presence of wort. With the new regulations introduced in West Germany in 1981 for spraying hops with EBDC's, the residues of ETU/PTU in beer are considerably reduced.

Registry No. EBDC, 34731-32-3; ETU, 96-45-7; PTU, 2055-46-1; propineb, 12071-83-9; copper, 7440-50-8.

LITERATURE CITED

Attar, A.; Bieniek, D.; Klein, W.; Korte, F. *GSF-Ber. O* **1982**, *665*, 29.
Graham, S. L.; Davis, K. J.; Hansen, W. H. *Food Cosmet. Toxicol.* **1975**, *13*, 493.
Graham, S. L.; Hansen, W. H. *Bull. Environ. Contam. Toxicol.* **1972**, *7*, 19.
Innes, J. R. M.; Ulland, B. M.; Valerio, M. G.; Petrucelli, L.; Fishbein, L.; Hart, E. R.; Pallota, A. J.; Bates, R. R.; Falk, H. L.; Gart, J. H.; Klein, M.; Mitchel, I.; Peters, J. *J. Natl. Cancer Inst. (U.S.)* **1969**, *42*, 1101.
Keppel, G. E. *J. Assoc. Off. Anal. Chem.* **1969**, *52*, 162.
Larsson, K. S.; Arnander, C.; Cekanova, E.; Kjellberg, M. *Tetralogy* **1976**, *14*, 171.
Lesage, S. *J. Agric. Food Chem.* **1980**, *28*, 787.
"Merkblätter des Amtlichen Pflanzenschutzdienstes der Bayerischen Landesanstalt für Bodenkunde und Pflanzenbau"; Wolnzach und Landesanstalt für Pflanzenschutz: Stuttgart, 1980; Section Hops.
"Merkblätter des Amtlichen Pflanzenschutzdienstes der Bayerischen Landesanstalt für Bodenkunde und Pflanzenbau"; Wolnzach und Landesanstalt für Pflanzenschutz: Stuttgart, 1981; Section Hops.
Newsome, W. H.; Laver, G. W. *Bull. Environ. Contam. Toxicol.* **1973**, *10*, 151.
Nitz, S.; Moza, P. N.; Korte, F. *J. Agric. Food Chem.* **1982**, *30*, 593.

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Metabolism of Tributyl Phosphate in Male Rats

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When rats were given a single oral dose of ^{14}C-labeled tributyl phosphate at 14 mg/kg, 50, 10, and 6% of the applied ^{14}C were excreted in urine, exhaled air, and feces, respectively, within 1 day. On the other hand, when rats were given a single intraperitoneal dose (same amount), 70, 7, and 4% of the applied ^{14}C were excreted in urine, exhaled air, and feces, respectively, within 1 day. After a single intraperitoneal dose of tributyl phosphate at 250 mg/kg, 11 phosphorus-containing metabolites were identified in the 24-h urine. Major metabolites were dibutyl hydrogen phosphate, butyl dihydrogen phosphate, and butyl bis(3-hydroxybutyl) phosphate as well as small amounts of derivatives hydroxylated at the butyl moieties. Administration of a probable intermediate, dibutyl 3-oxobutyl phosphate, gave a metabolic profile similar to that of dibutyl 3-hydroxybutyl phosphate, which is considered to be the major metabolic intermediate. This supports the view that dibutyl 3-oxobutyl phosphate is also on the main metabolic pathway. Furthermore, the metabolites from urine of rat administered butyl bis(3-hydroxybutyl) phosphate and dibutyl hydrogen phosphate were examined. Some of the possible metabolic pathways of tributyl phosphate are proposed.

Tributyl phosphate (TBP) (I) has been widely used as a solvent for extraction of metals and as a plasticizer. Recently it has also been examined for possible usage as a volatilization controller for insecticidal fumigants (Koezuka, 1979). The occurrence of this material in drinking water (Williams and LeBel, 1981), river water (Schou et al., 1981; Meijers and Van der Leer, 1976), and the edible parts of fish (Environmental Agency of Japan, 1978) has been demonstrated. It is toxic to fish (Sasaki et al., 1981), Daphnia (Bringmann and Kuehn, 1982), other types of aquatic organisms (Bringmann and Kuehn, 1980) and mammals (Oishi et al., 1980).

The metabolic fate of this compound has not been examined in detail, though Jones (1970) reported that I was metabolized to butyl-L-cysteine and dibutyl hydrogen phosphate in rodents. We now report the metabolic fate of I in male rats in order to provide a basis for assessing its toxicological impact.

MATERIALS AND METHODS

Treatment of Rats and Collection of Samples. For the identification and determination of the metabolites of I, male Wistar rats (180–210 g) were given a single intraperitoneal (ip) injection of I, dibutyl 3-hydroxybutyl phosphate (II), dibutyl 3-oxobutyl phosphate (III), butyl bis(3-hydroxybutyl) phosphate (IV), or dibutyl hydrogen phosphate (Va) dissolved 10% in corn oil at the dosages indicated in table III or under Extraction and Purification of Some Main Metabolites.

For the radioanalysis, male rats were given a single ip injection or a single oral dose of [^{14}C]TBP (specific activity 0.179 mCi/mmol) at a dosage of 14 mg/kg of body weight, dissolved in 0.1 mL of corn oil. The rats were kept in metabolic cages and supplied ad libitum with diet and water, both of which were proved to be free from TBP. Feces and urine were collected separately at regular intervals. The volume of each sample of urine was determined and aliquots were taken for radioassay. Each sample of feces collected was air-dried, weighed, and ground, and an appropriate quantity was taken for radioassay. The radioactivity fortified to the sample was recovered almost quantitatively.

Radioassay. Radioactivities in organic solvents or in the aqueous layer were determined by mixing the sample with Aquasol-2 (New England Nuclear). Radioactivity in fecal material was determined by using an Aloka ASC-113 automatic sample oxidizer. $^{14}CO_2$ formed was trapped in a scintillation solution, Oxifluor-CO_2 (New England Nuclear), and counted on an Aloka LSC-700 liquid scintilla-

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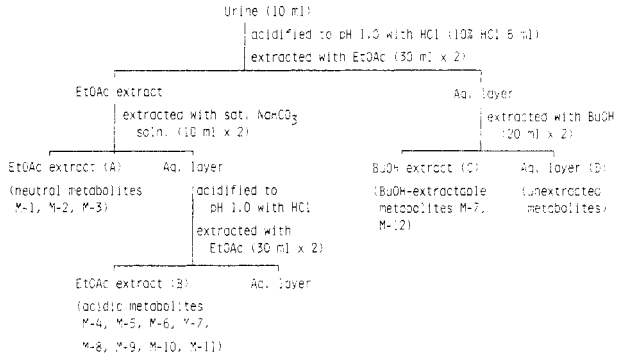

Figure 1. Extraction and separation of metabolites in urine.

tion spectrometer, with correction by the external standard method.

Gas–Liquid Chromatography. For the identification and determination of metabolites, a Shimadzu GC-4BM gas chromatograph equipped with flame photometric detectors (FPD, P mode or S mode) was used. The column packings used were 1% OV-225 on Chromosorb W (AW), 80–100 mesh. The OV-225 was packed in a glass column 1.5 m long and with a 3-mm i.d. Operating conditions were as follows: injector temperature 250 °C, detector temperature 250 °C, nitrogen gas pressure 1.0 kg/cm^2, air flow 80 mL/min, and hydrogen gas flow 180 mL/min. Column temperature and retention time (RT) are presented in each figure.

Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). NMR spectra were recorded on a JEOL JNX FX 200S NMR spectrometer (200 MHz) in $CDCl_3$ with tetramethylsilane as an internal standard (δ value) (s, singlet; d, doublet; t, triplet; q, quartet; br, broad).

Mass Spectroscopy (MS). Mass spectra were obtained by coupled GLC–MS with a Shimadzu LKB 9000 instrument in the electron impact (EI) mode at 70 eV. The column used for GLC–MS was the same as described under Gas–Liquid Chromatography. The column temperature was set at 90–210 °C.

Infrared (IR) Spectroscopy. IR spectra were measured with a JASCO A 102 spectrometer.

Enzymatic Hydrolysis with β-Glucuronidase. Urine (10 volumes) was adjusted to pH 1 and extracted with EtOAc (equal volume to urine) 3 times. The aqueous phase was taken up in a glass tube, flushed with nitrogen gas until EtOAc was removed, and adjusted to pH 5 with 10% $NaHCO_3$ solution, then 1 volume of β-glucuronidase (β-G, 13 000 Fishman units/mL) in 1 M acetate buffer (pH 5.0) was added, and the mixture was incubated at 37 °C for 18 h. After completion of incubation, the solution was treated according to Figure 1. The glucuronide was determined from the difference between sample urine and reference urine to which β-G had not been added.

Detection of *S*-Butyl-L-cysteine (VI) and *N*-Acetyl-*S*-butyl-L-cysteine (VII). According to the method described by James et al. (1968), an aliquot of urine was acetylated with acetic anhydride in the presence of NaOH. The EtOAc extract of this solution obtained under acidic conditions was methylated with ethereal CH_2N_2 and subjected to FPD-GC.

Acetylation. The sample was dissolved in acetic anhydride (10 mL), and two drops of pyridine were added to the solution. After standing at room temperature for 12 h, the solution was evaporated to dryness and the residue was purified on silica gel with $CHCl_3$ as the eluting solvent.

Methylation. A sample solution in MeOH or EtOEt was treated with excessive ethereal CH_2N_2 below 0 °C.

Thin-Layer Chromatography (TLC). Silica gel HF_{254} (E. Merck) was used.

Column Chromatography. Silica gel (100 mesh, Mallinckrodt) and aluminum oxide (neutral, M. Woelm) were used.

EXPERIMENTAL PROCEDURES

Extraction and Purification of Some Main Metabolites. The 24-h urine from five rats dosed intraperitoneally with I (250 mg/kg) was combined. After separation according to Figure 1, the acidic fraction (B) was methylated and then separated on silica gel (15 g) with $CHCl_3$ as the eluent. The first eluate (180 mL) contained several minor metabolites. Subsequent elution with 1% MeOH–$CHCl_3$ gave the methyl ester of M-7 (3 mg). The BuOH fraction (C) was evaporated, methylated, and chromatographed in the same manner as described for the acidic fraction. After elution of the methyl ester of M-7, the methyl ester of M-12 was obtained; the latter was acetylated and purified on silica gel with $CHCl_3$ as the mobile phase.

Radiosynthesis. ^{14}C-Labeled TBP was synthesized as follows. To [1-^{14}C] BuOH (250 μCi, 0.5 mCi/mmol) was added BuOH (250 mg) in dry dioxane (5 mL), followed by $POCl_3$ (180 mg) and triethylamine (500 mg) in dry dioxane (5 mL). The mixture was allowed to stand for 2 weeks, and then precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated under reduced pressure. The residue was taken up in H_2O (30 mL) and then the solution was extracted with EtOAc. The organic layer was washed with 10% $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and then evaporated to dryness. The residue was subjected to column chromatography on silica gel (15 g) ($CHCl_3$). The eluate from 50 to 70 mL was collected and concentrated to afford TBP (97 mg): 98% radiochemical purity by TLC (10% acetonitrile–benzene); 26% radiochemical yield; 33% chemical yield; specific activity 179 μCi/mmol.

Synthesis of Metabolic Standards. The NMR, mass, and IR spectral data for the purchased and synthesized compounds are listed in Table I. All organic solutions were dried over anhydrous Na_2SO_4 and concentrated in a rotary evaporator under reduced pressure.

TBP (I). TBP of >97% purity (GC) was purchased from Wako Pure Chemical Industries Ltd.

Dibutyl 3-Hydroxybutyl Phosphate (II). Triethylamine (28 g, 0.28 mol) in dry dioxane (200 mL) was added to a mixture of dibutyl chlorophosphate (VIII) [32.5 g, 0.14 mol; synthesized by the method of Sosnovsky and Zaret (1969)], 1,3-butanediol (25 g, 0.28 mol), and dry dioxane (50 mL) at room temperature under stirring, and the mixture was stirred for 4 days. After removal of triethylamine hydrochloride by filtration, the filtrate was concentrated and extracted with EtOAc. The EtOAc layer was washed with H_2O, dried, and evaporated. Distillation of the residue under reduced pressure gave crude II (33 g), bp 165–167 °C/1 mmHg. This product contained about 10% impurities, which were removed by chromatography on Al_2O_3 (activity I) with $CHCl_3$. Acetylation of II gave a colorless oil. Anal. Calcd for $C_{14}H_{29}O_6P$: C, 51.84, H, 9.01. Found: C, 51.96; H, 9.02.

Dibutyl 3-Oxobutyl Phosphate (III). A solution of II (2.8 g, 0.011 mol) in AcOH (10 mL) was treated with CrO_3 (1.7 g, 0.018 mol) in a small amount of H_2O, followed by 2 drops of H_2SO_4. After being allowed to stand for 12 h, the solution was kept at 50 °C for 1 h and then extracted with EtOAc. The extract was washed with H_2O, dried, and then evaporated to give an oily residue. Chromatography of the oily residue on Al_2O_3 (activity I, 90 g) and then on

silica gel (50 g) with $CHCl_3$ gave a colorless oil (2.1 g), III, which was very unstable even at −20 °C under a nitrogen atmosphere and decomposed to give dibutyl hydrogen phosphate (Va) in 100% yield on storage in $CHCl_3$ for 1 week at room temperature. Before use, III was chromatographed on silica gel ($CHCl_3$) to avoid contamination with Va.

Butyl Bis(3-hydroxybutyl) Phosphate (IV). Triethylamine (15.8 g, 0.16 mol) in dry dioxane (80 mL) was added to a mixture of butyl dichlorophosphate (IX) [15 g, 0.078 mol; synthesized by the method described by Mizuma et al. (1961)], 1,3-butanediol (28.2 g, 0.31 mol), and dry dioxane (80 mL) in the same way as described for II. Column chromatography of the netural extract on silica gel (80 g) with $CHCl_3$ gave an oily material, IV (4.2 g). Acetylation of IV (500 mg) gave IV-diacetate (420 mg). Anal. Calcd for $C_{16}H_{31}O_8P$: C, 50.25; H, 8.17. Found: C, 50.35; H, 8.19.

Dibutyl Hydrogen Phosphate (Va), Dibutyl Methyl Phosphate (Vb), and Butyl Dimethyl Phosphate (X). A mixture of dibutyl hydrogen phosphate (55%) and butyl dihydrogen phosphate (45%), purchased from Wako Pure Chemical Industries Ltd., was methylated and the products were used as authentic standards. For the purpose of administration, dibutyl hydrogen phosphate (Va) (>95%) was purchased from Wako Pure Chemical Industries Ltd.

S-Butyl-L-cysteine (VI). S-Butyl-L-cysteine (VI) was synthesized by the method described by Bray et al. (1959), mp 238–242 °C (lit. mp 238–242 °C). This sample was methylated to give the methyl ester of VI.

N-Acetyl-S-butyl-L-cysteine (VII). N-Acetyl-S-butyl-L-cysteine (VII) was synthesized by the method described by Bray et al. (1959), mp 68.5–69.5 °C (lit. mp 67 °C). This sample was methylated to give the methyl ester of VII.

Butyl (3-Methoxycarbonyl)propyl Methyl Phosphate (XI). MeOH (25 g, 0.78 mol) was added dropwise to a dry ethereal solution of IX (150 g, 0.78 mol) under stirring at room temperature, and the mixture was stirred for a further 5 h. The solvent was evaporated off, and then the residue was distilled to give an oily product, butyl methyl chlorophosphate (XII) (85 g), bp 98–101 °C/12 mmHg.

A mixture of 1,4-butanediol (2.8 g, 0.031 mol), triethylamine (2.7 g, 0.027 mol), and dry dioxane (20 mL) was added to a dry dioxane solution (50 mL) of XII (5.0 g, 0.027 mol) under stirring at 8–10 °C. Column chromatography of the neutral fraction, which was obtained in the same way as in the case of IV, gave butyl 4-hydroxybutyl methyl phosphate (XIII) as a pure oil (610 mg). An AcOH solution (5 mL) of XIII (610 mg, 0.0025 mol) was treated with CrO_3 (300 mg, 0.003 mol) in a small amount of H_2O. the mixture was kept at 55 °C for 15 min, then a small amount of MeOH was added, and the whole was concentrated nearly to dryness. H_2O (30 mL) and 10% HCl (3 mL) were added to the residue, and the solution was extracted with EtOAc. The extract was methylated, washed with H_2O, dried, and then evaporated. Column chromatography of the residue on silica gel (15 g) with $CHCl_3$ gave a colorless oil, XI (560 mg). Anal. Calcd for $C_{10}H_{21}O_6P$: C, 44.97; H, 7.89. Found: C, 45.24; H, 7.89.

Dibutyl (3-Methoxycarbonyl)propyl Phosphate (XIV). Dibutyl 4-hydroxybutyl phosphate (XV) (1.8 g) was obtained in the same way as described for II, from VIII (4.0 g, 0.014 mol), 1,4-butanediol (6.0 g, 0.066 mol), and triethylamine (1.4 g, 0.014 mol): NMR ($CDCl_3$) δ 0.94 (t, 6 H, J = 7.4 Hz, CH_3), 1.41 (6 lines, 4 H, J = 7.4 Hz, CH_2), 1.5–1.9 (m, 8 H, CH_2), 2.2–2.4 (br s, 1 H, OH), 2.66 (t, 2 H, J = 7.0 Hz, CH_2OH), 3.9–4.1 (m, 1 H, CH_2). Oxidation of XV (1 g) with CrO_3, followed by methylation of the product and purification on silica gel (10 g) ($CHCl_3$), gave a colorless oily material, XIV (750 mg). Anal. Calcd for $C_{13}H_{27}O_6P$: C, 50.31; H, 8.77. Found: C, 50.32; H, 8.77.

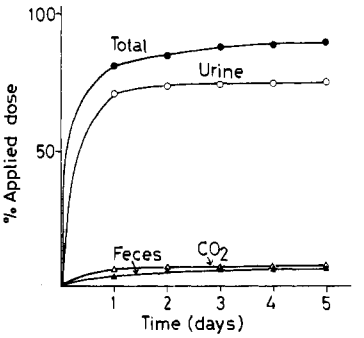

Figure 2. Elimination of radiolabel from male Wistar rats dosed intraperitoneally with [^{14}C]TBP (14 mg/kg).

Butyl 3-Hydroxybutyl (3-Methoxycarbonyl)propyl Phosphate (XVI). A neutral oily material (ca. 1 g) was obtained in the same way as described for IV, from IX (10.6 g, 0.055 mol), 1,4-butanediol (5 g, 0.055 mol), 1,3-butanediol (5 g, 0.055 mol), and triethylamine (11 g, 0.11 mol). This material was oxidized, methylated, and chromatographed in the same way as described for the synthesis of XIV to give an oily product, XVI (260 mg). XVI-acetate: Anal. Calcd for $C_{15}H_{29}O_8P$: C, 48.93; H, 7.94. Found: C, 49.01; H, 7.82.

Butyl 3-Hydroxybutyl Methyl Phosphate (XVII). IX (10 g, 0.052 mol), 1,3-butanediol (4.7 g, 0.052 mol), and triethylamine (5.2 g, 0.052 mol) were reacted in the same way as described for the synthesis of II, and the reaction mixture was filtered, concentrated, and then extracted with 10% $NaHCO_3$ solution. The alkaline extract was acidified with 10% HCl and then extracted with EtOAc. The organic layer was methylated and then evaporated to give an oily residue (1.1 g), which was chromatographed on Al_2O_3 (activity III) with $CHCl_3$ to afford a colorless oil, XVII (850 mg). Anal. Calcd for $C_9H_{21}O_5P$: C, 44.99; H, 8.81. Found: C, 45.00; H, 8.87. Acetylation of XVII gave a colorless oil, XVII-acetate.

Butyl Bis[(3-methoxycarbonyl)propyl] Phosphate (XVIII). IX (7 g, 0.036 mol), 1,4-butanediol (6.5 g, 0.072 mol), and triethylamine (7.3 g, 0.072 mol) were reacted and processed in the same way as described for the synthesis of IV. The crude diol thus obtained was oxidized in the same way as described for the synthesis of XIV, methylated, and purified on silica gel ($CHCl_3$) to give an oily material (1.5 g), XVIII. Anal. Calcd for $C_{14}H_{23}O_8P$: C, 47.46; H, 7.68. Found: C, 47.50; H, 7.69.

Butyl 2-Hydroxybutyl Methyl Phosphate (XIX). IX (11.4 g, 0.059 mol), 1,2-butanediol (5.3 g, 0.059 mol), and triethylamine (6.0 g, 0.059 mol) were reacted and processed in the same way as described for the synthesis of XVII. Purification on silica gel ($CHCl_3$) and then on silica gel (10% acetonitrile–benzene) gave a colorless oily material, XIX (150 mg). Acetylation of XIX gave a colorless oil (150 mg). Anal. Calcd for $C_{11}H_{23}O_6P$: C, 46.80; H, 8.12. Found: C, 46.85; H, 8.22.

RESULTS AND DISCUSSION

Radiolabel Balance. When a rat was given a single dose of [^{14}C]TBP intraperitoneally, 70% of the radiocarbon was found in urine, 7% in exhaled air, and 4% in feces. In total, 81% of administered radioactivity was recovered within 1 day and 90% within 5 days (Figure 2). On the other hand, when the radiolabeled compound was administered orally, 50% of the radiocarbon was found in urine, 10% in exhaled air, and 6% in feces; a total of 66%

Table I. Spectral Data

	NMR ($CDCl_3$)	MS, m/z (rel intensity)	IR, cm^{-1}
I	δ 0.94 (t, 9 H, J = 7.3 Hz, CH_3), 1.41 (6 lines, 6 H, J = 7.8 Hz, CH_3CH_2), 1.6-1.7 (m, 6 H, OCH_2CH_2), 4.0-4.1 (m, 6 H, OCH_2)	99 (100), 155 (17)	
II	δ 0.94 (t, 6 H, J = 7.2 Hz, CH_3CH_2), 1.24 [d, 3 H, J = 6.4 Hz, CH_3CH], 1.37 (6 lines, 4 H, J = 7.1 Hz, CH_3CH_2), 1.6-1.8 (m, 6 H, OCH_2CH_2), 2.72 (br s, 1 H, OH)	99 (100), 125 (10), 127 (12), 153 (6), 155 (10), 182 (2)	3420, 2960, 1460, 1375, 1253, 1140, 1030, 910 (film)
II-acetate	δ 0.94 (t, 6 H, J = 7.3 Hz, CH_3CH_2), 1.27 (d, 3 H, J = 6.3 Hz, CH_3CH), 1.39 (6 lines, 4 H, J = 7.3 Hz, CH_3CH_2), 1.62 (5 lines, 2 H, J = 8.3 Hz, $OCH_2CH_2CH_2$), 1.70-2.05 [m, 2 H, $CH_2CH(OCOCH_3)$], 2.03 (s, 3 H, $OCOCH_3$), 3.92-4.10 (m, 6 H, OCH_2), 4.83-5.08 (m, 1 H, CH_3CH)	61 (4), 71 (4), 72 (3), 73 (2), 99 (81), 114 (6), 115 (100), 116 (8), 125 (10), 127 (6), 137 (2), 153 (19), 155 (17), 183 (4), 212 (1)	1739 (film)
III	δ 0.94 (t, 6 H, J = 7.3 Hz, CH_3CH_2), 1.41 (6 lines, 4 H, J = 7.8 Hz, CH_3CH_2), 1.67 (5 lines, 4 H, J = 8.1 Hz, $CH_3CH_2CH_2$), 2.20 (s, 3 H, CH_3CO), 2.83 (t, 2 H, J = 6.1 Hz, CH_2CO), 4.07 (q, 4 H, J = 7.1 Hz, OCH_2-CH_2CH_2), 4.29 (q, 2 H, J = 6.4 Hz, OCH_2-CH_2CO)		2960, 2860, 1722, 1460, 1270, 1023 (film)
IV	δ 0.93 (t, 3 H, J = 7.3 Hz, CH_3CH_2), 1.23 [d, 6 H, J = 6.1 Hz, CH_3CH], 1.38 (6 lines, 2 H, J = 7.8 Hz, CH_3CH_2), 1.6-1.8 (m, 6 H, OCH_2CH_2CH), 2.70 (br s, 2 H, OH), 3.9-4.2 (m, 6 H, OCH_2), 4.2-4.4 (m, 2 H, CH_3-CH)	71 (7), 72 (11), 124 (65), 125 (11), 126 (100), 151 (19), 153 (17), 169 (12), 194 (11), 196 (40), 223 (21), 248 (2), 276 (6)	3400, 2960, 1460, 1373, 1248, 1138, 1023, 910 (film)
IV-diacetate	δ 0.94 (t, 3 H, J = 7.4 Hz, CH_3CH_2), 1.27 (d, 6 H, J = 6.7 Hz, CH_3CH), 1.41 (6 lines, 2 H, J = 7.7 Hz, CH_3CH_2), 1.5-1.8 (m, 2 H, $OCH_2CH_2CH_2$), 1.8-2.1 [m, 4 H, $CH_2CH(OCOCH_3)$], 2.04 (s, 6 H, $OCOCH_3$), 3.9-4.2 (m, 6 H, OCH_2), 5.02 [6 lines, 2 H, J = 3.2 Hz, $CH_2CH(OCOCH_3)$]		2960, 1739, 1456, 1372, 1240, 1023, 917, 862 (film)
Vb		95 (6), 113 (100), 125 (3), 139 (8), 169 (6)	
X		79 (6), 95 (6), 96 (5), 109 (38), 110 (5), 127 (100), 139 (3), 153 (6)	
XI	δ 0.94 (t, 3 H, J = 7.0 Hz, CH_3CH_2), 1.41 (6 lines, 2 H, J = 7.1 Hz, CH_3CH_2), 1.65 (5 lines, 2 H, J = 7.3 Hz, $CH_3CH_2CH_2$), 2.01 (5 lines, 2 H, J = 6.8 Hz, CH_2CH_2COO), 2.46 (t, 2 H, J = 7.3 Hz, CH_2COO), 3.68 (s, 3 H, $COOCH_3$), 3.76 (d, 3 H, J = 11.0 Hz, $POCH_3$), 4.0-4.2 (m, 4 H, OCH_2)	100 (2), 106 (4), 102 (100), 103 (4), 114 (30), 198 (13)	2958, 1735, 1435, 1260, 1170, 1025, 853 (film)
XIV	δ 0.94 (t, 6 H, J = 7.3 Hz, CH_3CH_2), 1.41 (6 lines, 4 H, J = 7.8 Hz, CH_3CH_2), 1.65 (5 lines, 4 H, J = 7.8 Hz, $CH_3CH_2CH_2$), 2.01 (5 lines, 2 H, J = 6.8 Hz, CH_2CH_2COO), 2.46 (t, 2 H, J = 7.3 Hz, CH_2COO), 3.68 (s, 3 H, $COOCH_3$), 3.9-4.1 (m, 6 H, OCH_2)	69 (59), 99 (40), 100 (10), 101 (100), 113 (6), 129 (9)	2960, 1740, 1460, 1433, 1270, 1170, 1028, 905 (film)
XVI		69 (63), 99 (100), 100 (13), 101 (84), 113 (14), 125 (14), 153 (2)	
XVI-acetate	δ 0.83 (t, 3 H, J = 6.0 Hz, CH_3CH_2), 1.13 (d, 3 H, J = 7.0 Hz, CH_3CH), 1.45-1.53 (m, 2 H, CH_3CH_2), 1.67-2.11 (m, 6 H, OCH_2CH_2), 1.92 (s, 3 H, $OCOCH_3$), 2.34 (t, 2 H, J = 6.0 Hz, CH_2COO), 3.57 (s, 3 H, $COOCH_3$), 3.72-4.16 (m, 6 H, OCH_2), 4.93 [6 lines, 1 H, J = 6.0 Hz, $CH(OCOCH_3)$]		2960, 1738, 1430, 1365, 1240, 1020
XVII	δ 0.94 (t, 3 H, J = 7.2 Hz, CH_3CH_2), 1.24 (d, 3 H, J = 6.4 Hz, CH_3CH), 1.42 (6 lines, 2 H, J = 7.5 Hz, $CH_3CH_2CH_2$], 1.68 (5 lines, 2 H, J = 7.0 Hz, $OCH_2CH_2CH_2$), 1.6-1.8 [m, 2 H, OCH_2CH_2CH], 2.80 (d, 1 H, J = 4.0 Hz, OH), 3.76 (d, J = 11.0 Hz, $POCH_3$), 4.01-4.13 (m, 4 H, OCH_2), 4.23-4.32 (m, 1 H, CH_3CH)	114 (100), 169 (7), 171 (4)	3410, 2950, 1450, 1375, 1030 ($CHCl_3$)
XVII-acetate	δ 0.94 (t, 3 H, J = 7.1 Hz, CH_3CH_2), 1.27 (d, 3 H, J = 6.3 Hz, CH_3CH), 1.40 (6 lines, 2 H, J = 8.1 Hz, CH_3CH_2), 1.66 (5 lines, 2 H, J = 6.6 Hz, $CH_2CH_2CH_3$), 1.7-2.1 [m, 2 H, $CH_2CH(OCOCH_3)$], 2.04 (s, 3 H, $OCOCH_3$), 3.75 (d, 3 H, J = 11.0 Hz, $POCH_3$), 3.8-4.1 (m, 4 H, OCH_2), 4.8-5.2 [m, 1 H, CH-$(OCOCH_3)$]		2960, 1724, 1450, 1370, 1250, 1030, 860, 830 ($CHCl_3$)

Table I (*Continued*)

	NMR ($CDCl_3$)	MS, m/z (rel intensity)	IR, cm^{-1}
XVIII	δ 0.92 (t, J = 6.0 Hz, 3 H, CH_3CH_2), 1.1-1.7 (m, 4 H, $CH_3CH_2CH_2$), 2.97 (5 lines, 4 H, J = 6.0 Hz, CH_2CH_2COO), 2.42 (t, 4 H, J = 6.0 Hz, CH_2COO), 3.64 (s, 6 H, $COOCH_3$), 3.8-4.3 (m, 6 H, OCH_2)		2950, 1738, 1430, 1258, 1165, 1020, 853 (film)
XIX		99 (100), 113 (7), 123 (15), 125 (8), 153 (14)	
XIX-acetate	δ 0.94 (t, 3 H, J = 5.8 Hz, $CH_3CH_2CH_2$), 0.97 (t, 3 H, J = 7.6 Hz, CH_3CH_2CH), 1.35-1.5 (m, 2 H, $CH_3CH_2CH_2$), 1.6-1.8 [m, 4 H, OCH_2CH_2 and $CH(OCOCH_3)CH_2CH_2$], 2.09 (s, 3 H, $OCOCH_3$), 3.76 (d, 3 H, J = 11.2 Hz, $POCH_3$), 3.9-4.3 (m, 4 H, OCH_2), 4.9-5.1 [m, 1 H, $CH(OCOCH_3)$]		2960, 1742, 1460, 1370, 1235, 1030, 860 (film)

of the radioactivity was recovered within 1 day and 82% within 5 days (Figure 3). The detection of radioactivity in the feces of rats given [^{14}C]TBP intraperitoneally means that some metabolites were excreted via the bile duct.

The general features of distribution of radiocarbon among the fractions indicated in Figure 1 are shown in Figure 4. In the urine of rats given [^{14}C]TBP orally (24 h), 3% of the radiocarbon was found in the neutral fraction (A), 40% in the ethyl acetate extractable (acidic) fraction (B) 25% in the BuOH-extractable fraction (C), and the remainder in the unextractable fraction (D). With the passage of time, ethyl acetate extractable (acidic) radiocarbon decreased whereas radiocarbon in the more polar fraction increased. On the other hand, in the case of ip administration 7% of the radiocarbon was found in the neutral fraction, which is larger than that after oral dosing, while the fraction that was unextractable with the organic solvent decreased. This phenomenon might be attributed to increased loading of substrate in the case of ip administration compared with oral administration, since the drug has easier access to the liver in the former case.

Identification of Metabolites. The comparison of retention times for identification of metabolites was made by analysis using a GLC photometric detector. GLC–MS was used for confirmation of it. The relative retention times of metabolites and standards are shown in Table II.

Neutral Metabolites. The gas chromatogram of the neutral fraction obtained according to Figure 1 is shown in Figure 5.

(*1*) *M-1.* The RT of M-1 was coincident with that of I and the mass spectrum of M-1 showed fragment peaks at m/z 99 (100) and 155 (19), which are characteristic of I (Table I).

(*2*) *M-2.* Although the RT of M-2 on the gas chromatogram was identical with that of dibutyl 3-hydroxybutyl phosphate (II), the quantity obtained was quite small, so only the base peak, m/z 99, which is specific to II, was observed in the mass spectrum.

(*3*) *M-3.* The gas chromatographic and mass spectral behavior of M-3, which gave the highest peak on gas chromatogram of the neutral fraction, were similar to those of authentic butyl bis(3-hydroxybutyl) phosphate (IV). On the basis of these data, M-3 was identified as IV.

(*4*) *Unknown 1.* An unidentified peak was observed before M-3 (Figure 5), but the structure was not investigated further.

Acidic Metabolites. A gas chromatogram of the acidic metabolites after methylation is shown in Figure 6.

(*1*) *M-4 and M-5.* The RT values on the gas chromatogram and the mass fragment patterns of methyl esters of M-4 and M-5 were identical with those of authentic butyl dimethyl phosphate (X) and dibutyl methyl phosphate (Vb), respectively. Thus, M-4 and M-5 after methylation were identified as X and Vb, respectively.

(*2*) *M-6.* Methylated M-6, which has a base peak at m/z 99, was identified as butyl 2-hydroxybutyl methyl phosphate by comparing the mass spectral data with those of authentic XIX.

(*3*) *M-7.* Methylated M-7, which was isolated and purified by silica gel column chromatography of the methylated acidic extract, showed a triplet methyl signal (3 H, J = 7.2 Hz) at 0.94 due to CH_3CH_2, a doublet methyl signal (3 H, J = 6.4 Hz) at 1.24 ascribed to $CH_3CH(OH)$, and a doublet methyl signal (3 H, J = 11.0 Hz) at 3.76 ppm assigned to $POCH_3$. The acetate of M-7 after methylation (M-7-acetate) showed a singlet methyl signal at 2.04 attributed to $OCOCH_3$ and a multiplet (1 H) at 4.8–5.2 ppm assigned to $CH(OCOCH_3)$. On the basis of the NMR data described above, methylated M-7 was assumed to be butyl 3-hydroxybutyl methyl phosphate, and this was confirmed by comparison of the mass spectra of methylated M-7 and an authentic sample of XVII, and the NMR spectra of M-7-acetate and XVII-acetate.

(*4*) *M-8.* Methylated M-8, which showed characteristic fragment peaks at m/z 102 (100), 114 (30), and 198 (13) in the mass spectrum, was identified as butyl (3-methoxycarbonyl)propyl methyl phosphate (XI).

(*5*) *M-9.* Methylated M-9 showed the same RT on the gas chromatogram as authentic dibutyl (3-methoxycarbonyl)propyl phosphate (XIV), but its identity was uncertain because of the small amount obtained and because of another overlapping peak in the chromatogram. However, the results of mass chromatography at m/z 99, 100, 101, and 113 confirmed that M-9 was identical with XIV.

The next peak, which followed M-9, was assumed to be M-3 (IV) based on the results of GLC and GLC–MS. This neutral metabolite may have been carried over into the acidic fraction because of its high solubility in water.

(*6*) *M-10.* Methylated M-10 showed fragment peaks at m/z 69 (95), 99 (100), 100 (32), 101 (68), 113 (17), 125 (19), and 153 (14) in its mass spectrum, which was identical with that of authentic butyl 3-hydroxybutyl (3-methoxycarbonyl)propyl phosphate (XVI).

(*7*) *M-11.* The RT of methylated M-11 on the gas chromatogram was identical with that of authentic butyl bis[(3-methoxycarbonyl)propyl] phosphate (XVIII), but further confirmation was not possible because of the small quantity of M-11 obtained.

Butanol-Extractable Metabolites. Gas chromatograms of the methylated butanol extracts of urine from rats given TBP (Figure 7a) and the diol IV (Figure 7b) are shown. Various kinds of metabolites that were not completely extracted with ethyl acetate were observed in this fraction, and the pattern was basically similar to that of the ethyl acetate extract. The more intense peaks observed in this

Table II. Relative Retention Times of TBP Metabolites and Standards[b]

metabolite	standard	RRT[a]
M-1	I, tributyl phosphate	1.0
M-2	II, dibutyl 3-hydroxybutyl phosphate	4.0
M-3	IV, butyl bis(3-hydroxybutyl) phosphate	18
methyl ester of M-4	X, butyl dimethyl phosphate	0.2
methyl ester of M-5	Vb, dibutyl methyl phosphate	0.5
methyl ester of M-6	XIX, butyl 2-hydroxybutyl methyl phosphate	1.6
methyl ester of M-7	XVII, butyl 3-hydroxybutyl methyl phosphate	2.2
methyl ester of M-8	XI, butyl (3-methoxycarbonyl)propyl methyl phosphate	3.8
methyl ester of M-9	XIV, dibutyl (3-methoxycarbonyl)propyl phosphate	5.3
methyl ester of M-10	XVI, butyl 3-hydroxybutyl (3-methoxycarbonyl)propyl phosphate	24
methyl ester of M-11	XVIII, butyl bis[(3-methoxycarbonyl)propyl] phosphate	36
methyl ester of M-12	3-hydroxybutyl dimethyl phosphate	0.8

[a] Relative to tributyl phosphate = 1.4 min. [b] The column of 1% OV-225 on Chromosorb W (AW), 3 mm i.d. × 1.5 m, was used.

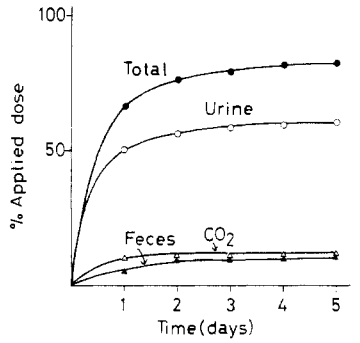

Figure 3. Elimination of radiolabel from male Wistar rats dosed orally with [^{14}C]TBP (14 mg/kg).

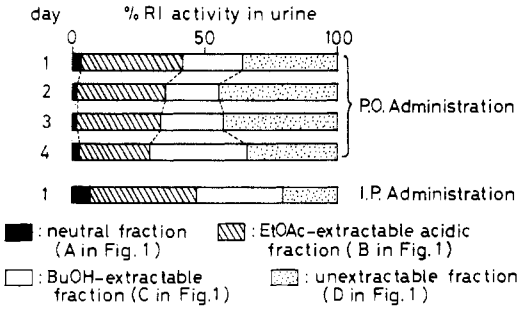

Figure 4. Fractionation of urine metabolites by solvent extraction.

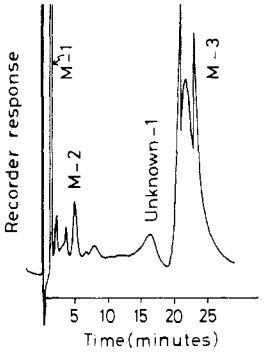

Figure 5. FPD (P-mode) gas chromatogram of neutral metabolites. Column temperature: 180 °C.

fraction are the methyl esters of M-4 and M-7, whose structures were already clarified. A minor product, the methyl ester of M-12, was also observed. The methyl ester of M-12 was also found on the gas chromatogram of the methylated butanol extract of urine from rats given the diol (IV) . The methyl ester of M-12-acetate showed a doublet methyl signal (3 H, J = 6.1 Hz) at 1.27 due to CH_3CH, a singlet methyl signal at 2.04 assigned to $OCO-CH_3$, a multiplet (2 H) at 1.9–2.2 ascribed to CH_2CH_2O, a doublet (6 H, J = 11.2 Hz) at 3.76 due to $POCH_3$, a multiplet (2 H) at 4.0–4.2 attributed to OCH_2, and a multiplet (1 H) at 4.9–5.1 ppm due to CHOCO in the NMR spectrum. Thus, M-12 after methylation was concluded to be 3-hydroxybutyl dimethyl phosphate.

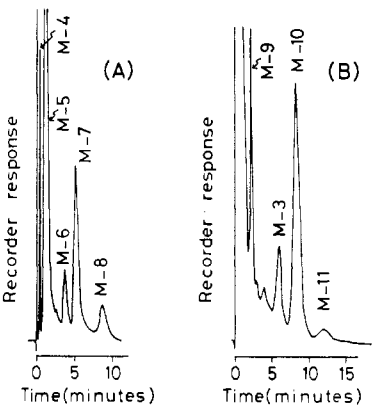

Figure 6. FPD (P-mode) gas chromatograms of acidic metabolites. Column temperature: (A) 140 °C; (B) 210 °C.

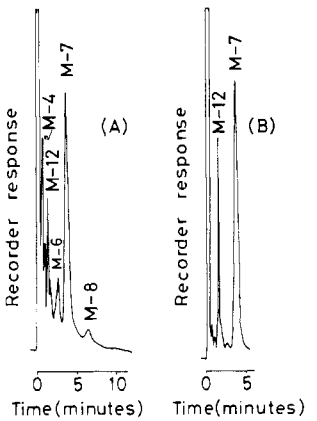

Figure 7. FPD (P-mode) gas chromatograms of butanol-extractable metabolites of TBP and butyl bis(3-hydroxybutyl) phosphate (IV). Column temperature: 150 °C. (A) Metabolites of TBP; (B) metabolites of butyl bis(3-hydroxybutyl) phosphate.

Detection of Glucuronide and Cysteine Conjugates. Glucuronide could not be detected (no difference was found between the control and test rats). Further, neither *S*-butyl-L-cysteine (VI) nor *N*-acetyl-*S*-butyl-L-cysteine (VII) could be detected, even though they could be recovered well from the urine.

Determination of Urinary Metabolites. The results of determination of metabolites, corrected for recoveries, are shown in Table III. A high dose gave a better total

Table III. Determination of Urinary Metabolites from Rats Given a Single Intraperitoneal Dose of TBP or Its Metabolic Intermediates[c]

metabolite	I (250 mg/kg)[a]			I (50 mg/kg)[a]			II (250 mg/kg)[a]			III (250 mg/kg)[a]			IV (250 mg/kg)[a]			Va (250 mg/kg),[a] 1,[b] 2,[b] and 3[b]
	1[b]	2[b]	3[b]	1[b]	2[b]	3[b]	1[b]	2[b]	3[b]	1[b]	2[b]	3[b]	1[b]	2[b]	3[b]	
M-1	0.02	tr[d]	ND[e]	0.01	ND	ND										
M-2	0.06	0.01	0.01	tr	ND	ND	0.06	0.01	tr	tr	ND	ND				
M-3	0.94	0.04	0.07	0.48	0.04	ND	1.7	0.19	0.01	0.07	0.02	ND	2.4	0.74	ND	
M-4	3.1	0.60	0.14	3.5	0.35	0.07	4.4	0.21	0.02	10.6	1.1	0.15	6.4	0.02	tr	0.03
M-5	15.9	1.59	0.29	4.6	0.46	0.08	22.2	2.58	0.40	20.6	2.1	2.8				47.6
M-6	0.11	0.06	0.02	0.24	0.03	ND	0.74	0.05	0.01	0.85	0.10	0.11				ND
M-7	1.28	0.66	0.07	0.80	0.06	ND	8.1	0.45	0.15	7.1	1.1	0.63	16.3	1.6	0.81	0.08
M-8	0.32	0.22	0.03	0.39	0.03	ND	1.9	0.08	ND	1.1	0.13	0.20				0.10
M-9	0.07	0.07	0.01	0.13	0.02	tr										
M-10	3.0	0.1	ND	1.5	ND	ND	0.42	0.05	ND	0.10	ND	ND				
M-11	tr	ND	ND	ND	ND	ND										
M-12	+[f]						+++			++++			++++			+
	24.8	3.35	0.64	11.7	0.99	0.15	39.5	3.62	0.59	41.0	4.55	3.89	25.1	2.36	0.81	
total	28.8			12.8			43.8			49.4			28.3			47.8

[a] Compound (dose). [b] Days after dose. [c] Values are expressed as percent of applied dose. [d] tr = trace. [e] ND = not detected. [f] (+) indicates detection of M-12; it could not be quantitated because authentic M-12 was not available.

recovery of metabolites but required a longer period for clearance. The low total recovery (percent of dose) of metabolites, especially at the low dose of I, as compared with the result in Figure 2, suggests the existence of further unidentified phosphorus-containing polar metabolites, e.g., H_3PO_4.

Analysis of the Metabolic Pathways by Administration of Probable Metabolic Intermediates. As can be seen from Table III, the main metabolites of I are M-5 and M-4. The dealkylation of trialkyl and dialkyl phosphate by enzymatic hydrolysis has not been reported, and TBP was also stable to the chemical hydrolysis at 37 °C in the neutral condition; consequently, the possibility that M-5 and M-4 were produced by simple hydrolysis of TBP could be excluded. On the other hand, TBP incubated with rat liver homogenate was converted to almost exculsively to one metabolite, dibutyl 3-hydroxybutyl phosphate (Sasaki et al., 1984).

The metabolites except M-5 and M-4 have butyl moieties hydroxylated at C-3. Only M-9 and M-11 are not hydroxylated at C-3, and they were obtained in small amounts. Therefore, it seems probable that hydroxylation at C-3 is an early metabolic process, which is followed by further metabolic reactions. Thus, dibutyl 3-hydroxybutyl phosphate (II), dibutyl 3-oxobutyl phosphate (III), butyl bis(3-hydroxybutyl) phosphate (IV), and dibutyl hydrogen phosphate (Va) were each administered intraperitoneally at the same dosage as I. The results showed that the metabolic patterns of II and III were quite similar. On the other hand, administration of I gave a poor recovery of all kinds of metabolites compared to those from II or III. This means that TBP might be metabolized through other pathways that are bypassed when II or III was administered directly. Administration of the oxo compound III gave only 0.07% of the dihydroxy compound M-3, while II gave 1.7% of M-3 and I afforded an intermediate value, 0.94%. This result indicates that only a small amount of the oxo compound III is transformed back to the 3-hydroxy compound M-2. Instead, the oxo compound undergoes α-oxidation or transalkylation to yield dibutyl hydrogen phosphate M-5. The susceptibility of III to further transformation (synthetic III was too unstable for elemental analysis to be possible) is clearly an important feature, and the reactivity of III with tissue components could be important in relation to the in vivo effects of I.

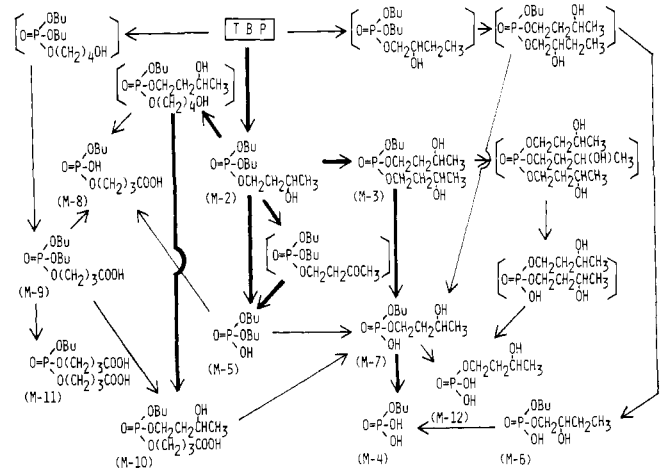

Figure 8. Proposed metabolic pathways of TBP.

However, there is no evidence that all of II is necessarily metabolized via III.

When the dihydroxy compound IV was administered, the main metabolites were only M-7, formed by loss of a 3-hydroxybutyl moiety, and M-4, produced by loss of the remaining 3-hydroxybutyl moiety from M-7.

There are three routes to 3-hydroxybutyl dihydrogen phosphate (M-12). One is by loss of butyl and 3-hydroxybutyl moieties from M-3, and another is by oxidation of M-3 to a triol, which is decomposed to M-12 through loss of two 3-hydroxybutyl moieties. The last is by oxidation of M-7 to bis(3-hydroxybutyl) hydrogen phosphate, followed by loss of a 3-hydroxybutyl moiety. The major route is not clear at present, but the kind of leaving group may play a decisive role. Administration of I gave 0.32% of M-8. However, the production of metabolites oxidized in the terminal methyl moiety was higher from II and III (1.9 and 1.1%, respectively). On the other hand, the amount of M-10 was less when II or III was administered. Removal of the alkyl moiety, which is low from I, should be smoother from II or III, and, as expected, more M-7 was formed after administration of II or III than after that of I.

When rats were dosed with dibutyl hydrogen phosphate (Va), much of it (47.6%) was recovered intact, and only small amounts of M-7 and M-8 were formed, as well as

some M-4. Thus, M-5 produced as an intermediate in the metabolism of I would be mostly excreted. On the other hand, it is expected that M-4 will be readily metabolized to further degradation products.

On the basis of the above results and discussion, some of the possible metabolic pathways of I are summarized in Figure 8. The results of further studies on the formation of mercapturate and on the unidentified products present in the acidic fraction will be reported elsewhere.

Registry No. I, 126-73-8; II, 89197-69-3; III, 89197-70-6; IV, 89197-71-7; Va, 107-66-4; Vb, 7242-59-3; VI, 4134-56-9; VII, 19216-62-7; VIII, 819-43-2; IX, 1498-52-8; X, 10463-06-6; XI, 89197-72-8; XII, 52551-81-2; XIII, 89197-73-9; XIV, 89197-74-0; XV, 89197-75-1; XVI, 89197-76-2; XVII, 89197-77-3; XVIII, 89197-78-4; XIX, 89197-79-5; M-4, 1623-15-0; M-5, 107-66-4; M-6, 89197-80-8; M-7, 89197-81-9; M-8, 89197-82-0; M-9, 89197-83-1; M-10, 89197-84-2; M-11, 89197-85-3; 1,3-butanediol, 107-88-0; 1,4-butanediol, 110-63-4; 1,2-butanediol, 584-03-2.

LITERATURE CITED

Bray, H. G.; Franklin, T. J.; James, S. P. *Biochem. J.* **1959**, *73*, 465.
Bringmann, G.; Kuehn, R. *Water Res.* **1980**, *14*, 231.
Bringmann, G.; Kuehn, R. *Z. Wasser Abwasser Forsch.* **1982**, *15*, 1.
Environment Agency of Japan. *Environ. Agency, Off. Health Stud. Rep. Ser.* **1978**, *No.* 4.
Koezuka, T. *Jpn. Kokai Tokkyo Koho* **1979**, 23129.
James, S. P.; Jeffery, D. A.; Waring, R. H.; Wood, P. B. *Biochem. J.* **1968**, *109*, 727.
Jones, A. R. *Experientia* **1970**, *26*, 492.
Meijers, A. P.; Van der Leer, R. C. *Water Res.* **1976**, *10*, 597.
Mizuma, T.; Minaki, T.; Toyoshima, S. *Yakugaku Zasshi* **1961**, *81*, 51.
Oishi, H.; Oishi, S.; Hiraga, K. *Toxicol. Lett.* **1980**, *6*, 81.
Sasaki, K.; Suzuki, T.; Takeda, M.; Uchiyama, M., submitted for publication in *Bull. Environ. Contam. Toxicol.*, **1984**.
Sasaki, K.; Takeda, M.; Uchiyama, M. *Bull. Environ. Contam. Toxicol.* **1981**, *27*, 775.
Schou, L.; Krane, J. E.; Carlberg, G. E. *Sci. Total Environ.* **1981**, *20*, 277.
Sosnovsky, G.; Zaret, E. H. *J. Org. Chem.* **1969**, *34*, 968.
Williams, D. T.; LeBel, G. L. *Bull. Environ. Contam. Toxicol.* **1981**, *27*, 450.

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Effects of Light Intensity and Temperature on the Uptake and Metabolism of Soil-Applied [^{14}C]Phorate by Plants

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The potential effects of light intensity [500 or 3000 foot-candles (ft-c)], temperature (13 or 28 °C), and combinations thereof were investigated relative the fate of [^{14}C]phorate in soils and its uptake and metabolism by oat, pea, and corn plants. Increasing the intensity of light, particularly at a temperature of 28 °C, resulted in decreased recoveries of phorate from soils and an increased translocation of ^{14}C compounds into plant tops. Increasing the temperature from 13 to 28 °C, particularly at the greater light intensity of 3000 ft-c, resulted in an accumulation of ^{14}C compounds in plant tops but also in an accelerated metabolism of the insecticide. This was indicated by increased production of water-soluble and bound ^{14}C compounds and an increased formation of $^{14}CO_2$ from ^{14}C compounds. At a temperature of 28 °C and a light intensity of 3000 ft-c the amounts of phorate and its metabolites in soils had decreased, while in plant tops the amounts of some phorate metabolites such as phorate sulfoxide, phoratoxon sulfoxide, and phoratoxon sulfone had increased.

Many interrelated factors such as temperature, light intensity, photoperiod, air humidity, air movement, plant characteristics, and others can affect the uptake and metabolism of soil-applied insecticides by plants, thus necessitating a knowledge of the effects and interrelation of each of these factors. Hacskaylo et al. (1961) reported that temperature was more important than relative humidity in its effects on the accumulation of soil-applied phorate by cotton plants, while Wheeler et al. (1967) did not observe differences in the quantities of dieldrin taken up by rye grown at 18 °C or at 24 °C for 21–24 days. Tietz (1954) found that *Coleus* plants exposed to 60% relative humidity (RH) in a closed system translocated larger quantities of demeton to the leaves and also released more volatile ^{14}C than plants exposed to 70% RH.

A study conducted previously in our laboratory dealt with differences in water transpiration by a number of C_3 and C_4 plants in relation to the uptake and metabolism of soil-applied [^{14}C]phorate (Anderegg and Lichtenstein, 1981). This present study was conducted in the Biotron of the University of Wisconsin to investigate potential effects of different combinations of light intensity and temperature on the fate of the insecticide phorate in soils and its uptake and metabolism by oat, pea, and corn plants.

MATERIALS AND METHODS

Chemicals. [*methylene*-^{14}C]Phorate (specific activity 9.7 mCi/mmol) was obtained through the courtesy of American Cyanamid Co. The insecticide was determined to be at least 97% pure by thin-layer chromatography (TLC) and autoradiography. The [^{14}C]phorate was diluted with nonradioactive phorate before its addition to soils. Phorate sulfoxide, phorate sulfone, phoratoxon, phoratoxon sulfoxide, and phoratoxon sulfone were also supplied by American Cyanamid Co. Solvents used were redistilled acetone, benzene, and hexane, as well as analytical-grade methanol, toluene, nitromethane, and acetonitrile.

Plant Material. Corn seeds (hybrid variety Funk G4444-0900, wilt resistant) were obtained through the

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