study), residues of mercury were greater in liver than in kidney.

In 1970, Panogen 15 was suspended for use as a seed treatment and such treated seeds were suspended from travel in Interstate Commerce. No tolerances are allowed on feed grains. It is still registered for use on cotton as a liquid or granular formulation applied in-furrow and covered at planting time. Registrations are also valid for nongrazed grass areas. The chances of an animal to be exposed to some grain treated with Panogen 15 are small; however, if such an exposure did occur, the consumption of tissues from this animal by humans could be hazardous.

LITERATURE CITED

Analytical Methods Committee, *Analyst* **85,** 643 (1960).
Buck, W. B., *J. Amer. Vet. Med. Ass.* **155,** 1928 (1969).
Dalton, E. F., Consumer and Marketing Service, U. S. Department of Agriculture, Beltsville, Md., personal communication, 1969.
Herberg, W. H., *Vet. Med.* **49,** 401 (1954).
Palmer, J. S., *J. Amer. Vet. Med. Ass.* **142,** 1385 (1963).
Palmer, J. S., Wright, F. C., Haufler, M., *Clin. Toxicol.* submitted for publication (1973).
Perkin-Elmer Corp., "Analytical Methods for Atomic Absorption Spectrophotometry," 1968.
Willis, J. B., *Anal. Chem.* **34,** 614 (1962).

Received for review November 30, 1972. Accepted February 9, 1973. This report reflects the results of research only. Mention of a proprietary product or a pesticide in this paper does not constitute an endorsement or a recommendation of this product by the USDA. Presented before Division of Pesticide Chemistry, 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972.

Metabolism of [^{14}C]Parathion and [^{14}C]Paraoxon with Fractions and Subfractions of Rat Liver Cells

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To obtain information relative to the metabolism of insecticides in mammalian systems, [2,6-ring-^{14}C]parathion and [2,6-ring-^{14}C]paraoxon were incubated with rat liver cell fractions obtained from the same liver homogenate and with highly purified subfractions from mitochondria and microsomes. Different enzymes in the rat liver were responsible for the degradation and detoxication of parathion and paraoxon. It was primarily with the soluble fractions obtained at 105,000 × *g* and 500,000 × *g* forces that parathion was degraded to water-soluble metabolites (20% of the applied radiocarbon) and only to a lesser extent by microsomes (1% of the applied radiocarbon). Based on analyses of the organic solvent extraction phases, enzyme activities in the soluble fraction resulted in a reduction of parathion to aminoparathion, but also in an oxidation of parathion to paraoxon. Microsomes showed some parathion-degrading activity and the amount of *p*-nitrophenol produced was relatively small. Paraoxon, however, was mostly degraded by particulate associated enzymes through hydrolysis, yielding *p*-nitrophenol in the organic solvent phase. The largest amounts of [^{14}C]paraoxon derived water-soluble metabolites (65% of the applied radiocarbon), though, were produced by the soluble fractions which also reduced paraoxon to aminoparaoxon. A biological assay of the water extraction phases from [^{14}C]parathion- or [^{14}C]paraoxon-treated incubation mixtures containing the 105,000 × *g* supernatant did not result in insect mortalities, thus indicating a detoxication of the insecticidal substances.

Detoxification mechanisms of insecticides in biological systems have been studied for years by many research workers. Of particular interest are those investigations that deal with the metabolism of insecticides through enzymatic reactions. In this respect, a number of studies were conducted relative to the degradation of parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate) and paraoxon (*O,O*-diethyl *O*-4-nitrophenyl phosphate) in mammalian systems. Nakatsugawa *et al.* (1969) showed that the arylphosphate cleavage of [^{35}S]parathion was primarily effected by rat liver microsomal oxidases and a minor portion was catalyzed by nonoxidative soluble enzymes requiring reduced glutathione. The authors concluded that "the metabolism of parathion in the rat is mostly initiated by liver microsomal oxidases." Neal (1967), working with microsomes from rat livers, reported that the major metabolites of [^{32}P]parathion were paraoxon, diethyl hydrogen phosphate, diethyl hydrogen phosphorothionate, and *p*-nitrophenol. Utilizing ^{3}H-labeled paraoxon, Kojima and O'Brien (1968) found that this compound was degraded by four distinct enzymatic pathways in soluble and particulate fractions of the liver cells. They reported that the soluble fraction produced *O*-desethylparaoxon. In another investigation, Fukami and Shishido (1966) demonstrated the major role of the soluble cell fraction in degrading methyl parathion. They showed that the supernatant fraction of tissue homogenates from rat liver and insect mid-gut cleaved methylparathion to desmethylparathion. Hollingworth (1970), using methylparaoxon labeled with ^{14}C in one methyl group, demonstrated that the addition of glutathione caused an increase in the degradation of methylparaoxon by the supernatant fraction from mice liver. He further stated that "it seems unlikely that microsomal oxidation plays a general role in detoxifying dimethyl esters in mouse liver." The enzymatic reduction of parathion was demonstrated with soluble, mitochondrial, and microsomal fractions from rat livers (Hitchcock and Murphy, 1967). Gaines *et al.* (1966) showed that parathion, after its "infusion into the hepatic portal system of rats, was more toxic than when infused by way of the femoral vein into the general circulation." The authors state that "parathion is converted to the highly toxic par-

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aoxon in the liver, but it is presumably in the liver also that it is eventually detoxified."

Most of the above mentioned *in vitro* studies were conducted with ^{32}P-, ^{35}S-, or ^{3}H-labeled insecticide. Studies with [2,6-ring-labeled ^{14}C]parathion (Lichtenstein and Fuhremann, 1971), however, showed that it was with the soluble fraction obtained at 150,000 × *g*/60 min from housefly abdomens that [^{14}C]parathion was reduced to aminoparathion, indicating the presence of a NADPH-dependent nitroreductase. Aminoparathion was the major metabolite detected, which over a prolonged incubation period degraded into water-soluble compounds.

In the present investigation [2,6-ring-^{14}C]parathion and [2,6-ring-^{14}C]paraoxon were incubated with rat liver cell fractions obtained from the same rat liver homogenate and also with subfractions from mitochondria and microsomes. In previous studies by other authors, experiments with parathion or paraoxon were conducted separately. The purity of the various cell fractions in our studies was confirmed by electron microscopy and by chemical means (Bloemendal *et al.*, 1967; Hochberg *et al.*, 1972b). These experiments with ring-labeled insecticides were intended to provide further information about the metabolism of parathion and paraoxon in the rat liver, in particular the breakdown of these compounds into organic solvent soluble compounds and into noninsecticidal water-soluble materials as tested by insect bioassay procedures. Also, the location of the enzyme(s) catalyzing these different reactions was investigated.

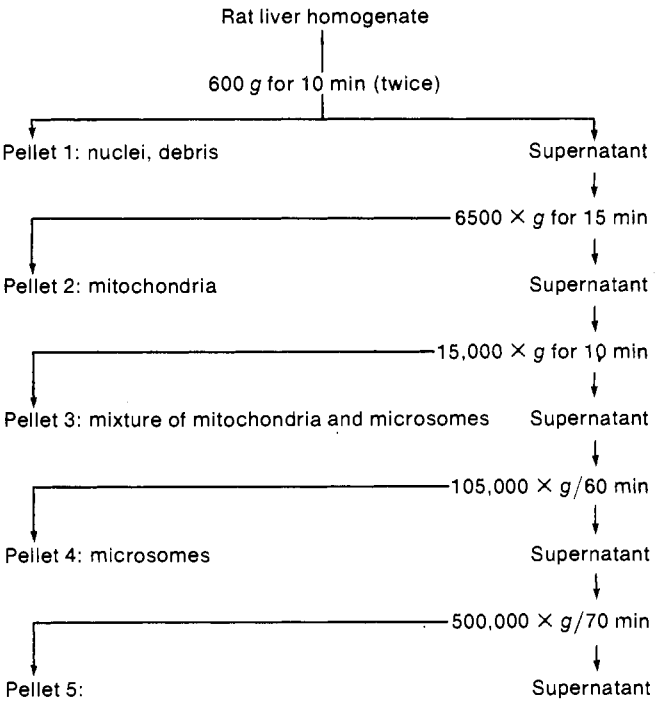

Figure 1. Preparation of subcellular fractions as enzyme source.

MATERIALS AND METHODS

Chemicals. [^{14}C]Parathion, labeled in the 2,6-ring position (International Chemical Nuclear Corporation, Irvine, Calif.) was diluted with nonradioactive parathion to give a specific activity of 0.58 mCi/mmol. [^{14}C]Paraoxon was prepared by oxidation of [^{14}C]parathion with bromine water. For this purpose, 5 mg of [^{14}C]parathion (9.86 μCi) was placed in a 15-ml, glass-stoppered test tube with 10 ml of distilled water and 5 drops of reagent grade bromine. The contents of the tube were shaken for 1 min. Nitrogen was bubbled through the solution until it became colorless. This solution was then extracted five times with 10-ml portions of chloroform, which were pooled and dried over anhydrous sodium sulfate. To verify the presence of [^{14}C]paraoxon, the chloroform solution was subjected to thin-layer chromatography (tlc) and autoradiography, as described below.

Isolates from the thin-layer plate that had the same R_f value as analytical grade paraoxon were eluted with acetone and analyzed by gas–liquid chromatography (glc) and liquid scintillation counting (lsc). Results by glc and lsc were in close agreement and indicated an 80% yield of [^{14}C]paraoxon.

Parathion, paraoxon, aminoparathion, and aminoparaoxon were obtained through the courtesy of Farbenfabriken-Bayer, Germany. NADPH was purchased from Nutritional Biochemical Co., Cleveland, Ohio. Solvents used were redistilled hexane and acetone, and absolute reagent grade ethanol, methanol, and diethyl ether.

The universal medium ("TKM") for homogenization of rat liver, resuspension of subcellular particles, and incubation of cell fractions with the insecticides consisted of 0.25 *M* sucrose in Tris(Cl) buffer (50 m*M*), KCl (25 m*M*), and $MgCl_2$ (10 m*M*), as described by Bloemendal *et al.* (1967) and modified by Hochberg *et al.* (1972a). The pH of TKM was adjusted to 7.5 with 6 *N* NaOH. This medium is suitable for obtaining metabolically active submicrosomal fractions. All chemicals used were of the highest purity available.

Preparation of Cell Fractions. Depending on the amount of liver needed, 8 to 12 female albino rats (Sprague–Dawley Corp., Madison, Wis.), 200–250 g each, were decapitated and their livers were homogenized in 3 vol of ice-cold TKM medium. The resulting homogenate was then fractionated by differential centrifugation, according to Figure 1. This procedure yielded relatively pure mitochondria and microsome pellets (Hochberg *et al.*, 1972b). Mitochondrial fractions are contaminated with microsomes if isolated with *g* forces greater than 6500 × *g* (Sottocasa *et al.*, 1967). We also found that pellet 3, obtained after a 10-min centrifugation at 15,000 × *g*, contained mitochondria and some microsomes. It was, therefore, not used for incubation with insecticides. Pellets were washed once and finally resuspended in minimal volume of TKM.

Preparation of Mitochondrial and Microsomal Subfractions. Subfractionation of mitochondria was according to methods described by Hochberg *et al.* (1972b). The procedure yielded three subfractions: inner membranes ("heavy" subfraction); outer membranes ("light" subfraction); and the mitochondrial soluble subfraction. Microsomes were subfractionated according to the method described by Hochberg *et al.* (1972a), yielding the rough endoplasmic reticulum (RER), the smooth endoplasmic reticulum (SER), and polyribosomes. Purity of the mitochondrial and microsomal subfractions was verified by marker enzyme (Hochberg *et al.*, 1972b) and by electron microscopy, respectively. RNA protein ratios for endoplasmic reticulum fractions were SER, 0.13 and RER, 0.44. The polysome E260/E280 ratio was 1.53. These determinations were comparable to those published by Bloemendal *et al.* (1967).

Protein of the cell fractions was determined according to the Biuret method (Layne, 1957) and that of subfractions from mitochondria and microsomes was determined by the method as described by Lowry *et al.* (1951).

Triplicate reaction mixtures were incubated in 10-ml Erlenmeyer flasks. The protein content of each reaction mixture was adjusted to identical concentrations, as noted in the footnotes to the tables. In all experiments, TKM medium was added to yield a final incubation volume of 2 ml, to which NADPH was added at 1.3×10^{-4} *M* and also 50 μg (0.1 μCi) of [^{14}C]parathion or 50 μg (0.1 μCi) of [^{14}C]paraoxon in 10 μl of ethanol. Controls were handled the same way, except that no cell material was added.

The Erlenmeyer flasks, covered with aluminum foil, were incubated at 37° in a Dubnoff shaker for 2 hr or less, as indicated. Reactions were terminated by adding 5 ml of acetone to each incubation mixture, followed by immediate extraction or freezing at −15° for future analyses.

The Experiments. Basically, three experimental series were conducted with rat liver cell fractions. 1. Cell fractions obtained from 8–12 rat livers, according to diagram 1, were incubated with [^{14}C]parathion or [^{14}C]paraoxon over a 2-hr period to determine the metabolism of the insecticides by the various cell fractions. 2. The 105,000 × *g* supernatant was incubated with [^{14}C]parathion for 15, 30, 60, and 120 min to determine the metabolism of this compound with time. 3. Microsomal and mitochondrial subfractions were incubated with [^{14}C]parathion or [^{14}C]paraoxon for a 2-hr period to determine the location of the insecticide metabolizing enzymes.

In each particular experimental series, all studies with [^{14}C]parathion or [^{14}C]paraoxon were conducted with cell fractions that had been isolated from the same pooled rat liver material. This was necessary to compare quantitative results obtained with either of the compounds.

Extraction and Analyses. For extraction, the contents of each incubation flask were quantitatively transferred with two 5-ml portions of water and 10 ml of acetone into a 60-ml separatory funnel. No acidification of the incubation mixture was performed. It was extracted with two 10-ml portions of hexane and with two 10-ml portions of diethyl ether, which was added to the hexane. Ninety-eight percent of the *p*-nitrophenol was partitioned into the hexane–ether phase. As shown below, aminoparathion also partitioned into the organic solvent extraction phase, while aminoparaoxon partitioned primarily and *p*-aminophenol partitioned totally into the water extraction phase. Traces of acetone were removed from the organic solvent phase with 15 ml of water containing 2% sodium sulfate to inhibit emulsion formation, thus finally resulting in a water–acetone phase and an organic solvent phase. Precipitated protein in the water phase was removed by filtering. After the extraction phases had been adjusted to volume, they were analyzed by liquid scintillation counting for their radiocarbon content. The organic solvent phases were also analyzed by gas–liquid chromatography, thin-layer chromatography, and autoradiography. In some cases, 10-ml aliquots of water extraction phases were bioassayed by placing 15 third-instar larvae of the mosquito *Aedes aegypti* (L.) into them to determine if materials toxic to these insects were present.

Liquid scintillation counting (lsc) was performed to determine the radiocarbon content of the various samples using a Packard model 3320 liquid scintillation spectrometer. One- or 2-ml aliquots of hexane–ether samples were placed in 14 ml of a solution consisting of 6 g of diphenyloxazole (PPO) and 0.25 g of 1,4-bis-2-(4-methyl-5-phenyloxasolyl)benzene (POPOP) in 1000 ml of toluene, while 1-ml aliquots of water phases were counted in 16 ml of a solution consisting of 12 g of PPO, 0.6 g of POPOP, 60 g of naphthalene, 200 ml of methyl cellosolve, and 1000 ml of dioxane. Data were corrected for background, counter efficiency (organic phase = 85%, aqueous phase = 65%), and dilutions, calculated as total disintegrations per minute (DPM) per extraction phase, and finally expressed as percent of applied radioactivity.

Gas–liquid chromatography (glc) was employed for the quantitative and qualitative analyses of parathion, paraoxon, and their potential degradation products in the organic solvent phase. Analyses for parathion and aminoparathion were accomplished with a Tracor Model 550 gas chromatograph, equipped with a flame photometric detector and a 5250 Å phosphorus filter. For the detection of parathion, the column (3% OV-1 on acid-washed dimethyl dichlorosilane-treated 80/100 Chromosorb W, 176 cm × 3 mm i.d., Pyrex glass) was kept at a temperature of 190° and a nitrogen carrier flow rate of 100 ml/min. Under these conditions, however, paraoxon and aminoparathion gave peaks with identical retention times. Therefore, a second column (10% DC 200 on 80/100 Gas Chrom Q, 176 cm × 3 mm, Pyrex glass), operated also at 190° and a nitrogen flow rate of 60 ml/min, was used for the analyses of aminoparathion, since under these conditions paraoxon did not interfere.

Analyses for paraoxon and *p*-nitrophenol were conducted with a Packard Model 7834 gas chromatograph, equipped with an electron affinity detector which does not respond to aminoparathion. The column (3% OV-1 on acid-washed dimethyl dichlorosilane-treated 80/100 Chromosorb W, 118 cm × 3 mm i.d., Pyrex glass) was maintained at 190° and a nitrogen flow rate of 100 ml/min for the detection of paraoxon. Analyses for *p*-nitrophenol were made possible by an on-column silanization of the compound to the less polar and more volatile trimethylsilyl ether, as described by Cranmer (1970). *p*-Nitrophenol was injected in a hexane solution containing 10% (v/v) of hexamethyldisilazane—on the same column used for paraoxon, except that the column temperature was maintained at 150°.

Colorimetric Analyses. The partitioning with the described extraction procedure of potential amino metabolites derived from the insecticides was also tested. To that effect aminoparathion, aminoparaoxon, or *p*-aminophenol was added to TKM, the medium used for homogenization of rat liver, and was extracted as described. The organic solvent phase was evaporated to dryness, followed by the addition of 2 ml of TKM, 10 ml of acetone, 10 ml of distilled water, and 15 ml of 2% Na_2SO_4. The water phase had the same composition. These extraction phases were then analyzed by the colorimetric method of Averell and Norris (1948), except that the reduction step was omitted, thus revealing the presence of previously reduced compounds. It was found that aminoparathion partitions into the organic solvent extraction phase, that it develops a purple color within 10 min after the addition of NED [*N*-(1-naphthyl)ethylenediamine dihydrochloride], and that the absorption maximum is at 556 mμ. Aminoparaoxon partitions primarily into the water extraction phase, but also develops a purple color within 10 min after the addition of NED with an absorption maxima at 556 mμ. Some color due to aminoparaoxon was also developed within the organic solvent extraction phase. *p*-Aminophenol partitions into the water and slowly develops an intense blue color within 12 hr after the addition of NED. This color has an absorption maximum of 582 mμ. Added amounts of parathion, paraoxon, *p*-nitrophenol, and NADPH did not yield any color with the described procedure.

Utilizing these color reactions, determinations of previously reduced compounds were made with the water extraction phase obtained from the 105,000 × *g* supernatant with which [^{14}C]parathion had been incubated and also with the water extraction phase from the soluble fraction to which no insecticide had been added. This was done to determine if compounds in this incubation mixture which were not derived from parathion would give color reactions with the described method.

Thin-layer chromatography (tlc) combined with autoradiography was employed to qualitatively and quantitatively evaluate the organic solvent extraction phases of the triplicate incubation mixtures. ^{14}C-Labeled metabolites were resolved by thin-layer chromatography. For this purpose 5-ml aliquots were taken from each of the three organic phases (25 ml each), pooled, concentrated, and spotted on silica gel, utilizing hexane–chloroform–methanol (7:2:1) as developing solvents. The metabolites were then detected by autoradiography. In addition, color reactions were utilized on selected samples by spraying the

Table I. Metabolism of [Ring-^{14}C]Parathion with Rat Liver Fractions[a]

	Recovered from rat liver cell fractions						
	None (control)	Homogenate	Mitochondria	15,000 × *g* supernatant	Microsomes	105,000 × *g* supernatant	500,000 × *g* supernatant
				^{14}C-Ring (in % of applied)			
Water-soluble	0.2 ± 0.0	1.93 ± 1.2[b]	0.73 ± 0.1	9.57 ± 0.2[b]	0.97 ± 0.3[c]	20.03 ± 1.5[b]	18.26 ± 0.3[b]
Hexane-soluble	106.6 ± 3.0	99.7 ± 1.1[b]	103.9 ± 0.5[c]	90.1 ± 1.8[b]	101.5 ± 1.5[c]	83.9 ± 1.5[b]	76.3 ± 1.1[b]
				Parathion			
Remaining, μg[d]	56.5 ± 0.7	51.8 ± 4.1	56.0 ± 0.9	27.7 ± 1.0[b]	49.8 ± 2.8[c]	35.7 ± 1.2[b]	33.0 ± 0.9[c]
% of control	100	91.8	99.1	49.0	88.2	63.1	69.0[e]
% degraded	0	8.2	0.9	51.0	11.8	36.9	31.0
				Metabolites (hexane-soluble, glc)			
Paraoxon, μg[d]	0	1.5	0.71	3.53	0.39	0.52	Trace
Aminoparathion, μg[d]	0			7.50		4.74	5.31
p-Nitrophenol, μg[d]	1.2 ± 0.3	1.2 ± 0.4	0.9 ± 0.3	1.4 ± 0.2	1.2 ± 0.2	0.2 ± 0.0	0.1 ± 0.03
				Tlc, ^{14}C, % of total[f]			
Parathion	97.8	91.1	96.1	67.1	94.1	85.5	85.7
Paraoxon	1.3	3.0	1.2	10.5	1.2	3.06	2.5
Aminoparathion				7.9		6.14	6.1
p-Nitrophenol	0.7	2.6	1.5	7.7	3.0	2.7	5.7
Aminoparaoxon	0.0	2.4	0.6	2.0	0.7	1.0	
p-Aminophenol	0.0	0.7	0.3	3.9	0.1	1.4	

[a] Triplicate reactions containing 46 ± 1 mg of protein for each cell fraction were incubated for 2 hr at 37°, as described in Materials and Methods section. Values reported for % of applied and μg of parathion or *p*-nitrophenol recovered by glc are means ± standard deviations. [b,c] Results are significantly different from the control at the 1% (b) or the 5% level (c). [d] μg recovered by gas–liquid chromatography (glc) per 2 ml of incubation mixture. [e] Compared to a different control in which 47.8 μg of parathion remained. [f] ^{14}C recovered from silica gel areas of thin-layer plate with R_f values identical to cochromatographed analytical grade material. Expressed in % of total radiocarbon recovered with a particular subcellular fraction.

thin-layer plates successively with the reagents of Averell and Norris (1948), except that the reduction step was eliminated. In this way the presence of previously reduced compounds was indicated by a positive color test. Additional spraying of the plates with a palladium chloride solution, followed by spraying with 5 *N* NaOH, resulted in spots of different colors for parathion, paraoxon, aminoparathion, *p*-nitrophenol, and *p*-aminophenol. To determine the radiocarbon content of isolated compounds, silica gel areas which had the same R_f value as cochromatographed analytical grade chemicals were scraped from the plates into 10 ml of methanol and extracted by vigorous shaking over an 18-hr period. Duplicate 2-ml aliquots of these extracts were placed into 14 ml of a toluene-based counting solution to determine their radiocarbon content by lsc.

Data obtained from triplicated samples were statistically analyzed by the *t* test and expressed in percent of applied radioactivity or in percent of applied micrograms of parathion or micrograms of paraoxon.

RESULTS AND DISCUSSION

Degradation of [^{14}C]Parathion with Rat Liver Cell Fractions. Data obtained with [ring-^{14}C]parathion incubated with rat liver cell fractions over a 2-hr period are summarized in Table I. Based on the radiocarbon content of the extraction phases, data indicate that it was primarily with the soluble fractions obtained at 105,000 × *g* and 500,000 × *g* that degradation of parathion had occurred. Eighteen to 20% of the applied radiocarbon was recovered from the water extraction phase, which indicated the presence of water-soluble compounds derived from ring-labeled parathion. All of the applied radiocarbon was accounted for, since 76 to 84% of the radiocarbon applied originally to the soluble cell fractions was associated with the hexane extraction phase. Utilizing the above mentioned color reactions, addition of NED to the water extraction phase from specially [^{14}C]parathion-treated incubation mixtures (originally containing a total of 560 mg of protein in 12 incubation flasks) resulted, within 10 min, in the development of a purple color, which yielded a clearly defined absorption peak at 556 mμ (optical density 0.568). Controls (incubation mixtures without [^{14}C]parathion and an original protein content of 592 mg) developed a similar but less intense pink color. However, this color did not yield a definite absorption peak but gave a flat curve with a maximum optical density of 0.160. Since aminoparathion partitions into the organic solvent extraction phase and *p*-aminophenol partitions into the water phase, where it yields a blue color with an absorption maximum of 582 mμ, it appears that the compound in the water extraction phase from [^{14}C]parathion-treated incubation mixtures was aminoparaoxon. Since both paraoxon and aminoparathion were detected in the organic solvent phase (Table I), aminoparaoxon could have been formed from these compounds. Further studies are underway to identify the water-soluble radioactivity obtained with the soluble fraction.

The water extraction phase obtained from incubation mixtures containing the 105,000 × *g* supernatant was also bioassayed with mosquito larvae. For this purpose, 15 third-instar larvae each were placed into 3 × 10 ml aliquots from each of the water extraction phases, which had been obtained from triplicated samples. The same was done with controls which also had been incubated with [^{14}C]parathion, but without the 105,000 × *g* supernatant. No mortality occurred in any of these tests over a 24-hr exposure period. Addition of 5 μg of parathion (0.5 ppm) at that time to one of the replicates resulted in a 65 ± 4% mortality of the insect larvae within one additional hour, while in the other test tubes all larvae remained alive. This then indicated that the ^{14}C-containing compounds in the water extraction phases were nontoxic to these insects. The amount of water-soluble ^{14}C compounds produced, amounting to 20% of the applied dosage in the 105,000 × *g* supernatant, can therefore serve as an indicator of detoxication of the originally applied [^{14}C]parathion by rat liver cell fractions.

Analyses of the organic solvent phase by glc for remaining parathion also showed that the major degradation of this insecticide had occurred with the soluble cell frac-

Table II. Metabolism of [Ring-^{14}C]Parathion with Subfractions of Mitochondria and Microsomes from Rat Liver[a]

	Recovered from rat liver cell subfractions								
			Mitochondrial				Endoplasmic reticulum		
	None (control)	Mitochondria	Outer membrane	Inner membrane	Soluble	Microsomes	Rough	Smooth	Polyribosomes
				^{14}C-Ring (in % of applied)					
Water-soluble	0.15 ± 0.2	0.97 ± 0.2[b]	0.37 ± 0.1[b]	0.37 ± 0.1[b]	0.53 ± 0.4	0.80 ± 0.0[b]	0.63 ± 0.1[b]	0.80 ± 0.1[b]	0.77 ± 0.1[b]
Specific activity[e]	0	*0.02*	*0.04*	*0.05*	*0.07*	*0.02*	*0.19*	*0.26*	*0.25*
Hexane-soluble	101.3 ± 0.7	98.6 ± 0.6[b]	100.5 ± 0.6	100.3 ± 1.0[c]	99.8 ± 0.7[c]	99.1 ± 0.4[b]	98.4 ± 1.2[c]	100.9 ± 0.5	100.1 ± 0.4[c]
Specific activity[f]	0	*0.07*	*0.15*	*0.22*	*0.27*	*0.06*	*1.16*	*0.12*	*0.48*
				Parathion (glc)					
Remaining, μg[g]	50.7 ± 0.4	49.9 ± 2.7	51.9 ± 0.2	46.8 ± 3.9	48.9 ± 1.7	48.8 ± 2.0	51.0 ± 4.4	51.6 ± 2.5	47.4 ± 2.0[d]
% of control	100	98.4	102.4	92.3	96.5	96.3	100.6	101.6	93.5
% degraded	0	1.6	0	7.7	3.5	3.7	0	0	6.5
Specific activity[f]	0	*0.04*	0	*1.67*	*0.64*	*0.10*	0	0	*2.62*
Metabolites		Not analyzed							
				tlc, ^{14}C, % of total[h]					
Parathion	97.0	95.7	96.6	96.7	96.3	96.8	95.9	94.4	94.4
Paraoxon	2.6	0.8	2.0	2.4	3.1	0.2	1.8	2.9	3.4
Aminoparathion	0	0.3	0	0	0	0.2	0.1	0	0
p-Nitrophenol	0.4	2.8	1.3	0.8	0.6	2.6	2.0	2.3	2.2
Aminoparaoxon *p*-Aminophenol	0.1	0.4	0.1	0.1	0	0.2	0.2	0.4	0

[a] Conditions identical to Table I, except that reaction mixtures with mitochondria or microsomes contained 39 mg of protein, with mitochondrial subfractions 5.1 mg of protein and with microsomal subfractions 2.5 mg of protein. [b,c,d] Results are significantly different from the control at the (b) 1%, (c) 5%, and (d) 10% level. [e] ^{14}C recovered per mg of protein in % of applied from the water extraction phase, relative to the control. [f] ^{14}C lost per mg of protein from the hexane extraction phase relative to controls or parathion degraded per mg of protein. [g] μg of parathion recovered by gas–liquid chromatography per 2 ml of incubation mixture. [h] ^{14}C recovered from silica gel areas of thin-layer plates with R_f values identical to analytical grade materials. Expressed in % of total radiocarbon recovered with a particular cell fraction.

Table III. Metabolism of [Ring-^{14}C]Paraoxon with Subfractions of Mitochondria and Microsomes from Rat Liver[a]

	Recovered from rat liver cell subfractions								
			Mitochondrial				Endoplasmic reticulum		
	None (control)	Mitochondria	Outer membrane	Inner membrane	Soluble	Microsomes	Rough	Smooth	Polyribosomes
				^{14}C-Ring (in % of applied)					
Water-soluble	2.8 ± 0.3	12.7 ± 1.5[b]	2.8 ± 0.2	3.2 ± 0.1	3.1 ± 0.1	24.9 ± 3.2[b]	6.0 ± 0.4[b]	5.5 ± 0.4[b]	5.0 ± 0.3[b]
Specific activity[d]	0	*0.27*	0	*0.09*	*0.06*	*0.58*	*1.28*	*1.08*	*0.89*
Hexane-soluble	102.0 ± 0.1	83.5 ± 0.6[b]	99.2 ± 0.3[b]	98.2 ± 1.5[c]	99.0 ± 1.1[c]	71.5 ± 3.9[b]	96.2 ± 1.3[c]	94.7 ± 1.7[b]	97.2 ± 1.7[c]
Specific activity[e]	0	*0.45*	*0.51*	*0.80*	*0.52*	*0.78*	*2.28*	*2.88*	*1.89*

	Paraoxon (glc)							
Remaining, μg^f	49.0 ± 2.8	10.9 ± 0.8[b]	49.6 ± 1.4	49.6 ± 1.1	49.5 ± 0.7	4.6 ± 1.0[b]	48.4 ± 0.8	48.3 ± 0.7	49.6 ± 0.5
% of control	100	22.2	101.2	101.2	101.0	9.4	98.8	98.7	101.2
% degraded	0	77.8	0	0	0	90.6	1.2	1.3	0
Specific activity[e]	0	*1.95*	0	0	0	*2.37*	*0.48*	*0.52*	0
	Metabolites (hexane-soluble)								
p-Nitrophenol, μg^f	0.55 ± 0.21	16.7 ± 0.9[b]	1.97 ± 0.05[b]	1.0 ± 0.08[b]	0.77 ± 0.05[b]	28.0 ± 1.6[b]	1.43 ± 0.09[b]	1.37 ± 0.12[b]	0.93 ± 0.12[c]
Specific activity[g]	0	*0.42*	*0.27*	*0.10*	*0.04*	*0.72*	*0.35*	*0.33*	*0.15*
	Tlc, ^{14}C, % of total[h]								
Paraoxon	97.9	26.7	94.2	97.7	98.2	19.6	93.6	93.8	96.1
"Aminoparathion" area	0.2	0.5	0.1	0.0	0.3	2.7	0.1	0.1	0.1
p-Nitrophenol	1.3	71.5	5.6	2.3	1.5	77.1	6.3	5.5	3.8
Aminoparaoxon	} 0.6	1.3	0.1	0.0	0.0	0.6	0.0	0.6	0.0
p-Aminophenol									

[a] Conditions identical to Table II. [b,c] Results are significantly different from the control at the (b) 1% and (c) 5% level. [d] ^{14}C recovered per mg of protein in % of applied from the water extraction phase, relative to the control. [e] ^{14}C lost per mg of protein from the hexane extraction phase relative to controls or paraoxon degraded per mg of protein. [f] μg of paraoxon or μg of *p*-nitrophenol recovered by gas–liquid chromatography per 2 ml of incubation mixture. [g] μg of *p*-nitrophenol produced per mg of protein, relative to controls. [h] ^{14}C recovered from silica gel areas of thin-layer plates with R_f values identical to analytical grade materials. Expressed in % of total radiocarbon recovered with a particular cell fraction.

tions (31 to 37% of applied) and some with microsomes (12%). The highest rate of degradation (51%) was noticed with the 15,000 × *g* supernatant. Since the radiocarbon content of the organic solvent phase of these fractions was higher than the amount of parathion recovered by glc, it appeared likely that organic solvent soluble, ring-labeled ^{14}C metabolites had been produced during a 2-hr incubation period. This indeed was demonstrated by further analyses by glc and tlc of the organic solvent phase, indicating that aminoparathion was the primary metabolite produced by the soluble cell fractions, followed by paraoxon. Only small amounts of *p*-nitrophenol were detected by glc. Color reactions on thin layer plates and autoradiography, together with determinations of the ^{14}C content of isolated areas, also confirmed these findings.

It appears, therefore, that parathion was reduced to aminoparathion, primarily by the soluble cell fractions. At the same time, the insecticide had also been oxidized to paraoxon. Based on colorimetric data, it would appear that aminoparaoxon was formed, which then appeared primarily in the water extraction phase. Based on tlc data, some *p*-nitrophenol was formed, accounting for 3 and 2.7% of the total radiocarbon recovered from thin-layer plates with microsomes or the 105,000 × *g* supernatant, respectively. However, *p*-nitrophenol was one of the major metabolites of paraoxon (as shown in Table IV). The homogenate was relatively inactive in degrading [^{14}C]parathion.

Degradation of [^{14}C]Parathion with Time. To determine the rate of degradation of [^{14}C]parathion, the insecticide was incubated as described with the rat liver 105,000 × *g* supernatant (45.5 mg of protein per incubation mixture) in each of 12 incubation flasks. The reaction in each of three mixtures was terminated after 15, 30, 60, or 120 min. Three control flasks without the rat liver fraction were incubated for 2 hr. Analytical results are presented in Figure 2. Based on lsc of the water and organic solvent extraction phases, a total of 95% of the applied radiocarbon was recovered from both phases at each time interval. However, a steady decrease in the radiocarbon content of the organic solvent phase had occurred, while a steady increase in the production of ^{14}C water-soluble metabolites had taken place. After analysis of the organic solvent phase by glc for parathion, results were also expressed in percent **of the** applied dosage of 50 μg per incubation mixture (dotted line, Figure 2). The total radiocarbon content in the hexane phase after a 2-hr incubation period was only slightly higher (68.3 ± 1.23% of applied) than the amount of parathion recovered (63.9 ± 0.75% of applied), indicating that only small amounts of organic solvent soluble metabolites had been produced. The primary organic solvent soluble metabolite was aminoparathion (3.45 ± 0.15% of applied parathion), since only trace amounts of paraoxon and *p*-nitrophenol could be detected. The major metabolites produced with the 105,000 × *g* supernatant, however, were water-soluble compounds derived from ^{14}C-ring-labeled parathion which, based on colorimetric tests, appeared to be aminoparaoxon. This water extraction phase was not insecticidal.

In controls, 95.8% of the recovered radiocarbon was associated with the organic solvent phase and only 0.4% with the water phase; 96.6% of the applied parathion was still present after 2 hr of incubation.

Degradation of [^{14}C]Parathion with Mitochondrial and Microsomal Subfractions. As shown previously (Table I), microsomes were more active in degrading parathion than were mitochondria. With microsomes, some indication of the formation of water-soluble metabolites had been evidenced, and although relatively small, a degradation of parathion (11.8% of the applied dosage) was noticed. Results obtained after incubation of [^{14}C]para-

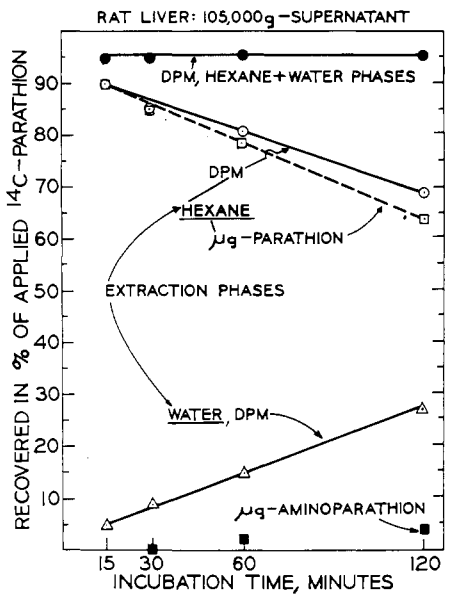

Figure 2. Metabolism of [^{14}C]parathion during a 2-hr incubation at 37° with the 105,000 × *g* supernatant from rat livers.

thion with subfractions from mitochondria and microsomes are summarized in Table II. Based on the amount of parathion remaining after incubation with the various subfractions, some significant degradation of the insecticide had occurred only with polyribosomes. Based on the production of water-soluble ^{14}C metabolites, however, all three microsomal subfractions had specific activities which were significantly higher ($p < 0.01$) than the one observed with intact microsomes. Analyses of the organic solvent extraction phases by tlc indicated that in all cases approximately 95% of the recovered radiocarbon was associated with areas that had R_f values identical with that of the cochromatographed analytical grade [^{14}C]parathion. This indicated that a limited metabolism of the insecticide had occurred with these fractions. Radioactivity attributable to paraoxon was found primarily with the polyribosome subfraction and that attributable to *p*-nitrophenol was found primarily with the mitochondria and microsome fractions.

Degradation of [^{14}C]Paraoxon with Rat Liver Cell Fractions. Data obtained with [^{14}C]paraoxon as the substrate indicated that this compound was more readily degradable than was parathion. In addition, various enzyme systems were responsible for its metabolism (Table IV). Our results show that the quantity of water-soluble ^{14}C compounds (58 to 65% of applied) formed from [^{14}C]paraoxon with the soluble cell fractions was considerably higher than the amounts formed from [^{14}C]parathion (Table I). Contrary to the findings with parathion, both mitochondria and microsomes also produced significant amounts (15–18% of applied) of water-soluble ^{14}C compounds which were derived from ^{14}C-ring-labeled paraoxon. Bioassay tests as previously described were also conducted with mosquito larvae that were exposed to water extraction phases obtained from incubation mixtures containing the 105,000 × *g* supernatant and [^{14}C]paraoxon. Although 65% of the originally applied radiocarbon was associated with this extraction phase, no insect mortalities were observed during a 24-hr exposure period to this "water." Addition of 5 μg of paraoxon (0.5 ppm) to one replicate each after the 24-hr exposure resulted in insect mortalities, but not as rapid as with parathion. This latter finding was surprising and could possibly have been associated with the relatively high water solubility of paraoxon (2400 ppm) as opposed to parathion (24 ppm). This could have made paraoxon less available, due to its reduced lipid solubility. The pH of these water samples ranged from 5.7 to 7.02. Based on these tests paraoxon had been metabolized, yielding noninsecticidal, water-soluble substances.

Most of the ^{14}C compounds that remained in the organic solvent extraction phase (80% with the particulate fraction and 30% with the soluble fraction) were metabolites of paraoxon. This was indicated by glc analyses, which showed that 92 to 95% of the applied paraoxon had been degraded by mitochondria and microsomes, and 75 to 85% was degraded by the soluble cell fractions. Further analyses showed that with the particulate fraction, *p*-nitrophenol was the primary metabolite recovered from the organic solvent phase. Since 24 μg of *p*-nitrophenol was recovered per incubation mixture, it accounted for nearly all of the applied (50 μg) paraoxon. This was also demonstrated by tlc and autoradiography (Table IV), indicating that 88% of the recovered radiocarbon was in an area which

Table IV. Metabolism of [Ring-^{14}C]Paraoxon with Rat Liver Fractions[a]

	Recovered from rat liver cell fractions						
	None (control)	Homogenate	Mitochondria	15,000 × *g* supernatant	Microsomes	105,000 × *g* supernatant	500,000 × *g* supernatant
				^{14}C-Ring (in % of applied)			
Water-soluble	2.9 ± 0.2	19.8 ± 0.9[b]	17.6 ± 0.8[b]	51.5 ± 2.8[b]	15.2 ± 0.6[b]	64.7 ± 2.9[b]	57.9 ± 1.2[b]
Hexane-soluble	102.3 ± 0.6	73.1 ± 1.0[b]	78.7 ± 1.4[b]	42.3 ± 1.7[b]	81.7 ± 1.2[b]	31.9 ± 0.7[b]	28.8 ± 2.3[b]
				Paraoxon			
Remaining, μg[c]	38.8 ± 0.0	14.8 ± 0.9[b]	3.3 ± 0.3[b]	5.3 ± 0.3[b]	1.9 ± 0.4[b]	5.9 ± 0.8[b]	12.4[a] ± 0.8[b]
% of control	100	38.2	8.5	13.7	5.0	15.2	24.4
% degraded		61.8	91.5	86.3	95.0	84.8	75.6
				Metabolites (hexane-soluble)			
p-Nitrophenol, μg[c]	1.7 ± 0.4	8.8 ± 0.3[b]	24.0 ± 1.3[b]	2.8 ± 0.8	24.0 ± 2.0[b]	1.9 ± 0.3	0.57 ± 0.03
				Tlc, ^{14}C, % of total[e]			
Paraoxon	96.7	61.0	7.9	50.5	8.6	72.7	88.3
"Aminoparathion" area	0.2	0.8	0.8	2.4	0.4	1.6	0.9
p-Nitrophenol	2.9	35.3	88.1	24.7	88.3	15.5	9.9
Aminoparaoxon	0.0	0.6	1.9	9.7	1.8	7.6	0.5
p-Aminophenol	0.0	2.1	1.0	12.6	0.6	2.4	0.4

[a] Conditions identical to Table I. [b] Results are significantly different from the control at the 1% level. [c] μg recovered per 2 ml of incubation mixture. [d] Compared to a different control in which 50.9 μg of paraoxon remained. [e] ^{14}C recovered from each area of the thin-layer plate with R_f values identical to analytical grade materials. Expressed in % of total radiocarbon recovered with a particular subcellular fraction.

Table V. Relative Activities of Subcellular Fractions from Rat Liver in Degrading [^{14}C]Parathion or [^{14}C]Paraoxon

	Relative activities [a]					
	Homogenate	Mitochondria	15,000 × *g* supernatant	Microsomes	105,000 × *g* supernatant	500,000 × *g* supernatant
H_2O-soluble ^{14}C metabolites produced from						
Parathion	0.04	0.02	0.20	0.02	0.43	0.40
Paraoxon	0.36	0.32	1.04	0.27	1.33	1.20
Degraded						
Lsc: parathion	0.18	0.09	0.36	0.14	0.49	0.46
paraoxon	0.61	0.50	1.25	0.44	1.48	1.56
Glc: parathion	0.18	0.02	1.09	0.26	0.79	0.46
paraoxon	1.32	1.96	1.84	2.06	1.82	1.67

[a] H_2O-soluble metabolites produced: ^{14}C recovered in % of applied from the water extraction phases (relative to controls) per mg of protein, after 2 hr of incubation at 37°. Degraded, lsc: ^{14}C lost from the hexane extraction phase per mg of protein relative to controls, after 2 hr of incubation at 37°. Glc: μg of parathion or paraoxon lost per mg of protein relative to controls after 2 hr of incubation at 37°.

had an R_f value identical with that of cochromatographed analytical grade *p*-nitrophenol. With mitochondria and microsomes, paraoxon was hydrolyzed, yielding *p*-nitrophenol and presumably diethylphosphoric acid. No analyses were conducted for the latter compound.

With soluble rat liver fractions, paraoxon had also been degraded but apparently by different enzymes. First of all, considerably more water-soluble ^{14}C compounds were formed than were with the particulate cell fractions, yet only 76 to 85% of the applied paraoxon had been degraded, in comparison to 92 to 95% with mitochondria and microsomes. Only small amounts of *p*-nitrophenol had been produced by the soluble fractions (glc analyses) and some indication of its presence was shown by tlc and autoradiography. Based on this latter method, weak spots appeared close to the origin with R_f values similar to aminoparaoxon (R_f 0.07) and *p*-aminophenol (R_f 0.00).

It appears, therefore, that paraoxon was hydrolyzed by mitochondrial and microsomal enzymes, yielding *p*-nitrophenol in the organic solvent phase. But it was also degraded by soluble enzymes which produced the largest amounts of water-soluble noninsecticidal ^{14}C compounds derived from [ring-^{14}C]paraoxon. These enzymes in the soluble liver fraction also appeared to have caused a reduction of paraoxon to aminoparaoxon.

Degradation of Paraoxon with Mitochondrial and Microsomal Subfractions. Results obtained after incubation of [^{14}C]paraoxon with mitochondrial and microsomal subfractions are presented in Table III. For proper evaluation of these data, it is important to realize that incubation mixtures with intact mitochondria or microsomes contained 39 mg of protein each, while the mitochondrial subfractions contained only 5.5 mg of protein and the microsomal subfractions contained 2.5 mg of protein each.

With intact mitochondria and microsomes, appreciable amounts of ^{14}C water-soluble compounds had been produced from [^{14}C]paraoxon, yet none were detected with any of the three mitochondrial subfractions. Conversely, all three microsomal subfractions had produced water-soluble ^{14}C metabolites, although these incubation mixtures contained the least amount of protein. Based on glc analyses of the organic solvent extraction phases, 90.6% of the applied paraoxon was degraded with intact microsomes and 77.8% with intact mitochondria. However, no significant degradation of paraoxon had occurred with any of the particulate subfractions. The amounts of *p*-nitrophenol produced by all cell fractions were all significantly higher than the amounts found in the controls. Specific activities (micrograms of *p*-nitrophenol produced per milligrams of protein) of intact mitochondria and microsomes were higher than those determined with the particulate subfractions, and polyribosomes were less active than RER and SER.

Comparison between Parathion and Paraoxon-Degrading Enzyme Activities. By expressing relative activities per milligrams of protein for each cell fraction, a comparison of parathion and paraoxon-degrading enzymes was made (Table V). It is evident that with the soluble cell fractions, the activities of paraoxon-degrading enzymes were about three times higher than those of parathion-degrading enzymes, while these differences were even larger with enzymes from the particulate cell fractions. In general, highest specific activities were observed with the soluble cell fractions. Only with paraoxon, based on the amount degraded as established by glc, was the highest specific activity encountered with mitochondria (1.96) and microsomes (2.06).

In conclusion, it becomes evident that different enzymes in the rat liver catalyze the degradation and detoxification of parathion and paraoxon. Parathion is degraded to water-soluble metabolites, primarily by the soluble fractions and to a lesser extent by microsomes. These results do not entirely support those of Neal (1967) and Nakatsugawa *et al.* (1969). In addition, enzyme activities in the soluble fraction of the rat liver resulted in a reduction of parathion to aminoparathion, but also in some oxidation to paraoxon, and possibly in a formation of aminoparaoxon. Microsomes showed some parathion-degrading activity. Paraoxon was mostly degraded by particulate-associated enzymes through hydrolysis, yielding *p*-nitrophenol. However, the largest amounts of water-soluble metabolites which were nontoxic to mosquito larvae were also produced by the soluble fractions. These fractions, in addition, reduced paraoxon to aminoparaoxon.

Water-soluble metabolites produced by rat livers from [^{14}C]parathion or [^{14}C]paraoxon are currently being further investigated.

ACKNOWLEDGMENT

The authors are indebted to B. N. Anderegg and T. T. Liang for their technical assistance.

LITERATURE CITED

Averell, P. R., Norris, M. V., *Anal. Chem.* **20,** 753 (1948).
Bloemendal, H., Bont, W. S., deVries, M., Benedetti, E. L., *Biochem. J.* **103,** 177 (1967).
Cranmer, M., *Bull. Environ. Contam. Toxicol.* **5,** 329 (1970).
Fukami, J., Shishido, T., *J. Econ. Entomol.* **59,** 1338 (1966).
Gaines, T. B., Meyer, Jr., W. J., Linder, R. E., *Nature (London)* **209,** 88 (1966).
Hitchcock, M., Murphy, S. D., *Biochem. Pharmacol.* **16,** 1801 (1967).
Hochberg, A. A., Stratman, F. W., Zahlten, R. N., Morris, H. P., Lardy, H. A., *Biochem. J.* **130,** 19 (1972a).
Hochberg, A. A., Zahlten, R. N., Stratman, F. W., Lardy, H. A., *Biochem. J.* **131,** 3143 (1972b).
Hollingworth, R. M., "Biochemistry and Toxicology of Insecticides," Academic Press, New York, N. Y., 1970, pp 75–88.
Kojima, K., O'Brien, R. D., *J. Agr. Food Chem.* **16,** 574 (1968).
Layne, E., *Methods Enzymol.* **3,** 540 (1957).
Lichtenstein, E. P., Fuhremann, T. W., *Science* **172,** 589 (1971).

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Nakatsugawa, T., Tolman, N. M., Dahm, P. A., *Biochem. Pharmacol.* **18**, 1103 (1969).
Neal, R. A., *Biochem. J.* **103**, 183 (1967).
Sottocasa, G. L., Kuylenstierna, B., Ernster, L., Bergstrand, A., *Methods Enzymol.* **10**, 448 (1967).

Received for review July 24, 1972. Accepted February 2, 1973. Research was supported in part by grants from the National Institutes of Health (AM 10334 and HD 51129) and U.W. Hatch Project 1387. Contribution from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Cooperative Research Project 96 entitled "Environmental Implications of Pesticide Usage."

Metabolism of Hexachlorocyclohexane to Chlorophenols and Effect of Isomer Pretreatment on Lindane Metabolism in Rat

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The role of γ-2,3,4,5,6-pentachlorocyclohex-1-ene (γ-PCCH) in the metabolism of lindane (γ-HCH) and the effect of pretreatment with various HCH isomers on lindane metabolism was evaluated in this study. Four groups of rats were treated perorally daily with one of four configurational isomers of hexachlorocyclohexane (α-, β-, γ-, or δ-HCH). One group of animals received γ-PCCH. After 1 week of pretreatment, all animals received an oral dose of γ-HCH. The daily urinary excretion of free chlorophenols was analyzed by glc. While β-HCH was metabolized to 2,4,6-trichlorophenol and γ-PCCH was metabolized to 2,4,5-trichlorophenol, the α- and δ-HCH were metabolized to both 2,4,5-, and 2,4,6-trichlorophenol. Rats treated with lindane, however, excreted 2,4,6-, 2,3,5-, and 2,4,5-trichlorophenol, 2,3,4,6- and 2,3,4,5-tetrachlorophenol, and 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol. If γ-PCCH is an intermediate, it is a minor one, and more efficient pathways are involved in the metabolism of lindane. This study also indicates that pretreatment with the isomers of HCH alters the metabolism of lindane in rats.

Grover and Sims (1965) reported the urinary excretion of the metabolites 2,4,5- and 2,3,5-trichlorophenol (2,4,5- and 2,3,5-TCP) in rats treated with γ-hexachlorocyclohexane (γ-HCH, lindane). They also administered γ-2,3,4,5,6-pentachlorocyclohex-1-ene (γ-PCCH) intraperitoneally and again detected these two trichlorophenols. Because this metabolic pattern resembled the degradation of 1,2,4-trichlorobenzene in rabbits, these investigators speculated that the metabolism of lindane in rats proceeds *via* dehydrochlorination through γ-PCCH to 1,2,4-trichlorobenzene and then to the chlorophenols. However, it has recently been reported that in addition to the chlorophenols identified by Grover and Sims, rats pretreated with lindane also excreted 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,5- and 2,3,4,6-tetrachlorophenol (2,3,4,5- and 2,3,4,6-TTCP), and 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol (PCCOL) (Chadwick and Freal, 1972a). These newly identified lindane metabolites are all excreted in greater quantities than either 2,3,5- or 2,4,5-trichlorophenol. If lindane is metabolized exclusively through γ-PCCH, then administration of this intermediate to rats should also result in the excretion of all the known lindane metabolites. However, Reed and Forgash (1968) have reported finding a second PCCH isomer from the exposure of houseflies to lindane. This isomer, referred to as iso-PCCH, is metabolized to a greater extent than γ-PCCH in the fly. Therefore, it is reasonable to assume that lindane metabolism may proceed through more than one intermediate. Furthermore, the metabolite excretion patterns resulting from the biodegradation of other hexachlorocyclohexane isomers might yield information on the type of pentachlorocyclohexene intermediates involved. Finally, pretreatment with other isomers of hexachlorocyclohexane could significantly alter the metabolism of a single oral dose of lindane.

The present study was undertaken to determine the type and quantity of metabolites excreted by rats treated with γ-PCCH and α-, β-, γ-, and δ-HCH. In addition, after 1 week of pretreatment, lindane was administered to all rats and the urine samples were analyzed to determine the comparative excretion of lindane-derived metabolites.

APPARATUS

The gas chromatograph employed was a Micro-Tek 220 using a Coulson electrolytic conductivity detector operated in the oxidative mode. A 6 ft × ¼ in. U-tube glass column containing 5% DEGS on 80/100 mesh Gas Chrom Q and isothermally maintained at 165° was used in the analysis. The temperatures of the combustion furnace, the transfer valves, and the inlet were 800, 225, and 200°, respectively. The nitrogen carrier gas flow was regulated at 90 ml/min.

REAGENTS

The isomers of HCH were obtained from the Primate and Pesticide Effects Laboratory Repository and were 99+% pure. The γ-PCCH was obtained from 98+% pure lindane, with mild NaOH dehydrochlorination (Nakajima *et al.*, 1949) and purified using a silicic acid column (Reed and Forgash, 1970). Technical grade chlorophenol standards (Aldrich Chemical Company) were purified by recrystallization.

PROCEDURE

Weanling female Sprague–Dawley rats were maintained on a purified vitamin A test diet (Nutritional Biochemicals Corporation, Cleveland, Ohio). The rats were kept in individual metabolism cages so that urine and feces could

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