.4%) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (0.01%). Tissue samples were counted by dissolving 50–100 mg of tissue in 1 ml of solubilizer (Soluene) at room temperature and then adding 15 ml of toluene scintillator solution. Quench corrections were made using an automatic external standard-channels ratio system.

Radioactive compounds were detected on thin-layer chromatograms by exposure to Kodak no-screen X-ray film.

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Table I. Distribution of Radioactivity throughout the Rat at Given Time Intervals after Dosing with DBP-7-^{14}C

	4 hr		8 hr		24 hr		48 hr	
	0.27 g/kg	2.31 g/kg	0.27 g/kg	2.31 g/kg	0.27 g/kg	2.31 g/kg	0.27 g/kg	2.31 g/kg
Blood	0.36[a] ± 0.21[b]	0.40 ± 0.16	0.41 ± 0.23	0.37 ± 0.28		0.39 ± 0.30	0	<0.01
Spleen	0.13 ± 0.05	0.28 ± 0.11	0.24 ± 0.13	0.25 ± 0.19	0.01 ± 0.01	0.17 ± 0.16	0	0
Kidney	0.66 ± 0.23	0.42 ± 0.11	0.53 ± 0.25	0.35 ± 0.23	0.04 ± 0.01	0.41 ± 0.28	<0.01	<0.01
Liver	0.20 ± 0.09	0.25 ± 0.09	0.24 ± 0.09	0.18 ± 0.18	0.02 ± 0.01	0.28 ± 0.22	<0.01	<0.01
Adipose	0.22 ± 0.08	0.39 ± 0.20	0.30 ± 0.11	0.57 ± 0.40	0.02 ± 0.01	0.18 ± 0.22	<0.01	<0.01
Muscle	0.11 ± 0.05	0.16 ± 0.07	0.20 ± 0.12	0.14 ± 0.11	<0.01	0.18 ± 0.15	<0.01	<0.01
Lungs	0.37 ± 0.16	0.38 ± 0.14	0.41 ± 0.12	0.36 ± 0.27	<0.01	0.27 ± 0.12	<0.01	<0.01
Testes	0.12 ± 0.05	0.25 ± 0.11	0.20 ± 0.12	0.20 ± 0.14	<0.01	0.18 ± 0.15	<0.01	<0.01
Heart	0.20 ± 0.10	0.26 ± 0.09	0.30 ± 0.10	0.22 ± 0.17	<0.01	0.20 ± 0.16	<0.01	<0.01
Brain	0.03 ± 0.02	0.06 ± 0.03	0.04 ± 0.02	0.07 ± 0.06	<0.01	0.07 ± 0.06	<0.01	<0.01
Urine	19.2 ± 5.1	10.7 ± 2.6	45.9 ± 7.8	19.6 ± 4.2	85.4 ± 16.7	61.3 ± 20.3	91.5 ± 6.3	82.5 ● 5.1
Feces			0.50 ± 0.45	0.09 ± 0.06	2.2 ± 0.22		3.3 ± 1.3	5.0 ± 1.7

[a] Expressed as percent of dose. [b] Standard deviation.

Table II. Percentages of DBP Metabolites Excreted in Rat Urine

Metabolite	R_f	%[a]
1	0.50	88.4
2	0.30	7.7
3	0.23	2.0
4	0.05	1.9

[a] Percentage calculated relative to radioactivity extracted by ether from acidified urine.

Dibutyl Phthalate-7-^{14}C. Phthalic anhydride-7-^{14}C (1.0 mg) was heated at 100° for 2 hr with 10% boron trichloride in 1-butanol (0.5 ml). The reaction mixture was cooled, diluted with hexane (10 ml), and washed with 0.5 *N* sodium hydroxide (10 ml) and 7% sodium sulfate (5 × 10 ml). Analysis by GLC (columns A and B) and by TLC–autoradiography (solvents A and C) showed that the DBP-7-^{14}C had a chemical and radiochemical purity of at least 99.5%. The overall yield was 75% and the specific activity was 12.88 mCi/mmol. Portions of this product were diluted with freshly distilled unlabeled DBP for use in the animal studies.

Methyl 4-Hydroxybutyl Phthalate and Methyl 3-Hydroxybutyl Phthalate. Phthalic anhydride (1 g) was heated at 100° for 4 hr with either 1,4-butanediol or 1,3-butanediol (20 ml). The monohydroxybutyl ester was purified by preparative TLC (1 mm silica gel, solvent B) and methylated with diazomethane in ether–5% methanol, and the methyl hydroxybutyl phthalate was purified by TLC (1 mm, silica gel, solvent C). 1,3-Butanediol also reacts with phthalic anhydride to give some 3-hydroxyisobutyl phthalate and this can be detected in the 3-hydroxybutyl phthalate by NMR of the methyl ester or by GLC of the silylated methyl esters. Pure methyl 3-hydroxybutyl phthalate is best prepared by sodium borohydride reduction of methyl 3-ketobutyl phthalate.

Methyl 3-Ketobutyl Phthalate. Methyl 3-hydroxybutyl phthalate (plus some methyl 3-hydroxyisobutyl phthalate) was oxidized with sodium dichromate in acid solution according to the general method of Hussey and Baker (1960). The reaction was followed by GLC (columns A and B) and stopped when the methyl 3-hydroxybutyl phthalate peak could no longer be detected. The reaction mixture was extracted with ether and the ether extract washed with base and water. The methyl 3-ketobutyl phthalate was characterized by NMR and MS.

Rat studies. All rats used were male Wistar strain (Biobreeding). (a) Two rats (79 and 105 g) were intubated with corn oil (0.5 ml) containing DBP (10.0 mg, 0.5 μCi) and kept individually in controlled ventilation cages. The air from the cage (300 ml/min) was pulled through two traps containing a solution of monoethanolamine–ethylene glycol monomethyl ether (1:2). The traps were changed at 24 and 48 hr and aliquots from these traps and from the excreted urine were analyzed for radioactivity. (b) Twenty-four rats (39 ± 3.6 g) were intubated with corn oil (0.5 ml) containing DBP (10.4 mg, 0.5 μCi). Six rats were killed (ether asphyxiation) at 4, 8, 24, and 48 hr and tissues, organs, urine, and feces were analyzed for radioactivity. (c) Twenty rats (41.5 ± 3.1 g) were intubated with corn oil (0.5 ml) containing DBP (96 mg, 0.48 μCi). Six rats were killed at 4 and 8 hr and four rats at 24 and 48 hr and tissues, organs, urine, and feces were analyzed for radioactivity. (d) Twenty-four rats (51.2 ± 4.7 g) were fed ground standard rat chow mixed with 2% corn oil and 0.1% DBP (1 g of DBP/kg of feed). Twelve control rats (51.7 ± 4.7 g) were fed ground rat chow mixed with 2% corn oil. Eight of the treated rats and four control rats were killed at 4, 8, and 12 weeks. For the 4-week study the diets of four of the treated rats also contained 10 μCi of DBP-7-^{14}C/kg of feed; the other four treated rats in the 4-week study were fed this radioactive diet only for the last 24 hr. For the 8- and 12-week studies the rats' diet contained 0.7 μCi of DBP-7-^{14}C/kg of feed for the last 24 hr. At the end of the studies the rats were weighed (treated rats 326.4 ± 29.4 g; control rats 308.6 ± 30.0 g) and killed and the organs and tissues removed, weighed, and frozen until analyzed.

RESULTS AND DISCUSSION

Preliminary experiments with rats under controlled ventilation conditions showed with single oral doses of DBP-7-^{14}C at 0.1 and 0.13 g/kg that 96% of the radioactivity was excreted in the urine and less than 0.1% was exhaled as $^{14}CO_2$ within 48 hr of dosing. Subsequent experiments were, therefore, carried out in open cages.

The distribution of radioactivity among the tissues and organs of rats given single oral doses of DBP-7-^{14}C at 0.27 and 2.31 g/kg was determined at 4, 8, 24, and 48 hr. The distribution of radioactivity was general throughout the body and was similar at both dose levels although the radioactivity was cleared from the body more quickly at the lower dose. Tissues and organs contained only traces of radioactivity 48 hr after dosing even with the higher dose (Table I).

Acidification (pH 1) of the urine and extraction with ethyl ether gave 93.5% extraction of the radioactivity

Table III. GLC Relative Retention Times of DBP Metabolite Derivatives

	OV-210		OV-101		SP-216-PS	
	165°, 24.4 min[a]	190°, 3.9 min	165°, 30 min	190°, 8.4 min	165°, 23 min	190°, 8.4 min
DBP	1.0	1.0	1.0	1.0	1.0	1.0
DMP	0.17	0.24	0.11	0.15	0.52	0.63
MBP	0.44	0.52	0.35	0.40	0.74	0.79
M(3-OHBu)P		1.36		0.77		
M(4-OHBu)P		2.00		1.09		
M(3-keto-Bu)P		1.94		0.56		
Silylated M(3-OHBu)P		1.09		1.37		
Silylated M(4-OHBu)P		1.52		1.51		
Silylated M(3-OH-*i*-Bu)P		0.94		1.29		

[a] Retention times refer to DBP.

Table IV. Principal Fragments in the Electron Impact Mass Spectra of DBP Metabolite Derivatives

	Relative abundance, %[a]						
m/e	MBP	DMP	M(4-OHBu)P	M(3-OHBu)P	M(3-ketoBu)P	Silyl M(4-OHBu)P	Silyl M(3-OHBu)P
309							3
280							12
253						9	16
237						23	35
236							
222			12				
221						5	26
217							
208				22	60		
207							18
194		11					
181	15		27				
176				37	36		
163	100	100	100	100	100	100	100
149	71	2	94	89	69		
117						13	42
89						45	37

[a] Relative to *m/e* 163 = 100%.

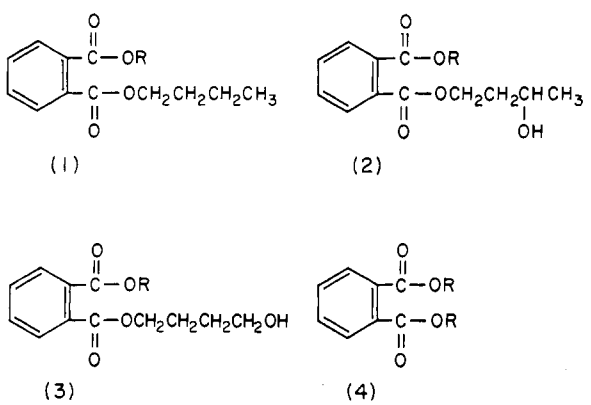

Figure 1. Metabolites of DBP as excreted in the urine of rats.

present in the urine. Analysis of this ether extract by TLC–autoradiography (solvent B) showed one major metabolite and three minor metabolites. Each radioactive metabolite was eluted from the TLC plate and estimated by liquid scintillation counting (Table II). Each metabolite on hydrolysis gave only one radioactive compound, identified as phthalic acid by TLC–autoradiography (solvent C) and confirmed after methylation with diazomethane as dimethyl phthalate by TLC–autoradiography (solvent C) and by GLC–MS. Each metabolite could be methylated with diazomethane and on electron impact mass spectrometric analysis gave a major fragment at *m/e* 163, indicative of a methyl phthalate ester. Two of the methylated derivatives were readily identified as methyl *n*-butyl phthalate (MBP) (Figure 1 (1), R = CH_3) and dimethyl phthalate (DMP) (Figure 1 (4), R = CH_3) by TLC–autoradiography (solvent C), GLC (columns A, B, and C), and GLC–MS. Metabolite 1 can, therefore, be identified as monobutyl phthalate (Figure 1 (1), R = H) and metabolite 4 as phthalic acid (Figure 1 (4), R = H). The methylated metabolites 2 and 3 were purified by preparative TLC (solvent C) and analyzed by NMR spectroscopy. Both compounds showed one methyl ester group (3.9 ppm) and no other three-proton singlet peak. Apart from the aromatic protons the only other distinctive peak in either spectrum was a three-proton doublet (1.23 ppm) in the spectrum of methylated metabolite 2. Reference to the NMR data presented by Albro et al. (1973) for the metabolites of bis(2-ethylhexyl) phthalate indicated that neither metabolite 2 nor 3 contained an aliphatic $COOCH_3$ group (3.64 ppm) or a $CH_3C{=}O$ group

Table V. NMR Spectra of Methyl Phthalate Esters[a]

ppm	Class	Number of protons				
		DMP	MBP	M(3-OHBu)P	M(4-OHBu)P	M(3-keto-Bu)P
7.6	Complex	4	4	4	4	4
4.58	Triplet					2
4.38	Triplet		2	2	2	
3.9	Singlet	6	3	3	3	3
3.85	Complex			1		
3.65	Triplet				2	
2.85	Triplet					2
2.2	Singlet					3
1.82	Complex			2		
1.75	Complex				4	
1.23	Doublet			3		
1.5	Complex		4			
0.95	Triplet		3			

[a] Run in C^2HCl_3 plus ^2H_2O.

Table VI. Radioactivity in Tissues and Organs of Rats Fed on a Diet Containing DBP-7-^{14}C

Tissue	cpm/g of tissue[a]	
	4 weeks on DBP-^{14}C	1 day on DBP-^{14}C
Spleen	32	49
Kidney	195	231
Adipose	224	167
Testes	31	6
Skeletal muscle	Trace	Trace
Heart	Trace	Trace
Lungs	Trace	Trace
Brain	Trace	Trace

[a] Corrected for background.

Table VII. DBP and BP Levels in Tissues and Organs of Rats Fed on a Diet Containing DBP

	DBP, μg/g of tissue		BP, μg/g of tissue	
	GLC	^{14}C	GLC	^{14}C
Spleen	0.5	<1.0	0.6	1.8
Heart	0.5	<1.0		
Kidney			6.9	8.0
Liver			2.2	3.9
Adipose				
Skeletal muscle				
Lungs				
Brain				

(2.12 ppm), but that metabolite 2 did contain a CH_3CHOH group (1.23 ppm, doublet). Both of the methylated metabolites could be readily trimethylsilylated. These data were consistent with the assignment of metabolite 2 as mono(3-hydroxybutyl) phthalate (Figure 1 (2), R = H) and metabolite 3 as mono(4-hydroxylbutyl) phthalate (Figure 1 (3), R = H), two of the compounds identified by Albro and Moore (1974) as metabolites of DBP. Synthesis of the methyl esters of these two compounds and comparison of the GLC retention times (Table III), mass spectra (Table IV), and NMR spectra (Table V) showed that this assignment was correct.

Albro and Moore (1974) have reported the presence of two other metabolites, 3-ketobutyl phthalate and 4-carboxybutyl phthalate, in the urine of rats dosed with DBP. During the preparative TLC purification of metabolites 2 and 3 trace amounts of other radioactive metabolites were detected but at levels too low to identify. Whether the differences in the percentages of metabolites excreted in the urine in our studies compared to those of Albro and Moore (1974) are due to the dosage or the age or strain of the rats is not clear.

To determine whether DBP or its metabolites would accumulate in tissues or organs rats were fed a diet containing 1 g (10 μCi) of DBP-7-^{14}C/kg of feed for a period of 4 weeks. In an attempt to differentiate between DBP ingested within the last 24 hr and not completely excreted from the rat (Table I) and that which had accumulated over the period of the study other rats were fed a diet containing unlabeled DBP until the last day of the study, at which time they were given the feed containing labeled DBP. Tissue samples and organs were collected from the rats and the counts per minute per gram of tissue were determined (Table VI). Under our counting conditions 20 cpm/g of tissue would be equivalent to 1 μg of DBP/g of tissue. Except possibly for the adipose tissue there appears to be no accumulation of DBP or its metabolites under these conditions.

Other rats were fed a diet containing unlabeled DBP at 1 g/kg feed for 8 and 12 weeks and a diet containing 1 g (0.7 μCi) of DBP/kg of feed for the last 24 hr of each study. For the 12-week study the tissues and organs were analyzed for DBP and BP by GLC and the values obtained compared with those calculated from radioactivity measurements (Table VII). One microgram of DBP/gram of tissue was equivalent in this study to only 1.4 cpm/g of tissue and due to the errors involved in measuring these low levels of radioactivity the values obtained by this method should be considered as approximate values. However, despite this limitation it can be seen that any DBP or BP detected in the organs and tissues was probably due to DBP ingested in the last 24 hr of the study and that there had been no substantial accumulation of these compounds. Analysis of the rats on the 8-week study gave similar results. There were no gross pathological changes nor significant differences in organ weights between the treated rats and control rats in either the 8- or 12-week study.

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Dissipation of Parathion and Related Compounds from Field-Sprayed Lettuce

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In June 1974 in Yolo County, California, duplicate plots of Climax lettuce were sprayed at the rate of application of active ingredient of 0.5 lb and 1 lb per acre 7 days before harvest with Thiophos parathion 4 E.C. A control plot was also established and sampled before the other plots were sprayed and daily thereafter with the sprayed plots. The sprayed lettuce was analyzed for parathion and related compounds as the field head, trimmed head, and trimmings from the initial day of pesticide application through the harvest sample, 7 days later at both rates of spray application. To determine the depth of penetration and amounts of residue present at each level within the head of the field lettuce, the outer 12 leaves were analyzed as a composite, the next 6 inner leaves, the next 6 inner leaves, and the remaining head. The sum total residues of parathion and related compounds on the untrimmed harvested lettuce samples 7 days after pesticide application were below the tolerance level of 1 ppm at the 0.5 lb rate of active ingredient per acre spray application. The trimmed heads at both rates of pesticide application were below 1 ppm residue levels at harvest.

Parathion applications have been recommended for the control of leaf-feeding aphids and leafminers on lettuce at the rate of 0.5 lb of actual material per acre not less than 7 days before harvest (*Calif. Agric. Exp. Stn. Bull.*, 1973). Storherr et al. (1964) have reported the identification and analysis of five organophosphate pesticides, including parathion, and their recoveries from crops fortified at 5.0, 1.0, and 0.1 ppm from eight different crops including lettuce. Coffin (1966) studied the oxidative metabolism and persistence of parathion and malathion on field-sprayed lettuce. Parathion was applied at a rate of 400 g of active ingredient per acre and was sampled at various intervals after spraying and was analyzed by paper chromatography for residues. Parathion residues decreased from 1.9 to 0.1 ppm in 4 days and detectable quantities were present at 15 days and longer. Small quantities of paraoxon and two unidentified metabolites were detected, at times within 2 days and longer after the parathion application. The effects of some climatic factors on the degradation of parathion and methylparathion residues on lettuce were investigated by Polizu et al. (1963). Parathion and methylparathion were sprayed in doses of 240 and 600 g/ha on lettuce in field and greenhouse tests followed by the determination of the toxic residues on the leaves. The degradation of the parathion and methylparathion was affected by exposure to sunshine and high temperature. The period between spraying and safe harvesting was 10–16 days in the field and 14–19 days in greenhouses.

Although parathion levels were determined by the above workers, most of the other possible related compounds were not determined. The purpose of the present investigations was to determine quantitatively the fate of the levels of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) as well as possible related compounds such as paraoxon (*O,O*-diethyl *O*-*p*-nitrophenyl phosphate), aminoparathion (*O,O*-diethyl *O*-*p*-aminophenyl phosphorothioate), *S*-phenylparathion (*O,O*-diethyl *S*-*p*-nitrophenyl phosphorothiolate), *S*-ethylparathion (*O,S*-diethyl *O*-*p*-nitrophenyl phosphorothiolate), *p*-nitrophenol, *O,O*-diethyl phosphate, and *O,O*-diethyl phosphorothioate in the environment on field-sprayed lettuce sampled daily from application through harvest.

EXPERIMENTAL SECTION

Lettuce Plots. In June 1974 in Yolo County, California, rows of Climax lettuce 40 in. wide and 48 ft in length were selected for duplicate spray applications of Thiophos parathion 4 E.C. at the rates of 0.5 (plot B) and 1 lb (plot C) of active ingredient per acre. Another plot of similar dimensions upwind from the others was established as a control plot (plot A). The lettuce selected was at the stage of maturity 7 days before harvest since the recommended spray application is 0.5 lb of active ingredient/acre 7 days before harvest. The first sampling of the control plot was taken before spraying the other plots. Each of the other plots was sampled immediately after spraying, and all plots were randomly sampled (approximately 6 kg) thereafter at the same time of day during the experiments' duration and extracted for analysis after subsampling composites. At the harvest sampling all the lettuce from each plot was completely harvested and random subsamples were immediately extracted for analysis. Samples were taken daily from time of pesticide application to time of harvest 7 days later. The

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