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Dioxathion Metabolites, Photoproducts, and Oxidative Degradation Products

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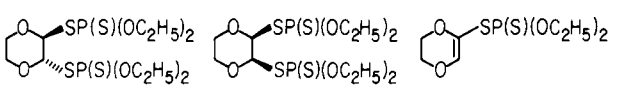
The trans and cis isomers of 2,3-*p*-dioxanedithiol *S*,*S*-bis(*O*,*O*-diethyl phosphorodithioate) (dioxathion acaricide and insecticide) are rapidly and extensively metabolized in rat liver microsome–NADPH systems and in rats in vivo by oxidation of the thionophosphorus moiety, forming oxons and dioxons, and probably also by oxidative *O*-deethylation and hydroxylation of the dioxane ring, the latter reaction forming labile intermediates that undergo ring cleavage and loss of both phosphorus moieties. The more toxic *cis*-dioxathion yields larger amounts of oxon and dioxon derivatives than the trans isomer in this liver enzyme system and on plant foliage and more $^{14}CO_2$ from the ethoxy group in rats. 2-*p*-Dioxenethiol *S*-(*O*,*O*-diethyl phosphorodithioate) is degraded enzymatically and on plants to products which are the same as or analogous to those formed from the dioxathion isomers. The three phosphorodithioates yield toxic oxon derivatives as photoproducts. Synthesis procedures are given for [^{14}C]ethoxy and [^{14}C]ring preparations of these compounds and for several of their unlabeled oxidative degradation products, some obtained by peracid oxidation.

Delnav is composed of two principal acaricidal and insecticidal components, the trans and cis isomers of 2,3-*p*-dioxanedithiol *S*,*S*-bis(*O*,*O*-diethyl phosphorodithioate) (referred to in this report as *t*- and *c*-dioxathion, respectively), a small amount of 2-*p*-dioxenethiol *S*-(*O*,*O*-diethyl phosphorodithioate) (referred to here as dioxenethion), and several minor constituents with little or no biological activity. Although *c*-dioxathion is present in smaller amount than *t*-dioxathion, the cis isomer is more toxic to mites, insects, and mammals (Arthur and Casida, 1959; Diveley et al., 1959). Studies with ^{32}P-labeled compounds established that *t*- and *c*-dioxathion and dioxenethion are cleaved to various phosphorus acids following administration to cows, mice, rats, and plants and that the degradation products on plant foliage probably include oxon derivatives (Arthur and Casida, 1959; Casida and Ahmed, 1959; Chamberlain et al., 1960; Plapp et al., 1960).



t-dioxathion *c*-dioxathion dioxenethion

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Further investigations were undertaken on dioxathion metabolites and photoproducts because of its continuing importance in pest control, the relatively small amount of information on this phosphorodithioate mixture, and the existence of geometrical isomers with different levels of potency. The products of peracid oxidation of dioxathion components were also examined since, on analogy with studies on other thionophosphorus compounds (Bellet and Casida, 1974), they might be identical with some of the dioxathion metabolites and photoproducts. *t*- and *c*-dioxathion and dioxenethion were prepared with ^{14}C labels in each of the ethoxy and ring moieties for use in these metabolic and environmental fate investigations.

METHODS

Spectroscopy. Infrared (ir) spectra were determined with a Perkin-Elmer 457 grating spectrophotometer using neat samples on NaCl plates. Nuclear magnetic resonance (NMR) spectra were obtained with a Perkin-Elmer R12B instrument using carbon tetrachloride as the solvent and tetramethylsilane (Me_4Si) as the internal standard. Chemical ionization mass spectra (CIMS) were obtained on a Finnigan 1015D mass spectrometer coupled to the System Industries Model 150 control system using methane at 1 Torr as the reagent gas. Samples introduced via a solid probe inlet were volatilized on slowly heating the probe. Radiocarbon was assayed by liquid scintillation

counting (lsc) with the Packard Tri-Carb Model 3003 or the Beckman Model LS-150 liquid scintillation spectrometer using 15 ml of 0.55% (w/v) 2,5-diphenyloxazole in toluene–methyl Cellosolve (2:1) as the scintillation mixture. The Packard Tri-Carb Model 306 sample oxidizer was used for determining total ^{14}C by combustion.

Thin-Layer Chromatography (TLC). TLC utilized 20 × 20 cm precoated silica gel chromatoplates with a layer thickness of 0.25 mm for analytical purposes (silica gel 60 F-254; EM Laboratories, Inc., Elmsford, N.Y.) and 0.5 mm (silica gel F-254; EM Laboratories) or 1.0 mm (silica gel GF; Analtech, Inc., Newark, Del.) for preparative separations. Chromogenic reagents used in detecting products and monitoring the reactions were as follows: 4-(*p*-nitrobenzyl)pyridine (Bellet and Casida, 1974), 2,6-dibromo-*N*-chloro-*p*-benzoquinone imine (Bellet and Casida, 1974), and palladium(II) chloride (Stahl, 1969) for thiophosphorus esters and other sulfur compounds; lead tetraacetate for ethylene glycol (Stahl, 1969); 2,6-dichlorophenol–indophenol, sodium salt, for glycolic acid (Stahl, 1969). ^{14}C-Labeled compounds were detected by autoradiography. The appropriate gel regions were scraped from the chromatoplates for quantitation of the labeled compounds by lsc or recovery of the chemicals by extraction of the gel with acetone–methanol (1:1).

TLC solvents used for product isolation and tentative identification of ^{14}C-labeled metabolites and ^{14}C-labeled degradation products by cochromatography were as follows: (A) benzene–hexane (17:3); (B) isopropyl ether–ethanol (19:1); (C) hexane–ethyl acetate–methanol (12:5:2); (D) ethyl acetate–methanol–water (13:3:1); (E) benzene–ether (3:1); (F) 1-butanol–1-propanol–0.12 N NH_4OH (2:1:1). Reference to solvent systems for two-dimensional development is illustrated by (D × C), which indicates development in the first direction with D and in the second direction with C. A designation such as 2 × A indicates two developments in the same direction with solvent system A.

Metabolism by Rat Liver Microsomes. The microsomal and post-microsomal supernatant (soluble) fractions were prepared from the livers of male albino Sprague-Dawley rats (150–160 g; Horton Laboratories, Oakland, Calif.) at 10% (w/v) fresh tissue weight equivalent in Tris-HCl buffer (50 mM, pH 7.5) by a described procedure (Abernathy et al., 1971). The incubation mixtures of 2-ml volume in 25-ml Erlenmeyer flasks contained: 1 ml of freshly prepared microsome preparation (1.7–2.0 mg of protein) in Tris-HCl buffer; 1 ml of Tris-HCl buffer or 1 ml of freshly prepared soluble fraction (6.4–6.6 mg of protein) at 0 °C; reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0 or 4 μmol); ^{14}C-labeled substrate (22 nmol) in ethanol (20 μl) injected with a syringe directly into the reaction medium. The flasks were immediately incubated with shaking in air at 37 °C for 1 h.

The reaction mixtures were extracted with ether (3 × 5 ml), the aqueous phase was acidified to pH 1 with 3 N HCl, $(NH_4)_2SO_4$ (0.5 g) was added, and the mixture was finally extracted with ether–ethanol (3:1) (3 × 5 ml). The extracts were individually dried (Na_2SO_4, 18 h, 5 °C) and concentrated under N_2 for TLC analysis, using solvent system C for the ether-extractable products and D for the ether–ethanol-extractable products. The aqueous phase was analyzed for protein-bound and water-soluble ^{14}C-labeled compounds. Protein was precipitated by addition of 20% (w/v) aqueous trichloroacetic acid (Cl_3CCOOH) (2 ml) and centrifugation followed by washing the precipitate in sequence with Cl_3CCOOH solution (2 ml), acetone (2 ml), and ethanol (2 ml), the washings being combined with the original aqueous phase to obtain the water-soluble fraction. Protein-bound ^{14}C was determined after dissolving the sample in Soluene 100 sample solubilizer (1 ml; Packard Instrument Co., Downers Grove, Ill.).

Metabolism in Rats. Male albino rats (see above) were individually treated orally by stomach tube with 1.6–3.2 mg/kg of each labeled compound using methoxy triglycol (90 μl) as the administration vehicle and as a wash (250 μl) for the stomach tube. The rats were held for 96 h in metabolism cages allowing for separate collection of urine, feces, and expired $^{14}CO_2$ (Krishna and Casida, 1966). Food and water were available at all times.

The 0–24-h urine (collected over ice and stored at −15 °C) was analyzed by directly spotting an aliquot (100 μl) for two-dimensional TLC (D × C). The 0–24-h feces (stored at −15 °C) was extracted by addition of methanol (4 ml/g of feces), homogenization with a Virtis Homogenizer (Virtis Research Equipment, Gardner, N.Y.) for 30 s, vacuum filtration, rehomogenization of the filter cake with an additional portion of methanol, and filtration once again. The filter cake was finally soaked for 18 h in methanol at 5 °C and then homogenized and filtered. The three filtrates were combined and stored at 5 °C until an aliquot equivalent to 40–90 mg of feces was analyzed by TLC as with the urine. Radiocarbon remaining in the dried filter cake was determined by combustion.

Each rat was sacrificed 96 h after treatment and the total ^{14}C content of tissue samples was determined by combustion.

Fate on Glass and Silica Gel Surfaces and on Bean Leaves. Clean microscope cover slips were individually treated with the ^{14}C-labeled compounds in methanol to yield a deposit of 1.3 or 4.0 nmol/cm^2. The treated surfaces were held at 25 °C in the laboratory under normal lighting conditions. For determining the residual ^{14}C, the entire cover-glass was placed into a scintillation vial for lsc. For product analysis, the residual ^{14}C-labeled compounds were washed from the glass with dioxane (4 × 250 μl) and subjected to TLC analysis (benzene or D × C). Triplicate determinations were made in each case.

Silica gel chromatoplates (0.25 mm layer thickness) spotted at the origin with the ^{14}C-labeled compounds to yield deposits of 50 nmol/cm^2 were exposed in the dark or to a sunlamp (275-W G.E. sunlamp at a distance of 30 cm, temperature at the gel surface approximately 35–40 °C) for various periods of time and then developed with solvent system C or D. The resolved ^{14}C-labeled products were extracted from the gel for tentative identification by cochromatography.

Snapbeans (Contender variety, 20–30 cm height, grown in a greenhouse and then moved outdoors a few days before use) were individually treated with the ^{14}C-labeled compounds (10 nmol) in absolute ethanol (40 μl, an amount that did not harm the foliage) applied with a syringe in a series of streaks across one of the trifoliate leaves. After appropriate exposure times to ambient outdoor conditions in October, the leaves were cut from the plant and individually washed with chloroform–methanol (3:1) (10 ml, then 2 × 5 ml), each time holding the leaf in the solvent mixture for 10 min with swirling every few minutes. Finally, the leaf was allowed to stand 30 min in scintillation fluid to remove the "polar and penetrated" ^{14}C-labeled compounds. The combined chloroform–methanol washes were concentrated under N_2 and the methanol-soluble products were separated by TLC (D × C) in order to recover individual compounds for TLC cochromatography in appropriate solvent systems. Unextractable ^{14}C in the

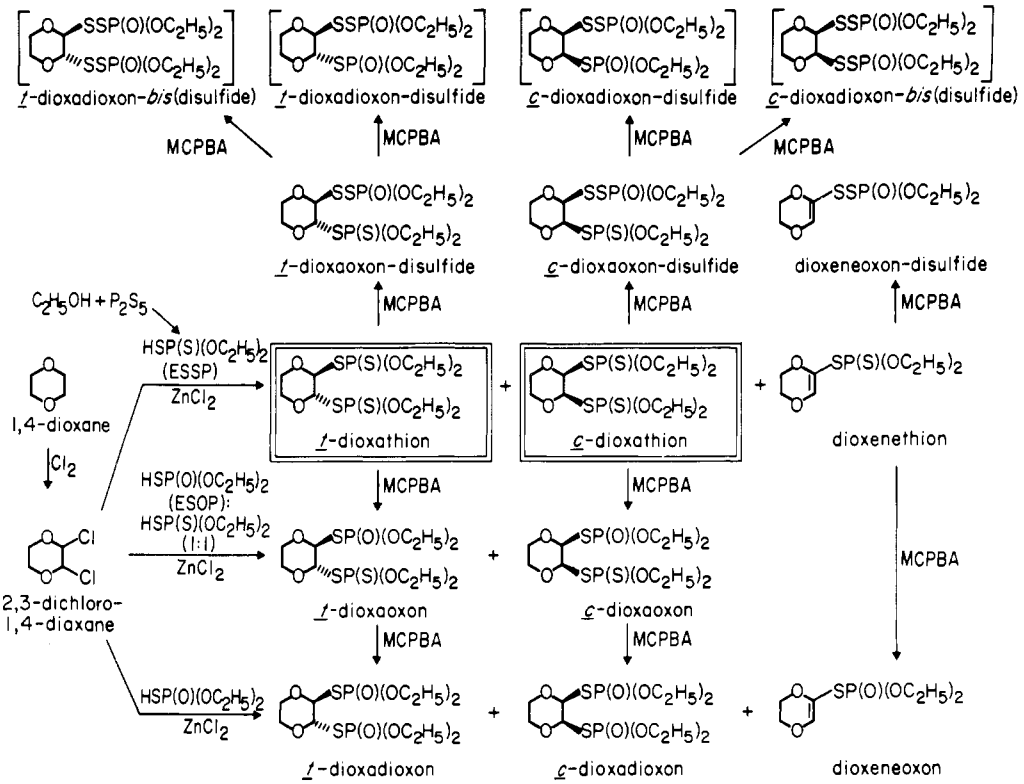

Figure 1. Preparation and designation of dioxathion components and their oxidation products. Products not isolated in pure form but tentatively identified by CIMS are shown in brackets.

leaves was determined by combustion.

Mouse Toxicity Assays. Male albino Swiss-Webster mice (20–22 g; Horton Laboratories) were administered the test compounds intraperitoneally (ip) using methoxy triglycol (50 μl) as the vehicle. Mortality observations were made after 24 h.

CHEMICALS

Figure 1 gives the reaction sequences used to obtain the principal dioxathion derivatives and the designations used for these compounds. They were prepared by coupling 2,3-dichloro-1,4-dioxane with the appropriate phosphorothioic acid or by oxidation of the phosphorodithioate with *m*-chloroperoxybenzoic acid (MCPBA) as described below. Their spectral and chromatographic properties are given in Table I.

Intermediates. 2,3-Dichloro-1,4-dioxane, synthesized by the method of Boeseken et al. (1931), was distilled as a colorless liquid collected at 44–48 °C (4–5 mm) (lit. 82.4 °C (14 mm)) and it gave appropriate spectra. ESSP was prepared from P_2S_5 and ethanol by the procedure of Diveley et al. (1959) while ESOP and *O,O*-diethylphosphoric acid (EOOP) were made according to the general method of Mastin et al. (1945) by treating *O,O,O*-triethyl phosphorotrithioate and *O,O,O*-triethyl phosphate, respectively, with KOH, followed by treatment with aqueous HCl.

Separation and Synthesis of Delnav Components. Chromatography of Delnav (2.0 g; Hercules Inc., Wilmington, Del.) on a silicic acid (100 mesh, 250 g) column with benzene–hexane mixtures (2:1 to 3:1) and TLC monitoring (A) of the eluate gave the following major products in the order of their elution: ESSP disulfide $[(C_2H_5O)_2P(S)SSP(S)(OC_2H_5)_2]$ (0.08 g), a trithioanhydride $[(C_2H_5O)_2P(S)SP(S)(OC_2H_5)_2]$ (0.03 g), pure *t*-dioxathion (0.59 g), a mixture of *t*- and *c*-dioxathion (0.10 g), and pure *c*-dioxathion (0.31 g). A higher yield (65%) of an unresolved mixture of *t*- and *c*-dioxathion was obtained on adding Delnav (10 g) on Florisil (60–100 mesh, 20 g; Floridin Co., Berkeley Springs, W.Va.) to a column containing dry Florisil (140 g) and developing rapidly with benzene–hexane mixtures (1:1 to 3:1) with monitoring as above. ESSP disulfide was also obtained in 85% yield from the sodium salt of ESSP by the procedure of Bartlett et al. (1955). Dioxenethion, which was only a trace component of the Delnav used above, was prepared by the general procedure for dioxathion (Diveley et al., 1959) except that equimolar ESSP and dichlorodioxane were used and the reaction was quenched with 5% aqueous NaOH. The reaction mixture was purified on a silicic acid column developed with benzene–hexane (2:1) to obtain pure dioxenethion.

Synthesis and Separation of [^{14}C]Delnav Components. The method of Diveley et al. (1959) was modified for radiosynthesis of ethoxy- (E*) and ring- (R*) labeled preparations of each of *t*- and *c*-dioxathion and dioxenethion.

To prepare the ethoxy-labeled compounds, [1-^{14}C]-ethanol (2.0 mmol, 1.0 mCi/mmol; New England Nuclear, Boston, Mass.) was sealed in a heavy-walled glass tube containing P_2S_5 (0.5 mmol), dry benzene (300 μl), and an 8-mm Teflon-coated stirring bar. The stirred solution was heated at 80 °C until all of the P_2S_5 had reacted (about 16 h), then the tube was cooled and opened, and the benzene and H_2S were removed with a gentle stream of dry N_2. Following addition of dry benzene (100 μl) and then anhydrous $ZnCl_2$ (1 mg), the mixture was stirred until the $ZnCl_2$ had reacted. 2,3-Dichloro-1,4-dioxane (0.45 mmol) was added and the tube walls were rinsed down with dry benzene (200 μl). The reaction mixture was stirred in an unsealed tube (the generated HCl under pressure in a sealed tube causes product decomposition) for 4 h at 80 °C and chromatographed on silicic acid (40 g) with benzene–hexane (2:1) for elution. The eluted ^{14}C-labeled products and unlabeled material in preliminary cold runs gave appropriate TLC chromatographic patterns

Table I. Chromatographic and Spectral Properties of Dioxathion Components and Some of Their Metabolites and Oxidation Products

Compd	TLC R_f with indicated solvent systems[a] A	B	C	D	ir, cm^{-1}	NMR (CCl_4), δ (Me_4Si)	CIMS, *m/e* (rel intensity)
t-Dioxathion	0.31	0.62	0.62	0.84	2975 (s), 1130 (s), 900 (m), 870 (m), 657 (s), 637 (s)	1.38 (t, 12 H, J = 7.5 Hz), 3.50-4.50 (m, 12 H), 5.57 (d, 2 H, J = 13 Hz)	457 (10) (M + 1), 456 (10) (M), 271 (100), 187 (2), 185 (3), 153 (7), 125 (3)
c-Dioxathion	0.26	0.59	0.61	0.84	2975 (s), 1095 (s), 890 (m), 860 (m), 655 (s)	1.38 (t, 12 H, J = 7.5 Hz), 3.50-4.50 (m, 12 H), 5.45 (d of d, 2 H, J = 1, 14 Hz)	457 (2) (M + 1), 271 (100), 187 (4), 185 (3), 153 (6)
t-Dioxaoxon	0.00	0.27	0.38	0.79	2980 (s), 1260 (s), 1135 (s), 898 (m), 868 (s), 648 (s)	1.39 (t, 12 H, J = 7 Hz), 3.50-4.50 (m, 12 H), 5.55 (d, 1 H, J = 13 Hz), 5.64 (d, 1 H, J = 11.5 Hz)	271 (9), 255 (7), 187 (12), 185 (12), 171 (76), 169 (17), 153 (100), 125 (38), 117 (12)
c-Dioxaoxon	0.00	0.24	0.33	0.77	2980 (s), 1265 (s), 1095 (s), 890 (m), 860 (m), 662 (s)	1.37 (t, 12 H, J = 7 Hz), 3.50-4.50 (m, 12 H), 5.45 (d of d, 1 H, J = 2.5, 14.5 Hz), 5.52 (d of d, 1 H, J = 2.5, 12.5 Hz)	271 (21), 171 (55), 169 (100), 153 (15), 125 (15), 117 (15)
t-Dioxadioxon	0.00	0.11	0.20	0.67	2980 (s), 1258 (s), 1132 (s), 896 (s), 868 (s)	1.37 (t, 12 H, J = 7.5 Hz), 3.55-4.45 (m, 12 H), 5.63 (d, 2 H, J = 11 Hz)	425 (4) (M + 1), 255 (100), 227 (26), 171 (13), 153 (12), 125 (6), 117 (2)
c-Dioxadioxon	0.00	0.07	0.17	0.63	2980 (s), 1260 (s), 1095 (s), 890 (m), 860 (m)	1.38 (t, 12 H, J = 7 Hz), 3.60-4.45 (m, 12 H), 5.50 (d, 2 H, J = 12.5 Hz)	255 (4), 199 (12), 171 (100), 153 (16), 125 (5), 117 (17)
t-Dioxaoxon disulfide[b]		0.36	0.43	0.79	2980 (s), 1257 (s), 1125 (s), 894 (s), 868 (s), 645 (s)	1.40 (t, 12 H, J = 7 Hz), 3.50-4.40 (m, 12 H), 5.40 (s, 1 H), 5.64 (d, 1 H, J = 12 Hz)	303 (39), 287 (18), 285 (15), 187 (17), 171 (100), 153 (21)
c-Dioxaoxon disulfide (impure)		0.32	0.33	0.77	2980 (s), 1255 (s), 1093 (s), 887 (m), 858 (w)	1.37 (t, 12 H, J = 6.5 Hz), 3.60-4.50 (m, 12 H), 5.25 (d, 1 H, J = 3 Hz), 5.53 (d of d, 1 H, J = 3, 14 Hz)	303 (100), 287 (89), 271 (74), 171 (47), 153 (16), 117 (89)
Dioxenethion	0.33	0.61	0.61	0.85	2980 (s), 1632 (s), 1305 (s), 1150 (s), 918 (s), 879 (m), 660 (s)	1.37 (t, 6 H, J = 7 Hz), 3.85-4.50 (m, 8 H), 6.25 (d, 1 H, J = 6 Hz)	271 (100) (M + 1), 185 (20), 153 (74), 125 (18), 117 (8)
Dioxeneoxon	0.00	0.23	0.30		2980 (s), 1632 (s), 1259 (s), 1146 (s), 1109 (s), 912 (s), 876 (m)	1.37 (t, 6 H, J = 7.5 Hz), 3.90-4.45 (m, 8 H), 6.28 (d, 1 H, J = 6 Hz)	255 (7) (M + 1), 227 (14), 171 (7), 169 (12), 153 (100), 125 (67), 117 (14)
Dioxeneoxon disulfide[c]		0.29			2980 (s), 1610 (s), 1252 (s), 1150 (s), 1012 (s), 913 (s), 880 (s)	1.37 (t, 6 H, J = 7 Hz), 4.11 (m, 8 H), 6.58 (s, 1 H)	171 (19), 153 (7), 125 (23), 117 (100)
ESSP disulfide	0.55		0.68	0.93	2980 (s), 1100 (m), 645 (s)	1.37 (t, 12 H, J = 7 Hz), 4.24 (d of quart, 8 H, J = 7, 10 Hz)	411 (7) (M + 41), 399 (23) (M + 29), 371 (100) (M + 1), 187 (52), 185 (34), 153 (20), 125 (7)
$(C_2H_5O)_2P(S)SP(S)(OC_2H_5)_2$	0.47				2980 (s), 1098 (m), 633 (s)	1.40 (t, 12 H, J = 7 Hz), 4.15 (d of quart, 8 H, J = 7, 10 Hz)	379 (5) (M + 41), 367 (15) (M + 29), 339 (100) (M + 1), 187 (52), 185 (22), 153 (34), 125 (20)

[a] TLC R_f values for additional compounds are as follows: ESSP, 0.00 (A), 0.06 (C), 0.40 (D), and 0.62 (F); ESOP, 0.00 (A), 0.00 (C), 0.30 (D), and 0.50 (F); EOOP, 0.00 (A), 0.00 (C), 0.07 (D), and 0.19 (F); methyl ester of EOOP, 0.25 (C) and 0.07 (E); methyl esters of ESOP, two isomers, 0.32 and 0.60 (C) and 0.12 and 0.59 (E); *t*-dioxadioxon disulfide and *t*-dioxadioxon bis(disulfide), 0.12 (B) and 0.22 (C), i.e., these compounds are not separated from *t*-dioxadioxon; *c*-dioxadioxon disulfide and *c*-dioxadioxan bis(disulfide), 0.07 (B) and 0.17 (C), i.e., these compounds are not separated from *c*-dioxadioxon. [b] Anal. Calcd for $C_{12}H_{26}S_4P_2O_7$: C, 30.50; H, 5.55; S, 27.14; P, 13.11. Found: C, 31.67; H, 5.40; S, 26.68; P, 12.88. [c] Anal. Calcd for $C_8H_{15}S_2PO_5$: C, 33.56; H, 5.28; S, 22.40; P, 10.82. Found: C, 33.61; H, 5.08; S, 22.04; P, 10.48.

(A) for Delnav with yields based on ethanol of 40 and 44% for [^{14}C]Delnav (^{14}C content) and for unlabeled Delnav (weight), respectively. Since column chromatography provided incomplete separation of dioxenethion and *t*- and *c*-dioxathion (TLC monitoring, A, autoradiography), appropriate fractions were further purified by preparative TLC (spotted as a band on 0.5-mm plates, 3 × A) to obtain the desired materials in a radiochemical purity of >99% with yields based on [^{14}C]ethanol as follows: dioxenethion (0.074 mmol, 7.4%, 2.0 mCi/mmol); *t*-dioxathion (0.050 mmol, 10.0%, 4.0 mCi/mmol); *c*-dioxathion (0.042 mmol, 8.4%, 4.0 mCi/mmol).

To prepare the ring-labeled compounds, 1,4-[^{14}C]dioxane (0.79 mmol, 2.5 mCi/mmol; New England Nuclear; contained in a glass tube with a small bulb at the lower end and cooled at the upper end by a wick saturated with pentane) was rapidly heated to 85 °C in an oil bath and Cl_2 was immediately added via a capillary at a rate and in an amount determined by cold runs to give the optimum yield of 2,3-dichloro-1,4-dioxane. The walls of the reaction vessel were then rinsed down with carbon tetrachloride (2 ml), this solvent and residual Cl_2 were removed with a gentle stream of dry N_2, the process was repeated using a benzene (1 ml) rinse, and finally a small stirring bar (8 mm) and dry benzene (300 μl) were added. The reaction vessel was heated to 80 °C, a solution of ESSP (1.6 mmol) and anhydrous $ZnCl_2$ (1 mg) in benzene (400 μl) was added dropwise with stirring, the walls were rinsed down with benzene (300 μl), and the reaction was allowed to stir for 4 h and then left for 12 h at 5 °C. The resulting mixture was placed on a neutral alumina- (dry column grade; M Woelm, Eschwege, Germany) 2% water column (40 g) which was developed with benzene (100 ml). The labeled Delnav and unlabeled material in preliminary trials gave appropriate TLC patterns (see above) in yields based on dioxane of 12 and 60%, respectively. The ring-labeled preparation was subjected to TLC as with the [^{14}C]ethoxy preparation (see above) to recover dioxenethion (0.0040 mmol, 0.5%), *t*-dioxathion (0.0142 mmol, 1.8%), and *c*-dioxathion (0.0047 mmol, 0.6%), each with a specific activity of 2.5 mCi/mmol and a radiochemical purity for *t*- and *c*-dioxathion of >99%. The [^{14}C]dioxenethion was further purified by TLC (C) to remove an impurity with identical R_f with the desired dioxenethion in solvent systems A and B.

The labeled compounds were stored as solutions in hexane–benzene (9:1) at −5 °C, conditions in which they were stable for at least 1 year.

Synthesis of *t*- and *c*-Dioxaoxon, *t*- and *c*-Dioxadioxon, and Dioxeneoxon. Dioxaoxon was prepared by an adaptation of the dioxathion procedure (Diveley et al., 1959) using a 1:1 mixture of ESSP and ESOP for coupling followed by chromatography on a silicic acid column eluted with benzene–hexane (9:1) to remove dioxathion and then benzene–ethyl acetate (9:1 and 4:1) to elute the separated *t*- and *c*-dioxaoxons. The individual isomers were further purified by preparative TLC (1 mm, B) to obtain pure *t*- and *c*-dioxaoxon in 2.8 and 2.2% yields, respectively. *t*- and *c*-dioxadioxon and dioxeneoxon were prepared in a manner similar to that of dioxathion except that ESOP was used and the silicic acid column was eluted with benzene–ethyl acetate (1:1) to obtain the desired products in the order: dioxeneoxon (42%), *t*-dioxadioxon (14%), and *c*-dioxadioxon (10%). *t*- and *c*-dioxaoxons appear to be less stable than the corresponding dioxons or dioxeneoxon on storage of neat samples at −10 °C.

***t*- and *c*-Dioxaoxon Disulfides and Other Products from Peracid Oxidation.** A complex mixture of products was obtained on treating milligram amounts of *t*- and *c*-dioxathion with MCPBA (0.5–4.0 equiv) in chloroform or methylene chloride at 0 or 26 °C followed by TLC separation (B) of the products and then CIMS analysis. In each case, the reaction products consisted of unreacted starting material, elemental sulfur, the appropriate dioxaoxon and dioxaoxon disulfide isomer (TLC (B) and CIMS comparisons with authentic standards described elsewhere in this report), a mixture of materials at low R_f discussed below, and polar material at the origin. The mixture of low R_f materials gave TLC R_f values (B and C) almost identical with those for the corresponding dioxadioxons (Table I) but CIMS examination revealed the presence of three sets of (M + 1) and (M + 29) peaks appropriate for dioxadioxon, dioxadioxon disulfide, and dioxadioxon bis(disulfide). The disulfide and bis(disulfide) fragmented by S–S bond cleavage, a characteristic of disulfides of this type (see below). The major product, the dioxaoxon disulfide, decomposed to products of high polarity, more readily than the corresponding dioxaoxon, on two-dimensional TLC and on storage of neat samples at −10 °C. The disulfide isomers were stable for many weeks when held as dilute solutions in a hexane–benzene mixture at −10 °C.

To obtain larger amounts of the oxidation products, a mixture of *t*- and *c*-dioxathion (4.0 g) in chloroform (45 ml) at 23 °C was treated slowly and with stirring by addition of MCPBA (85%, 1.5 equiv) in chloroform (10 ml). The reaction was stirred 15 min and washed with 5% $NaHCO_3$ (3 × 20 ml), the organic phase was dried ($MgSO_4$), the solvent evaporated, and 2.5 g of the product mixture was subjected to column chromatography (silicic acid, developed with benzene–ethyl acetate mixtures varying from 99:1 to 3:2) with TLC monitoring (B) of the eluted fractions. The products obtained, in the order of elution, were: several compounds less polar than dioxathion; unreacted dioxathion; *t*-dioxaoxon disulfide (3.6%); an unidentified compound containing both P=O and C=O groups (ir); impure *c*-dioxaoxon disulfide (5.2%); a second unidentified compound containing both P=O and C=O groups; two additional materials with TLC, ir, and NMR features appropriate for trans and cis isomers, respectively, of the mixture of one or more of dioxadioxon, dioxadioxon disulfide, and dioxadioxon bis(disulfide).

Oxidation of dioxenethion (0.5 g) and chromatographic separation of the products in a similar manner gave dioxeneoxon (7%) and dioxeneoxon disulfide (25%). The neat disulfide underwent extensive decomposition within 48 h at 25 °C.

The disulfides are easily differentiated by NMR (Table I) since the doublet splitting pattern produced by phosphorus coupling with the methine ring proton (PSCH in dioxathion and dioxaoxon) or with the vinyl proton (PSC=CH in dioxenethion and dioxeneoxon) was absent for the corresponding protons in the disulfide; this is the expected effect if the phosphorus is moved one atom further away from the proton as in the disulfides. The cleavage of the S–S bond under CIMS conditions (Table I) is also a useful diagnostic tool for determining the presence of oxon disulfides, especially when a parent ion is not observed. Thus, both dioxaoxon disulfide isomers gave a major CIMS ion at *m/e* 303 corresponding to cleavage of the S–S bond, whereas no ions of comparable mass arose from dioxathion, its oxon, and its dioxon (by P–S cleavage), these compounds undergoing instead S–C bond cleavage under CIMS conditions. As previously noted, the dioxadioxon disulfide and bis(disulfide) also undergo S–S cleavage. Dioxenethion and dioxeneoxon

Table II. ^{14}C-Labeled Compounds in Rat Liver Enzyme Systems Incubated with E*- and R*-*t*- and *c*-Dioxathion and E*- and R*-Dioxenethion

^{14}C-Labeled compd	TLC solvent system	% of initial radiocarbon with indicated reaction mixture[a]											
		t-Dioxathion				*c*-Dioxathion				Dioxenethion			
		M	M + NADPH	M + S	M + S + NADPH	M	M + NADPH	M + S	M + S + NADPH	M	M + NADPH	M + S	M + S + NADPH
Compounds Retaining Both Ethoxy and Ring Labels													
Original substrate[b]	C	88.3	3.3	59.8	3.9	92.8	9.6	71.6	3.8	83.6	1.4	17.3	0.9
Oxon[b]	C	0.5	0.6	2.2	0.2	0.6	12.8	5.2	2.4	0.6	1.4	1.2	0.5
Dioxon[b]	C	0.0	6.1	0.4	3.0	0.0	12.8	0.6	18.8				
Unknowns above origin[b,d]	C	0.0	1.6	0.4	0.5	0.0	3.7	0.9	1.5	0.0	0.0	0.0	0.0
Metabolites Retaining Ethoxy Label Only													
ESSP[c,e]	D	0.3	1.1	0.5	1.3	0.1	1.0	0.2	0.7	0.2	1.5	1.0	0.7
ESOP[c]	D	0.3	43.4	4.3	46.9	0.1	21.4	1.7	27.3	1.0	52.2	23.0	54.6
EOOP[c]	D	1.4	15.8	8.7	9.1	0.7	10.3	5.1	8.3	0.7	11.5	9.7	5.2
Unknowns[c]													
R_f 0.82	D	0.0	0.0	2.2	0.0	0.0	0.0	0.9	0.0	0.0	0.0	4.9	0.0
R_f 0.70	D	0.0	4.3	1.8	10.9	0.0	3.0	0.7	4.3	0.0	10.6	7.5	17.0
R_f 0.00	D	0.1	1.1	0.8	1.7	0.1	1.4	0.5	1.9	0.3	1.0	1.0	0.6
Protein bound		0.0	0.7	0.5	0.8	0.2	1.1	0.4	1.7	0.1	1.4	2.0	1.6
Water soluble		0.5	4.2	2.6	1.7	0.3	5.5	3.1	5.7	0.3	2.2	2.1	1.7
Loss[f]		8.6	17.8	15.8	20.0	5.1	17.4	9.1	23.6	13.2	16.8	30.3	17.2
Metabolites Retaining Ring Label Only													
Unknowns													
R_f 0.07[b]	C	0.0	0.0	0.3	1.2	0.0	0.0	0.4	1.0	0.0	0.0	0.0	0.0
R_f 0.64[c]	D	0.0	5.2	0.0	6.3	0.0	3.9	0.0	3.1	0.8	6.7	1.4	2.7
R_f 0.49[c]	D	0.0	5.5	1.8	5.6	0.0	2.4	0.0	2.8	0.0	8.7	1.2	1.9
R_f 0.41[c]	D	0.0	6.2	1.4	7.2	0.0	3.3	0.9	3.0	0.0	11.9	3.9	4.9
R_f 0.30[c]	D	0.8	2.4	0.5	0.0	0.6	2.1	0.5	2.1	0.0	0.0	0.0	0.0
R_f 0.11[c]	D	0.2	6.4	0.0	0.9	0.1	6.9	0.0	5.3	0.4	17.1	22.3	29.1
R_f 0.00[c]	D	0.8	10.7	2.9	9.4	0.7	6.0	2.5	7.6	0.1	2.5	1.4	1.5
Protein bound		0.9	16.8	9.7	28.7	0.5	6.5	4.8	14.0	0.5	2.8	5.8	6.0
Water soluble		0.7	12.7	4.9	10.5	0.5	11.8	3.5	15.8	1.0	22.2	30.5	33.5
Loss[f]		7.8	22.5	15.7	22.6	4.2	18.2	9.1	18.8	13.0	25.3	15.0	19.0

[a] Average of results from two separate experiments. M = microsome fraction and S = soluble fraction. [b] These compounds appear in the ether extracts only. [c] These metabolites appear in the ether–ethanol extracts only. [d] The *t*-dioxathion unknowns give R_f values of: 0.31 (all systems except M alone) and 0.52 (M + S only). Comparable values for the *c*-dioxathion unknowns are: 0.27 (M + NADPH only), 0.29 (all systems except M alone), 0.47 (M + NADPH only), and 0.53 (all systems except M alone). [e] This metabolite was detected as ESSP disulfide formed on oxidation of ESSP during workup and analysis. If ESSP disulfide was present in the original incubation mixture, it would appear in the ether extract. [f] Losses on TLC workup and analysis, some of which may be due to volatile metabolites.

gave an ion of m/e 117 (relative intensity, 8 and 14%, respectively), corresponding to S–P bond cleavage to yield the stabilized thioallyl cation, while dioxeneoxon disulfide had m/e 117 as the base peak from S–S bond cleavage.

The yields of various products in the *t*-dioxathion–MCPBA reactions in chloroform at 0 °C are dependent on both the ratio and the concentration of the reactants, based on studies quantitated by using E*-*t*-dioxathion added to unlabeled *t*-dioxathion, TLC analysis (D for products containing phosphorus acids; C for dioxaoxon and dioxaoxon disulfide), and lsc. With *t*-dioxathion at 1 mg/ml, the maximum yields of monooxons (41%, dioxaoxon and dioxaoxon disulfide) were obtained at the 2:1 MCPBA: dioxathion ratio, of dioxons (56%, dioxadioxon and dioxadioxon disulfide) at a 4:1 to 8:1 ratio, of ESOP (14%) at a 4:1 to 8:1 ratio, and of EOOP (9%) at the 8:1 ratio. With the 2:1 ratio of peracid to dioxathion and dioxathion concentrations of 0.01–1.0 mg/ml, the yields of dioxaoxon and dioxaoxon disulfide were 21–31 and 3–7%, respectively, at 3.3 mg/ml they were 20 and 10%, respectively, and at 10 mg/ml they were 8 and 23%, respectively. Thus, dilute solutions are most favorable for dioxaoxon recovery and concentrated solutions favor dioxaoxon disulfide formation.

METABOLISM AND DEGRADATION OF SURFACE DEPOSITS

Metabolism by Rat Liver Microsomes. *t*- and *c*-dioxathion and dioxenethion undergo little or no metabolism when incubated with microsomal preparations unless these mixtures are fortified with the soluble fraction (which contains endogenous NADPH), with NADPH, or with both the soluble fraction and NADPH (Table II). Controls consisting of boiled microsomes, soluble fraction alone, and boiled microsomes and soluble fraction gave no metabolism, with or without NADPH fortification. These findings indicate that microsomal oxidases are important in metabolism of *t*- and *c*-dioxathion and dioxenethion.

Metabolites retaining at least one ester linkage (detected with both the E*- and R*-labeled substrates) appeared only in the ether extracts and were tentatively identified by TLC cochromatography as follows: original substrate (A); *t*- and *c*-dioxaoxon, *t*- and *c*-dioxadioxon, and dioxeneoxon (one-dimensional development with each of B, C, and ethyl acetate). The yield of oxons was greatest with *c*-dioxathion, intermediate with *t*-dioxathion, and least with dioxenethion. Two unidentified ester metabolites (unknowns) from *t*-dioxathion and four from *c*-dioxathion were formed only in the presence of NADPH and they decreased in amount when the soluble fraction was also present. Not any of these metabolites cochromatographed on TLC (B or C) with the corresponding dioxaoxon disulfide or dioxadioxon disulfide. There is some indication that in the formation of two of these unidentified ester metabolites of *c*-dioxathion (R_f 0.27 and 0.53 in C) by

NADPH-fortified microsomes (the only system that forms all of these metabolites), there is a loss of one or more of the ethoxy groups. Thus, in five experiments, the average radiocarbon recoveries for these metabolites were much lower than anticipated from E*-dioxathion as compared to the recoveries from R*-dioxathion. These metabolites might arise, therefore, by O-deethylation or monodephosphorylation of dioxathion or its diester metabolites. However, their extractability into ether and their high R_f values on TLC (C) might not be appropriate for such derivatives.

Metabolites retaining the ethoxy label only (detected with E*- but not with R*-dioxathion and -dioxenethion) were detected in the ether–ethanol but not in the ether extracts. Formation of each of these metabolites was dependent on fortification of the microsomes with one or both of NADPH and the soluble fraction. Two phosphorus acid metabolites, ESOP and EOOP, comprising up to 64% of the original radiocarbon, were tentatively identified by TLC cochromatography both before (D) and after methylation with diazomethane (C and E). A portion of the ESOP was oxidized to EOOP in the process of recovery from the chromatoplates and rechromatography for identification. Small amounts of ESSP were also detected, this material being analyzed as ESSP disulfide (identified by cochromatography in A) as a result of oxidation during workup and analysis (see Table II, footnote *e*). Two labeled unknowns (R_f 0.70 and 0.82) from each ethoxy-labeled substrate extracted and chromatographed in a manner which may be appropriate for ESOP disulfide and the mixed disulfide of ESSP and ESOP, respectively; formation of these disulfides as artifacts would be analogous to the formation of ESSP disulfide as described above. Protein-bound and water-soluble metabolites were relatively minor with the ethoxy-labeled substrates.

Metabolites retaining the ring label only (detected with R*- but not with E*-dioxathion and -dioxenethion) appeared in both the ether and ether–ethanol extracts, totaling at least seven separate compounds most of which were derived from each of the R*-labeled substrates. None of these metabolites was identified but there is some information on their properties and on conditions for their formation. Only one of these metabolites appeared in the ether extracts and it was in small amount and gave a low R_f value (0.07, C); it appears possible that this material is the same as one of the less polar metabolites recovered in the ether–ethanol extract but this point was not established. The remaining metabolites retaining the ring label only were recovered in the ether–ethanol extracts and generally they did not chromatograph as distinct spots, suggesting some decomposition during development. When recovered from TLC plates and rechromatographed, most of the materials decomposed to a number of products of both higher and lower R_f. Two of these metabolites (R_f 0.07, C; R_f 0.30, D) are not formed from dioxenethion suggesting that they are unique to the dioxane moiety whereas the others might arise by ring cleavage of either the dioxane or dioxene moiety. Protein-bound metabolites were major with the ring-labeled substrates, requiring NADPH fortification for high yields and increasing in amount in the order of dioxenethion < *c*-dioxathion < *t*-dioxathion. The greater amount of protein-bound metabolite(s) from *t*-dioxathion relative to *c*-dioxathion may be related to the lower yield of oxon and dioxon in the former case, both the binding of the ring fragments and the formation of oxons apparently resulting from oxidative attack. Water-soluble metabolites are also important with the R*-substrates, particularly with dioxenethion. Oxalic acid was either minor or not present as a metabolite of *t*-dioxathion and dioxenethion in the microsome–NADPH system since fortification of the aqueous fraction from these reactions with oxalic acid and precipitation of this carrier by addition of calcium ion yielded only 0.2–0.6% of the radiocarbon in the precipitate.

Table III. Radiocarbon in the Urine, Feces, Carbon Dioxide, and Tissues of Male Rats 96 h after Oral Administration of E*- and R*-*t*- and -*c*-Dioxathion

	t-Dioxathion		*c*-Dioxathion	
Sample analyzed	E*	R*	E*	R*
Administered Dose, mg/kg	1.59	2.05	2.99	3.24
% of Administered Radiocarbon				
Urine				
0-1 day				
Identified[a]	73.5	0.0	67.7	0.0
Unidentified	1.7	52.6	1.0	70.9
1-4 days	4.2	2.3	5.9	2.8
Feces				
0-1 day				
Methanol extract				
Identified[a]	5.1	2.1	9.2	4.7
Unidentified	0.5	5.1	0.1	2.3
Unextractable	5.8	3.6	0.5	4.8
1-4 days	4.5	2.7	1.7	1.1
$^{14}CO_2$				
0-1 day	2.2	6.3	8.7	6.0
1-4 days	0.3	0.7	0.5	0.6
Total				
0-4 days	97.8	75.4	95.3	93.2
Tissue Residues, ppm of Dioxathion Equiv.				
Blood	0.02	0.54	0.06	0.34
Bone	0.02	0.11	0.04	0.12
Brain	0.01	0.03	0.04	0.04
Fat	0.07	0.24	0.18	0.24
Heart	0.01	0.11	0.05	0.13
Kidney	0.10	0.18	0.31	0.32
Liver	0.46	0.29	0.66	0.60
Lung	0.03	0.14	0.12	0.18
Muscle	0.01	0.06	0.03	0.09
Spleen	0.02	0.20	0.06	0.16
Testes	0.02	0.06	0.09	0.09

[a] See Table IV.

Metabolism in Rats. Table III gives the radiocarbon balance sheet following oral administration of E*- and R*-*t*- and -*c*-dioxathion. Most of the radiocarbon excreted within 96 h was produced during the first 24-h period following administration, i.e., 88–95% for $^{14}CO_2$, 92–96% for urine, and 72–91% for feces (methanol extract and unextractable). A very large portion (80–87%) of the radiocarbon excreted over 96 h from both isomers and labels was in the urine. The tissue retention of radiocarbon at 96 h was at a maximum in the liver and kidney with E*-*t*- and -*c*-dioxathion and in the blood and liver with lesser amounts in the fat, kidney, and spleen with R*-*t*- and -*c*-dioxathion.

Unmetabolized dioxathion appeared in the feces, more with the cis than with the trans isomer, but not in the urine (Table IV). No cis–trans isomerization was detected. The identifications were based on one-dimensional cochromatography in three different solvent systems (2 × A, C and D) and two-dimensional cochromatography (D × A).

Most of the urinary and fecal metabolites retaining the ethoxy label only are identified (Table III) as ESSP, ESOP, and EOOP (Table IV), in each case the tentative identifications being based on TLC cochromatography (D and F) and comparison of TLC R_f values on two-dimensional TLC (D × C). A small portion of the ESSP chromatographed as ESSP disulfide on TLC (A) but is

Table IV. ^{14}C-Labeled Compounds in the Urine and in the Methanol Extract of Feces of Male Rats 24 h after Oral Administration of E*- and R*-*t*- and *c*-Dioxathion

	% of administered radiocarbon[a]			
	t-Dioxathion		*c*-Dioxathion	
^{14}C-Labeled compd	Urine	Feces	Urine	Feces
Compounds Retaining Both Ethoxy and Ring Labels				
Dioxathion	0.0	2.1	0.0	4.7
Metabolites Retaining Ethoxy Label Only				
ESSP	14.5	0.0	2.6	0.0
ESOP	45.7	0.5	47.4	1.1
EOOP	13.3	2.5	17.7	3.4
Unknowns at origin[b]	1.7	0.5	1.0	0.1
Metabolites Retaining Ring Label Only				
Unknowns				
Above origin	32.8[c]	1.8	54.5[c]	1.4
At origin[b]	19.8	3.3	16.4	0.9

[a] Corrected for losses (average and standard deviation) on TLC analyses of 8.4 ± 3.1% from urine and 2.1 ± 0.8% from feces. [b] Two-dimensional TLC development (D × C). [c] Includes six metabolites from each of *t*- and *c*-dioxathion. The R*-*t*-dioxathion metabolites give R_f values in D × C of: 0.07 × 0.00 (major); 0.11 × 0.00 (major); 0.23 × 0.04; 0.40 × 0.06; 0.56 × 0.10; 0.64 × 0.16. Comparable values for the R*-*c*-dioxathion metabolites are: 0.11 × 0.00 (major); 0.28 × 0.01 (major); 0.37 × 0.03; 0.41 × 0.04 (major); 0.51 × 0.08; 0.56 × 0.05.

tabulated along with the ESSP (see previous section on microsomal metabolism). On comparing urine collected at 0–6 h vs. 6–24 h after treatment, it was found that the proportionate amount of EOOP relative to ESOP and ESSP was greater in the later urine samples.

No urinary or fecal metabolites were identified which retained the ring label only (Table IV). Most of these 0–24-h metabolites (91–97%) appeared in the urine and they were composed of a complex mixture of at least seven materials from each of *t*- and *c*-dioxathion, only two or three of which were major products. Some of these ^{14}C-labeled metabolites are probably the same compounds as derived from either *t*- or *c*-dioxathion based on similarities in their chromatographic properties on two-dimensional (D × C) and one-dimensional TLC development [1-propanol–17 N NH_4OH (7:3) and ethanol–9.7 N NH_4OH (39:11)]. Not any of these metabolites was sufficiently stable or well resolved for isolation by TLC and rechromatography, probably due to decomposition during chromatography and recovery from the silica gel. On comparing 0–6, 6–12, 12–24, and 24–36 h urine samples, it was found that the metabolites of lower R_f increased proportionately in amount with increasing residence time in the animal before excretion. Solvent system D separates ethylene glycol (R_f 0.46) and glycolic acid (R_f 0.13) but these materials did not cochromatograph with any metabolites of *t*-dioxathion. Cochromatography with glycolic acid (R_f 0.43) and oxalic acid (R_f 0.39) in 1-propanol–17 N NH_4OH (7:3) on cellulose TLC gave a poorly resolved radioactive area at an R_f value greater than either acid, with only trace levels of radioactivity remaining at the origin. The methanol-extractable feces metabolites of R*-*t*- and -*c*-dioxathion, except for the esters, remained at or near the origin on TLC (D).

No further attempts were made to solubilize and identify the unextractable fecal metabolites, which accounted for 3.6–5.8% of the administered radiocarbon at 0–24 h with E*- and R*-*t*-dioxathion and R*-*c*-dioxathion but only 0.5% with E*-*c*-dioxathion.

Fate on Glass and Silica Gel Surfaces and on Bean Leaves. The volatilization half-life values (total radiocarbon) were <30 min for E*- and R*-dioxenethion and about 32 days for either E*- or R*-*t*- and -*c*-dioxathion as 1.3 nmol/cm^2 deposits on glass surfaces. The residual labeled products on the glass surfaces after application of E*- and R*-*t*-dioxathion at 4.0 nmol/cm^2 were tentatively identified by R_f comparisons with authentic standards (benzene and D × C). They consisted of the following compounds and percentages of the applied radiocarbon at 21 and 47 days, respectively: *t*-dioxathion, 73.4 and 58.2%; *t*-dioxaoxon, 7.0 and 8.7%; *t*-dioxadioxon, 1.4 and 2.4%; EOOP, 4.6 and 5.0%; unidentified products, 2.1 and 2.3%.

The following products from exposure of the ^{14}C-labeled compounds on silica gel to sunlamp irradiation for 27 h were tentatively identified by TLC cochromatography in the indicated solvent systems: dioxaoxon [5–10%, C and ethyl acetate (R_f 0.60 for trans and 0.55 for cis)] and dioxadioxon [2–4%, ethyl acetate (R_f 0.34 for trans and 0.24 for cis)] from both *t*- and *c*-dioxathion; dioxeneoxon [5–10%, ethyl acetate (R_f 0.44)] from dioxenethion; ESSP, chromatographed as ESSP disulfide (A), as a major product from *t*-dioxathion and dioxenethion but minor or absent from *c*-dioxathion; ESOP and EOOP (D) as major products from all ethoxy-labeled compounds; ethylene glycol (D) as a major product from R*-*t*-dioxathion; an additional unidentified product from R*- but not from E*-*t*-dioxathion at R_f 0.09 (D, more polar than glycolic acid); large amounts of unidentified material at the origin (D) from both E*- and R*-*t*-dioxathion and much smaller levels of radiocarbon at the origin with *c*-dioxathion and dioxenethion. A larger amount of each of these products was detected on exposure to light as compared to holding in the dark for a comparable period of time, with the exception of *t*- and *c*-dioxadioxon which did not appear to accumulate in either case. Thus, most of the products formed from dioxathion and dioxenethion on exposure to light on silica gel appear to be photoproducts.

E*- and R*-dioxenethion volatilized rapidly from bean leaves, 60–70% of the applied radiocarbon having disappeared within the first day. The remaining chloroform–methanol extractable material consisted of dioxenethion, dioxeneoxon, and EOOP. *t*- and *c*-dioxathion (ethoxy and ring labeled) were much more persistent, undergoing some loss by volatilization and/or mechanical weathering and slow oxidation or metabolism to *t*- and *c*-dioxaoxon and -dioxadioxon (Table V), with a small amount of EOOP also being detected with the ethoxy-labeled preparations. These products were identified by TLC cochromatography as with the microsomal metabolites. More oxons and dioxons were found with *c*-dioxathion than with *t*-dioxathion. The chloroform–methanol extractable ^{14}C-labeled compounds designated as unknowns were in relatively small amounts, the ring-labeled unknowns being in greater abundance than the ethoxy-labeled unknowns. The polar and penetrated residues were very small whereas the unextractable material determined by combustion increased progressively with time. Radiocarbon moving through the rest of the plant (determined by combustion) was negligible with <0.5% in the roots and <1% in the untreated leaves and stem.

Toxicity of Dioxathion and Dioxenethion Derivatives. The mouse ip LD_{50} values (mg/kg) are as follows: *t*-dioxathion, >125; *c*-dioxathion, 38–75; dioxenethion, 50; *t*-dioxadioxon, 13; *c*-dioxadioxon, 5; dioxeneoxon, 7.

DISCUSSION

Figure 2 gives a partial metabolic pathway for *t*- and *c*-dioxathion in the rat liver microsome–NADPH system

Table V. ^{14}C-Labeled Compounds on or in Bean Leaves up to 8 Days after Application of E*- and R*-*t*- and -*c*-Dioxathion

^{14}C-Labeled compd	Label position	% of applied radiocarbon[a] at indicated day: *t*-Dioxathion 0	2	4	8	*c*-Dioxathion 0	2	4	8
Dioxathion and Ester Products									
Dioxathion	E*	99.0	89.2	67.8	54.9	99.2	99.6	48.5	27.2
	R*	94.3	78.3	61.3	42.9	88.1	74.8	64.4	50.6
Dioxaoxon	E*	1.8	4.4	7.5	7.5	1.7	6.3	8.9	9.0
	R*	2.7	8.4	8.0	7.1	3.4	10.4	12.0	9.1
Dioxadioxon	E*	0.1	0.9	1.6	1.3	0.0	0.6	1.6	2.6
	R*	0.0	1.7	1.7	2.1	0.9	1.4	2.1	1.9
Other Products									
EOOP	E*	0.5	1.7	5.1	5.0	0.2	1.0	2.6	4.4
Unknowns									
Above origin[b]	E*	0.0	0.0	1.0	1.3	0.0	0.5	1.4	1.9
	R*	0.3	1.8	2.4	2.7	0.7	2.6	5.0	5.1
At origin[c]	E*	0.1	0.3	0.4	0.8	0.1	0.3	0.5	0.9
	R*	0.3	0.6	1.1	2.4	0.1	0.6	1.7	1.7
Polar and penetrated	E*	0.5	0.6	0.7	0.6	0.3	0.2	0.6	1.3
	R*	0.4	0.5	0.4	1.7	0.2	0.2	0.5	0.5
Unextractable	E*	0.2	0.7	2.1	2.5	0.1	0.5	1.7	7.6
	R*	0.3	1.2	2.9	6.9	0.2	0.9	3.0	4.0
Dioxathion and Degradation Products									
Total	E*	102.2	97.8	86.2	73.9	101.6	109.0	65.8	54.9
	R*	98.3	92.5	77.8	65.8	93.6	90.9	88.7	72.9

[a] Average of three replicates. Corrected for losses (average and standard deviation) on TLC analysis of 17.6 ± 6.9%. [b] Includes two or three products from each labeled preparation which give R_f 0.00 in solvent system C and the following R_f values in solvent system D: E*-*t*-dioxathion, 0.15 and 0.25; R*-*t*-dioxathion, 0.03 and 0.29; E*-*c*-dioxathion, 0.10 and 0.24; R*-*c*-dioxathion, 0.03, 0.11, and 0.24. [c] Two-dimensional TLC development (D × C).

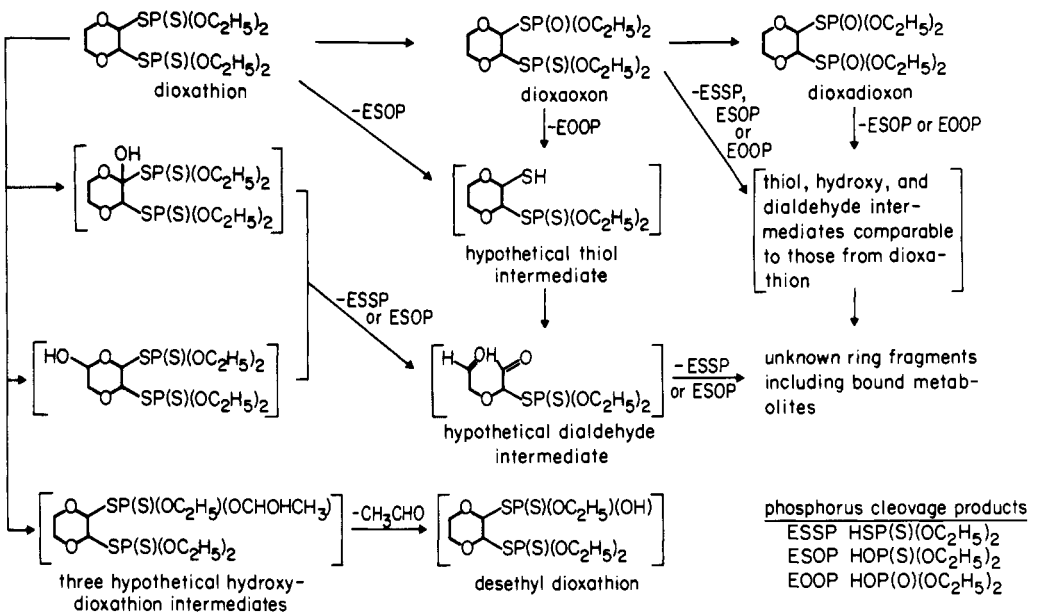

Figure 2. Partial metabolic pathway for dioxathion with hypothetical products or intermediates shown in brackets. Many of the reactions indicated involve multiple steps. This pathway is applicable to both *t*- and *c*-dioxathion in the rat liver microsome-NADPH system and a portion of the scheme is also appropriate for rats in vivo and the dissipation of residues from bean leaf and glass surfaces.

in vitro and in rats in vivo. Many parts of this metabolic scheme are also suitable for dioxenethion, with appropriate structural substitutions. Some are also applicable to the fate of dioxathion and dioxenethion on glass and silica gel surfaces, on bean leaves, and in the peracid system.

The reaction of greatest toxicological significance involves phosphorothionate oxidation to form dioxaoxon and dioxadioxon from dioxathion and dioxeneoxon from dioxenethion. There is a higher oxon and dioxon yield with *c*- than with *t*-dioxathion in microsome–NADPH systems and on bean leaves. The structural features leading to the higher oxon levels from the cis isomer may contribute to its greater biological activity. Such an isomer effect on the oxon yield might result from differences in the rate of oxon formation as opposed to oxidative attack at other molecular sites or from differences in the stability of the oxon once formed. No trans–cis isomerization was observed with dioxathion nor were products of lesser polarity than dioxathion encountered. The oxon disulfides were not found as metabolites in the present study, indicating either that they are not formed or that they are not sufficiently stable to survive the analytical procedures.

Peracid oxidation of dioxathion and dioxenethion gives not only the corresponding oxon but also significant levels of their respective oxon disulfides (such as dioxaoxon disulfide) (Figure 1), much more of the disulfides being

detected than with other phosphorodithioates previously examined (malathion, phosmet, and *S*-phenylparathion; see Bellet and Casida, 1974). We also found large amounts of oxon disulfide on MCPBA oxidation of another phosphorodithioate, *O,O*-diethyl *S*-isopropyl phosphorodithioate, the disulfide product being identified by CIMS. These oxon disulfides might arise from either rearrangement or cleavage of an intermediate "phosphorus oxythionate", in the latter case the fragments recombining to form the disulfide (Bellet and Casida, 1974). The present study provides direct evidence that at least some of the oxon disulfide formation occurs by oxidative coupling of the thiol fragments [ESOP and the "thiol intermediate" (Figure 2)] rather than by a concerted or simple rearrangement. Thus, there is more oxon disulfide formed in concentrated solutions than in dilute solutions, perhaps because the component fragments have more opportunity for recombination or due to the higher acid concentration in the former case. On this basis, the compounds giving high oxon disulfide yields may be those that form thiol fragments from both the phosphorus- and non-phosphorus-containing moieties that are both sufficiently reactive for oxidative coupling and produce relatively stable disulfide linkages. Dioxathion is not an appropriate compound for studying the mechanistic aspects of these reactions because it yields several fragments that undergo further oxidation as evidenced by the large amount of peracid consumed.

Dioxathion is rapidly metabolized in microsome–NADPH systems and in rats but ethoxy-containing metabolites other than the oxon, dioxon, and phosphorus acids (ESSP, ESOP, and EOOP) appear only in small or trace levels. A small portion of the dioxathion may undergo hydroxylation of the alkoxy phosphate group and cleavage to give acetaldehyde and the dealkyl phosphorus compound (Figure 2), since ethoxy-labeled *t*- and *c*-dioxathions yield $^{14}CO_2$ and two of the microsomal metabolites of *c*-dioxathion appear to retain at least one of the phosphorus ester groups but have reduced ethoxy:dioxane ratios. However, neither deethyldioxathion nor deethyldioxaoxon has been identified as a metabolite in the present or in other studies (Arthur and Casida, 1959; Chamberlain et al., 1960; Plapp et al., 1960). With the possible exception of these deethyl derivatives, there are no metabolites other than the oxons in significant levels which retain the dioxane ring and at least one phosphate moiety.

Dioxathion metabolism involves extensive ring fragmentation but the fragments are not identified. Thus, once there is hydrolytic or oxidative cleavage of one phosphorus-containing moiety the second such group is rapidly eliminated, before or after ring scission. There are several possible routes by which this might occur. One route involves enzymatic cleavage of dioxaoxon or dioxadioxon at the P–S bond to liberate EOOP and form a hypothetical thiol intermediate which then decomposes or is further metabolized via a hypothetical dialdehyde intermediate with the release of the additional phosphate moiety (Figure 2). The phosphorothiolates are most likely to undergo this type of cleavage since dioxathion itself appears to be relatively resistant to attack by esterases in liver microsomal and soluble fractions. Two other routes involve hydroxylation of the dioxane ring to form hypothetical hydroxydioxathion intermediates, hydroxylated at the 2 or 6 position. These intermediates should rapidly lose one phosphate moiety on forming the dialdehyde and the second on further degradation of the dialdehyde (Figure 2). Reactions of these types provide the most convenient routes to explain the formation of ESSP as a dioxathion metabolite. Many unstable intermediates are involved in these hypothetical ring cleavage and dephosphorylation reactions, possibly contributing to the large number of unidentified metabolites derived from the dioxane moiety. Attempts to identify ethylene glycol, glycolic acid, and oxalic acid as ring-cleavage fragments in microsomal systems or urine were unsuccessful. The two-carbon fragments such as glycolate and glyoxalate are readily converted in rats to a myriad of other products (Weinhouse, 1955). Aldehyde intermediates in dioxathion metabolism may form stable complexes or covalently bonded derivatives with microsomal components, on analogy with studies on other compounds (Ueda et al., 1975). This type of reaction may account for the binding of the ring radiocarbon in microsomal constituents and the retention of some of the ring radiocarbon in tissues.

Tissue levels of dioxathion and its metabolites are relatively low in rats and steers a few days after treatment (Arthur and Casida, 1959; Plapp et al., 1960; this study). Large differences in tissue specificity for retaining the ethyl vs. the ring label can arise only from metabolites not having both labels in common, i.e. both phosphate groups must be cleaved from the ring or ring fragments. Radiocarbon from the ethoxy group is located primarily in the liver and to a lesser extent in the kidney and fat, in agreement with earlier studies with [^{32}P]dioxathion in the rat and steer. These terminal metabolites are probably a mixture of diethoxyphosphoryl proteins and normal tissue constituents which have reincorporated the label from [^{14}C]-acetate. The dioxane label is incorporated into all tissues except liver to a greater extent than the ethoxy label, a differential which is greater with *t*-dioxathion than with *c*-dioxathion.

Dioxathion and dioxenethion undergo photooxidation on silica gel surfaces, and probably on glass, to form the corresponding oxons and a variety of cleavage products from both the phosphorus- and non-phosphorus-containing moieties. Similar products are encountered when dioxathion or dioxenethion residues on bean leaves are exposed to sunlight. Earlier work (Casida and Ahmed, 1959) has shown that dioxathion and dioxenethion undergo hydrolysis after absorption through the roots of bean plants. A portion of the foliage residues in the present study may have penetrated and undergone metabolism since the amount of polar and unextractable residues increased progressively with time after treatment of the leaves.

Dioxathion contains multiple sites for oxidative attack, leading to toxic oxons and dioxons and a variety of other, presumably inactive detoxification products. The principal sites of metabolic oxidation are at the thionophosphorus moiety and, probably, at carbon atoms adjacent to oxygen or sulfur leading to unstable intermediates that rapidly decompose with concomitant dephosphorylation. Thus, the toxic residues from use of dioxathion are likely to consist of only six compounds, i.e. *t*- and *c*-dioxathion and their respective mono- and dioxons.

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Synthesis of Bioactive Compounds. A Structure-Activity Study of Aryl Terpenes as Juvenile Hormone Mimics

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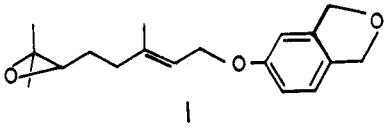
A series of aryl terpene ethers has been synthesized and bioassayed for juvenile hormone (JH) activity by induction of oviposition in the adult prediapause cereal leaf beetle [*Oulema melanopus* (L.), Chrysomelidae]. The selection of these JH mimics was based on bit by bit variations on the structure of 6,7-epoxygeranyl 4-ethylphenyl ether. Hansch type quantitative structure–activity relationships were investigated using polar, lipophilic, and steric parameters. Multiple regression analysis indicated that lipophilic and steric factors alone are responsible for the observed biological activity of these compounds. A hypothetical receptor site and its dimensions for these JH mimics are inferred from qualitative observations of the steric effect on JH response. Complete oviposition and mortality data upon treatment of the beetles are given for all compounds.

Of the hundreds of chemicals that have been investigated as to their juvenile hormone mimicking properties, probably the largest single group of these is the aryl acyclic monoterpenes. Two of these, namely (*E*)-6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)phenoxy]-2-octene (**1**, Bowers, 1969) and (*E*)-6,7-epoxy-1-(*p*-ethylphenoxy)-3,-7-dimethyl-2-octene (**2**, Pallos et al., 1971), have high orders of activity in many insect species. It was the latter of these which attracted our attention in an earlier study (Nilles et al., 1973) since this compound and C-16 juvenile hormone (**3**) have virtually the same order of activity with regard to diapause prevention in the adult cereal leaf beetle, *Oulema melanopus* (L.).



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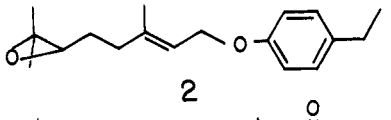

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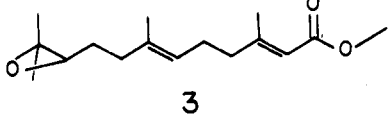

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Large numbers of analogues of **2** have been bioassayed (Jacobsen et al., 1972). Results of these studies indicate that the maximum juvenile hormone (JH) like response was obtained when the benzene ring was para substituted and the terminal double bond was epoxidized.

Quantitative structure–activity relationships (QSAR) have been modestly successful in predicting substituent effect on biological response in many series of pharmaceuticals (Dunn, 1973; Hansch et al., 1973). Metcalf (1974) has used QSAR in his studies of DDT analogues. Such correlations, if extended to JH mimics, should prove useful in the design of new hormonal materials.

If we make the somewhat liberal assumption that the variation in the number and position of introduced alkyl groups on or near the aryl portion of **2** will not greatly affect JH response arising from lipophilicity, electronic factors, or secondary structure in the molecule, then what effect remains should be due to steric factors, i.e., the bit by bit introduction and variation in the position of a methyl group, e.g., could give us a rough picture of the steric requirements of a hypothetical receptor site for **2** and its analogues.

Earlier (Nilles et al., 1973) we had postulated that one requirement for biological response in the JH molecule or its mimics was the placement of a group with a certain π-electron density 11 ± 1 Å away from the epoxy oxygen, assuming conformations in which hydrogen–hydrogen repulsions were minimized. Alternatively, we felt that the requirement may be only for a group having the approximate steric parameters of the *p*-ethylphenyl residue. We now wish to indicate that it is primarily the later alternative which prevails in analogues of **2**. In addition, correlations between JH response in terms of induced oviposition and diapause prevention and lipophilicity, steric parameters, and polar effects of the mimics will be