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Comparative Metabolism of 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane and Toxaphene in Six Mammalian Species and Chickens

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The feces of mice, rats, hamsters, guinea pigs, rabbits, monkeys, and chickens orally administered 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane contain the corresponding hexachlorobornane isomers and hexachlorobornene formed by reductive dechlorination and dehydrochlorination, respectively, at the *gem*-dichloro group. Yields of the two reductive dechlorination products are 20% in rabbits and monkeys and 3–9% in the other species and yields of the hexachlorobornene are 0.2–1.5% in each case. Toxaphene, as with the heptachlorobornane, is metabolized least extensively by chickens and most by monkeys. Most of the 29 glass capillary column gas chromatographic peaks characteristic of toxaphene are evident in the fat of all the treated animals and in the feces except for monkeys. The feces of toxaphene-treated monkeys contains the three metabolites of the heptachlorobornane discussed above as established by glass capillary column gas chromatographic comparison with authentic standards by cochromatography and electron impact mass spectrometry. Toxaphene-derived products in the liver 3 days after treatment vary with the species and in the most part are not toxaphene components.

In comparison with most pesticides, relatively little is known about the identity of the metabolites of toxaphene, a major chlorinated insecticide. This is not surprising since toxaphene is a complex mixture of related chlorinated terpenes, probably hepta-, octa-, and nonachlorobornanes in the most part, with no component making up more than 5% of the total (Saleh and Casida, 1977, 1978, 1979; Saleh et al., 1977). Some components of toxaphene are metabolized more rapidly than others, based on analyses of the fat, liver, and feces of treated rats, and new chlorinated derivatives are formed on metabolism (Saleh and Casida, 1978), a major complication in recognizing toxaphene residues in treated animals. By examining one major toxaphene component as a model, it is established that heptachlorobornane I undergoes reductive dechlorination and dehydrochlorination in rats, forming hexachlorobornanes II and III and hexachlorobornene IV, respectively (Figure 1); metabolites II–IV are less toxic than heptachlorobornane I to houseflies and goldfish (Saleh and Casida, 1978; Saleh et al., 1977).

The present investigation compares six mammalian species and chickens relative to their fecal metabolites of heptachlorobornane I and their toxaphene-derived products in fat, feces, and liver at 72 h after oral treatment. Particular attention is given to identification of three metabolites in the feces of monkeys treated with heptachlorobornane I or toxaphene.

MATERIALS AND METHODS

Chemicals. The toxaphene sample used was Lot. No. X16189-49 produced by Hercules Inc. (Wilmington, DE). Compounds I–IV were obtained as previously described (Saleh and Casida, 1978).

Treatment of Mammals and Chickens. The following test animals were treated orally with either toxaphene (~13 mg/kg) or heptachlorobornane I (~3 mg/kg): male rabbits (478–610 g) and female white leghorn chickens (1.1–1.5 kg) from Western Scientific Supply Co. (West Sacramento, CA); male Swiss-Webster mice (18–20 g), male albino Sprague-Dawley rats (150-165 g), male

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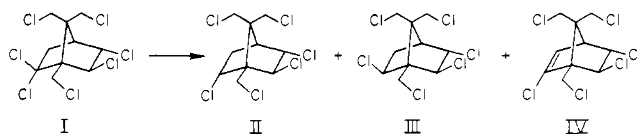

Figure 1. Metabolism of heptachlorobornane I to hexachlorobornanes II and III and hexachlorobornene IV.

Hartley guinea pigs (224–260 g), and male hamsters (70–85 g) from Simonsen Laboratories, Inc. (Gilroy, CA); male long-tailed monkeys (*Macaca fascicularis*) (~8 kg) colony born and handled at the Primate Research Center (University of California, Davis). With mice, rats, monkeys, and chickens, soybean oil was the administration vehicle and rinse for the stomach tube. With the other species, the compounds in soybean oil were applied to lettuce which was quickly consumed by the animals. Feces were collected in each case for 72 h and then the toxaphene-treated animals were sacrificed for removal of the liver and a sample of fat except for the monkeys where tissue samples were taken by biopsy.

Analyses. Toxaphene, heptachlorobornane I, and their metabolites were analyzed by a described gas chromatographic (GC) procedure (Saleh and Casida, 1977, 1978) using the Hewlett-Packard Model 5840 A gas chromatograph with an electron-capture (EC) detector, an SE 30-coated glass capillary column (0.25 mm i.d. × 30 m), helium as the carrier gas, and argon/methane (95:5) as the make-up gas. For analysis of toxaphene the oven temperature was maintained at 170 °C for 60 min, followed by programming from 170 to 200 °C at 0.5 °C/min and finally a constant temperature of 200 °C for 30 min. With heptachlorobornane I metabolites, the column temperature was isothermal at 200 °C.

The fat, liver, and feces, as appropriate, from treated and control animals were extracted with acetone (~10 mL/g; containing a total of 5 μg of mirex as internal standard in studies with heptachlorobornane I), the acetone was evaporated, and the products in hexane solution were subjected to cleanup on the sulfuric acid–Celite column and GC analysis as before (Saleh and Casida, 1978). It is assumed that acetone extraction gives essentially quantitative recoveries of compounds I–IV. The subsequent cleanup and analysis steps provide >98% recovery values for these compounds relative to the internal standard.

For more detailed metabolite analysis, the Finnigan Model 4023 GC/MS/computer system was used with an SP-2100 wall-coated glass capillary column (0.25 mm i.d. × 30 m) and helium as the carrier gas (30 cm/s). One microliter of each sample was injected splitless, and data were acquired at the rate of one scan/second. Chromatographic conditions were as follows: injection port and interface temperatures of 260 °C, column temperature for analysis of heptachlorobornane I metabolites of 200 °C for 3 min and then 2 °C/min programmed to 270 °C, and for analysis of toxaphene metabolites of 170 °C for 3 min and then 1 °C/min programmed to 270 °C.

RESULTS

Metabolism of Heptachlorobornane I. Amounts of unmetabolized I and metabolite yields in feces are given in Table I. Chickens excrete large amounts of I, whereas mice, guinea pigs, and rabbits excrete intermediate amounts and rats, hamsters, and monkeys excrete little or no unmetabolized compound. Each species excretes compounds II–IV, but metabolite IV is minor relative to II and III. The yields of II + III are highest with rabbits and monkeys, intermediate with chickens, rats, and guinea pigs, and lowest with mice and hamsters. The ratio of

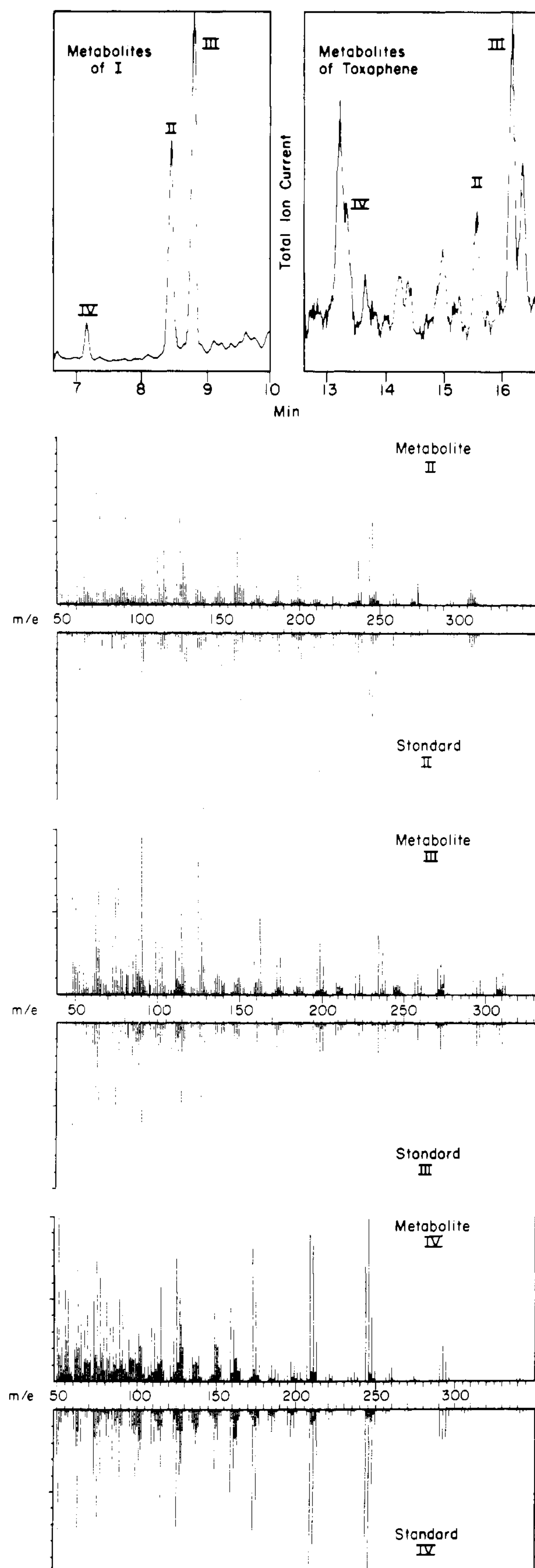

Figure 2. Glass capillary column GC analysis of metabolites II–IV in the feces of monkeys 72 h after oral administration of heptachlorobornane I and of toxaphene. Metabolites of the heptachlorobornane and of toxaphene are chromatographed with two different conditions of temperature programming (see Methods). Electron impact MS are compared for each metabolite of heptachlorobornane I and the standard with which it cochromatographs. The same MS are obtained for the corresponding peaks in the chromatogram of toxaphene metabolites.

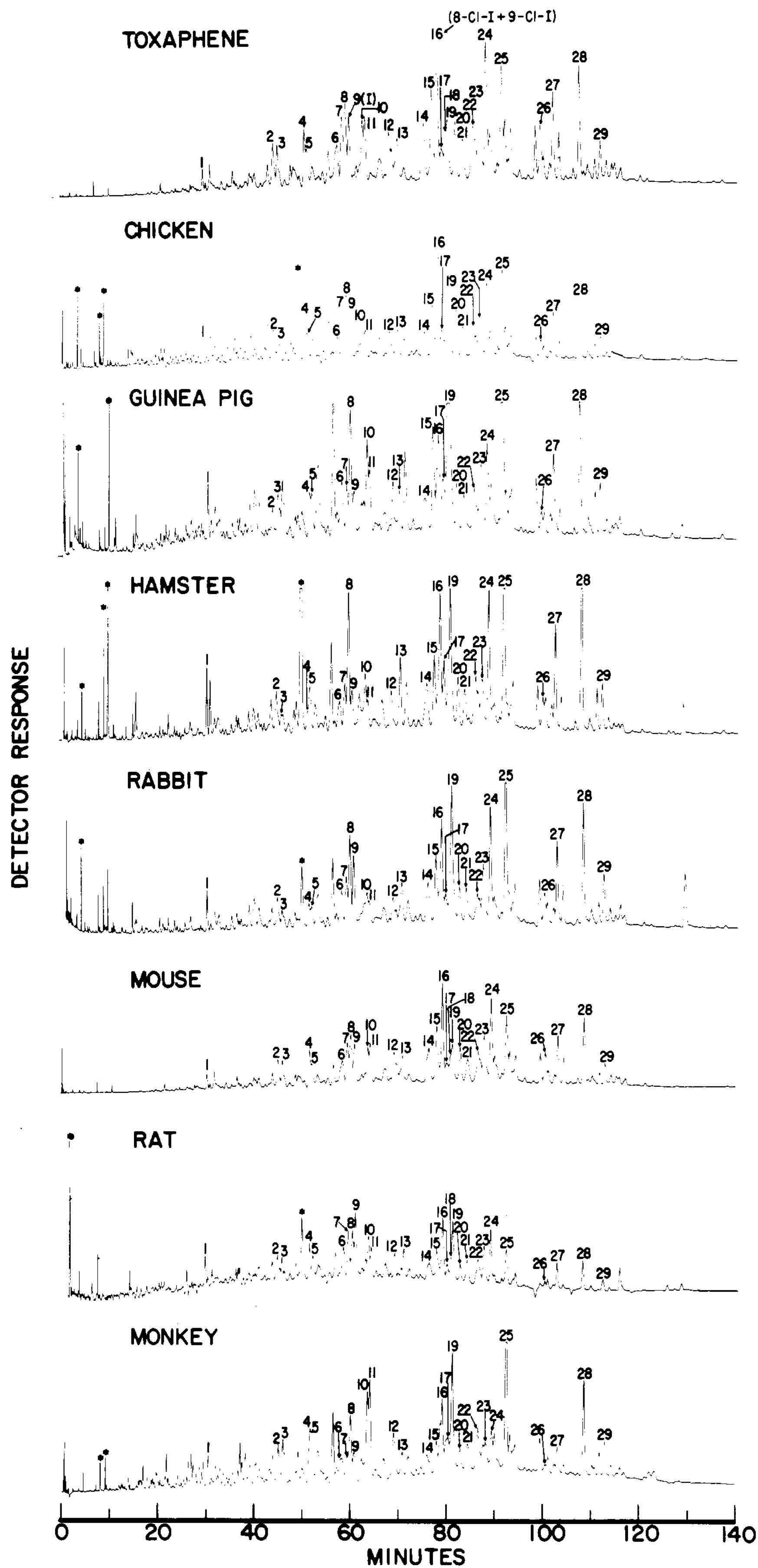

Figure 3. Glass capillary column GC analysis of toxaphene and of toxaphene-derived products in the fat of mammals and chickens 72 h after oral administration of toxaphene. The arabic numerals refer to toxaphene components present in ≥1% amounts as designated by Saleh and Casida (1977). The chromatographic positions of heptachlorobornane I (peak 9) and its 8- and 9-chloro derivatives (8-Cl-I plus 9-Cl-I) (peak 16) are indicated. Asterisks designate interfering materials of biological origin.

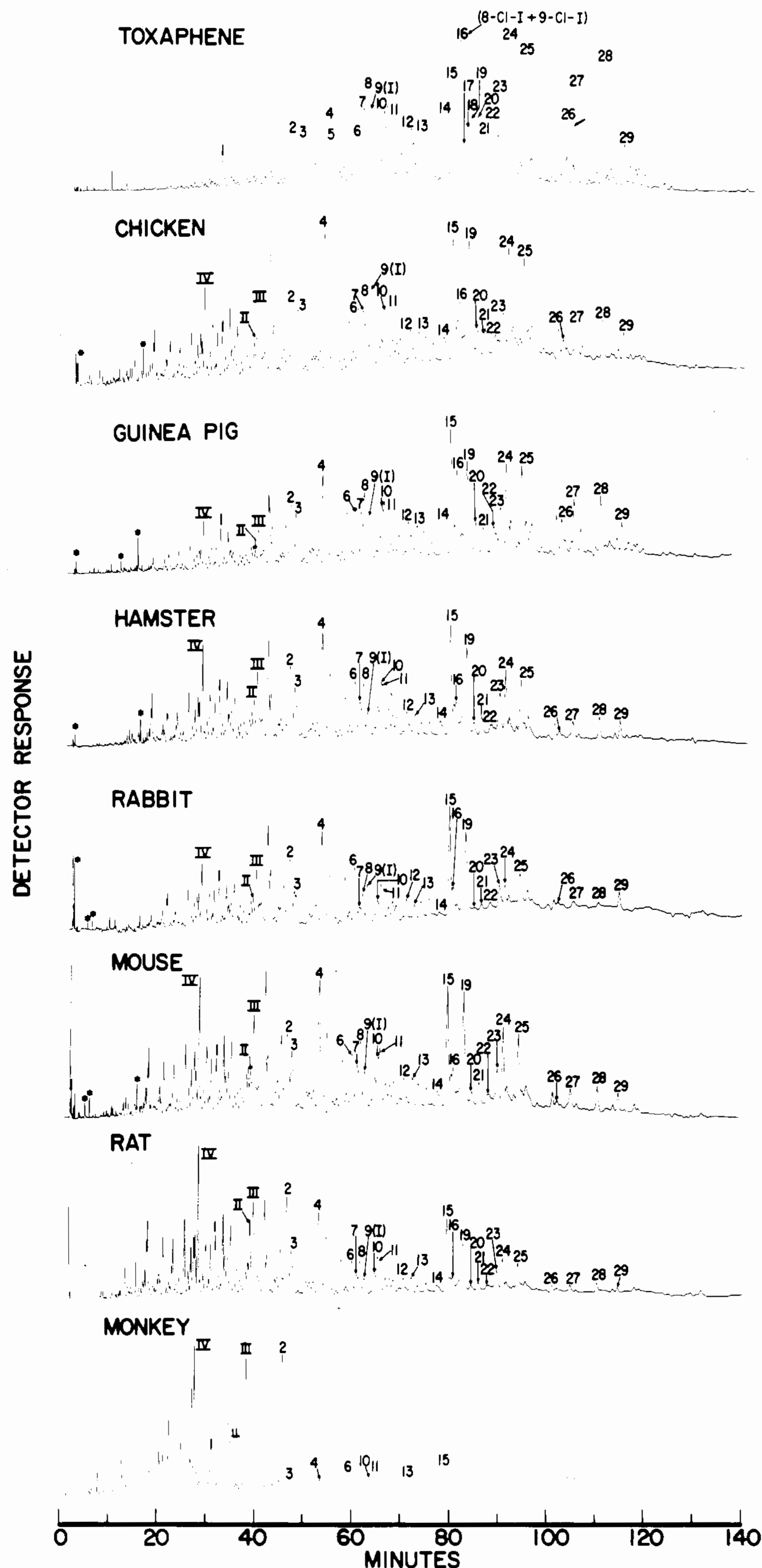

Figure 4. Glass capillary column GC analysis of toxaphene and of toxaphene-derived products in the feces of mammals and chickens 72 h after oral administration of toxaphene. The arabic numerals refer to toxaphene components present in ≥1% amounts as designated by Saleh and Casida (1977). The chromatographic positions of heptachlorobornane I (peak 9) and its 8- and 9-chloro derivatives (8-Cl-I plus 9-Cl-I) (peak 16) are indicated. The chromatographic positions of metabolites II–IV of heptachlorobornane I as defined by GC–MS with monkeys and by analogy with other species are also given. Asterisks designated interfering materials of biological origin.

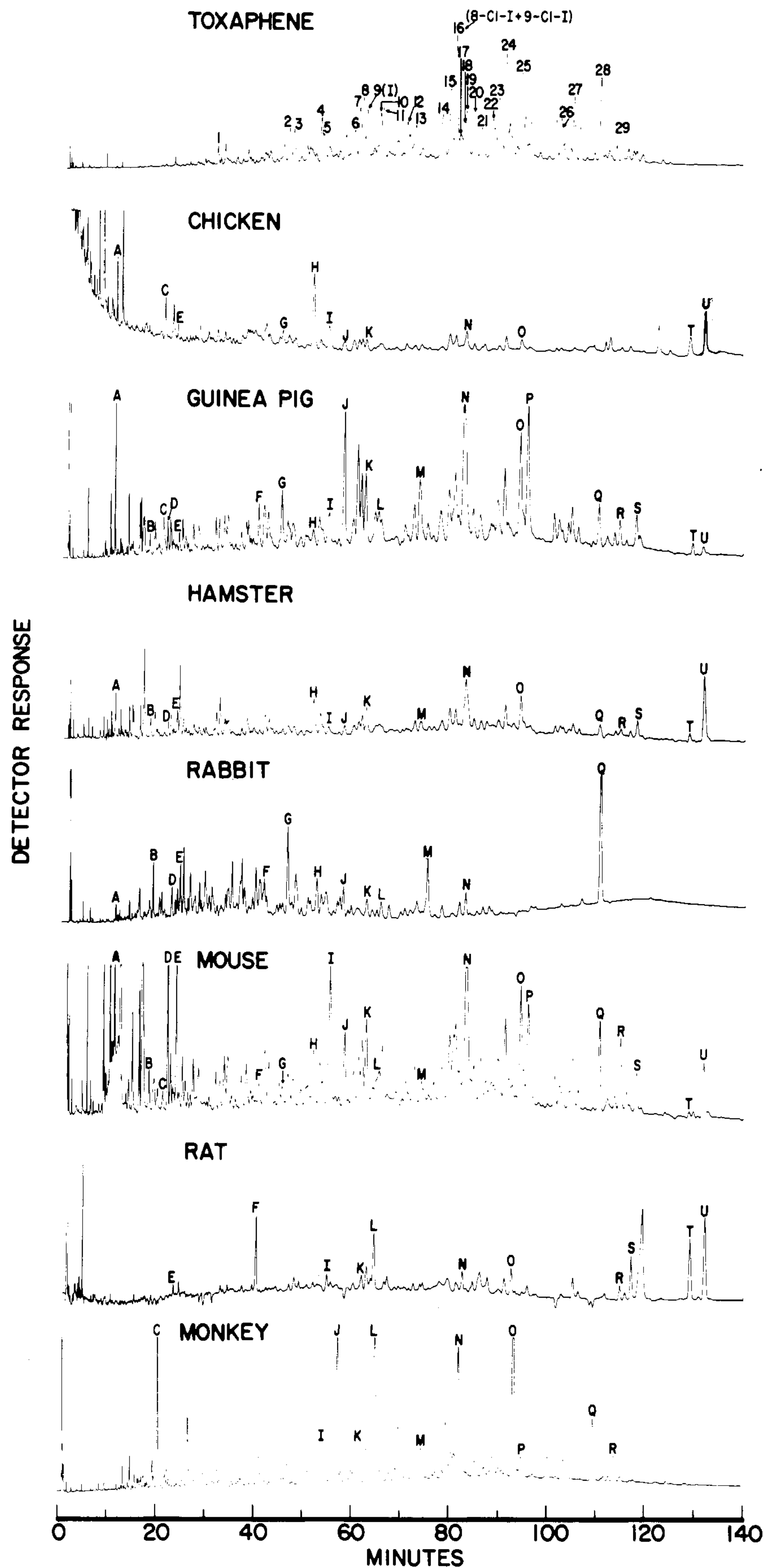

Figure 5. Glass capillary column GC analysis of toxaphene and of toxaphene-derived products in the liver of mammals and chickens 72 h after oral administration of toxaphene. The arabic numerals refer to toxaphene components present in ≥1% amounts as designated by Saleh and Casida (1977). The chromatographic positions of heptachlorobornane I (peak 9) and its 8- and 9-chloro derivatives (8-Cl-I plus 9-Cl-I) (peak 16) are indicated. Letter designations (A–U) refer to toxaphene-derived products in liver, many of which are likely to be toxaphene components. Asterisks designate interfering materials of biological origin.

Table I. Percent of Heptachlorobornane I and Its Metabolites in Feces within 72 h after Oral Administration of I at ~ 3 mg/kg

species	compound, % of administered dose[a]				product ratio, III/II
	I	II	III	IV	
chicken[b]	17.7 ± 2.7	3.8 ± 0.7	5.2 ± 0.9	0.9 ± 0.2	1.4
mouse[c]	2.8 ± 0.3	1.8 ± 0.1	2.2 ± 0.3	0.3 ± 0.0	1.2
rat	0.2 ± 0.1	2.1 ± 0.6	5.3 ± 1.6	1.0 ± 0.3	2.5
hamster	0.6 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	0.2 ± 0.0	1.1
guinea pig	2.3 ± 0.5	3.6 ± 0.5	4.0 ± 0.7	0.7 ± 0.1	1.1
rabbit	2.5 ± 0.0	10.1 ± 0.1	10.1 ± 0.1	1.5 ± 0.2	1.0
monkey[d]	0.0	8.8	10.7	0.9	1.2

[a] Average and standard error based on three experiments except with rats where five experiments were involved and monkeys where a single animal was used. [b] Feces combined with urine. [c] Comparable values for mice treated intraperitoneally with piperonyl butoxide (150 mg/kg) in dimethyl sulfoxide 1.0 h prior to oral administration of heptachlorobornane I in soybean oil are (% of administered dose): I, 7.5 ± 0.9; II, 4.3 ± 0.1; III, 5.5 ± 0.8; and IV, 0.8 ± 0.0. The product ratio III/II is 1.3. [d] The percentage of the total II + III + IV excreted at intervals up to 72 h were as follows: 0-12 h, 0%; 12-24 h, 7.3%; 24-48 h, 10.2%; 48-72 h, 2.9%. The metabolite ratio was the same at each of these time intervals.

metabolites III/II is nearly constant (1.0–1.4) for all species except rats where metabolite III definitely predominates. A monkey treated with heptachlorobornane I at 3.0 mg/kg contains the following ppb levels of I, II, III, and IV, respectively, in tissues at 72 h: 255, 0, 0, and 0 in fat; 50, 0, 450 and 0 in liver.

The identity of metabolites II, III, and IV in the feces of monkeys suggested by GC cochromatography is established by GC–MS (Figure 2).

Products Derived from Toxaphene in Fat, Liver, and Feces of Other Mammals and Chickens. The fat of each species gives a GC product pattern very similar to that of toxaphene with most or all of the 29 designated toxaphene components clearly evident although with some alterations in peak ratios (Figure 3).

The feces chromatograms differ greatly depending on the species, with chickens and guinea pigs showing the closest similarity to toxaphene and rats and monkeys the largest difference (Figure 4). This relationship parallels in the most part the amount of unmetabolized heptachlorobornane I excreted by each species (Table I). The identities of the monkey fecal products from toxaphene cochromatographing with heptachlorobornane I metabolites II, III, and IV are confirmed by GC–MS (Figure 2). Detailed examination of toxaphene by glass capillary column GC–MS establishes that it does not contain II, III, or IV so these compounds in the feces are metabolites and not toxaphene components.

The liver chromatogram patterns differ with each species and vary from relatively few major peaks (chicken, rat, and monkey) to many major peaks (the other species) (Figure 5). Several products retained for 72 h in liver give similar t_R values in two or more species (A–U). It is not established which of the designated liver peaks are toxaphene components and which are metabolites.

DISCUSSION

Reductive dechlorination and dehydrochlorination are major pathways in metabolism of heptachlorobornane I in rats (Saleh and Casida, 1978) and five other species of mammals and in chickens. Species variations in the yields of reductive dechlorination products in feces, ranging from relatively low (3–4% in hamsters and mice) to moderately high (20% in rabbits and monkeys), may be due to differences in either ease of formation or further metabolism of II and III. Thus, piperonyl butoxide, an inhibitor of microsomal cytochrome P450-mediated reactions which increases the toxicity of heptachlorobornane I to mice by eightfold (Saleh and Casida, 1978), increases the amounts of excreted I and the levels of metabolites II–IV by 2.4–2.7-fold. The dehydrochlorination product, IV, is generally present in about one-tenth the amount of II plus III. These three products are the only metabolites of heptachlorobornane I evident in the feces of any of the treated animals. Other metabolites are either not in the feces or are degraded in the course of analysis, such as on passage through the sulfuric acid–Celite column.

Toxaphene analysis by glass capillary column GC reveals a complex but reproducible chromatogram with 29 peaks each of which represents at least 1% of the total EC detector response (Saleh and Casida, 1977). Some or all of these components are detected in the fat and feces of each of the treated animals but there are also new EC-sensitive products, presumably chlorinated hydrocarbons, in these samples. Many or most of them are probably metabolites of toxaphene components. Three of these compounds in the feces of toxaphene-treated monkeys are metabolites II, III, and IV of heptachlorobornane I. The relatively high yields of these compounds and absence of I are surprising, but consistent with studies on the individual heptachlorobornane in monkeys. The fat chromatograms are most similar to toxaphene but still vary among the species possibly due to both component differences in rates of partitioning into the fat and of metabolism. Previous studies with rats (Saleh and Casida, 1978) indicate that the fat levels of heptachlorobornane I and its metabolites are similar at 7 and 72 h after oral treatment.

The toxaphene-derived products persisting in liver are of interest since toxaphene produces liver damage in rats and dogs (Lehman, 1965) and the related compound strobane is a hepatocarcinogen in mice (Innes et al., 1969). The liver chromatograms are completely different than the fat or feces chromatograms and those of toxaphene itself. The pattern depends on the species and is largely attributable to toxaphene metabolites since analogous peaks are absent in toxaphene and in the liver of control animals. The chemical nature of these compounds in liver and their contribution, if any, to the toxicological properties of toxaphene are not known.

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Uptake and Disposition of Endrin in Insecticide-Susceptible and -Resistant Mosquitofish (*Gambusia affinis*)

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Endrin uptake and tissue distribution was studied in insecticide-susceptible (S) and -resistant (R) mosquitofish (*Gambusia affinis*). Fish were exposed to equal (10.2 ppb) and equitoxic (0.6 ppb for S and 314 ppb for R) levels of [^{14}C]endrin for up to 8 h. The accumulation and tissue disposition patterns of endrin were significantly different between the two fish populations studied. A 13.5-fold greater [^{14}C]endrin concentration in gills of S fish than R fish at the equal exposure levels suggested a barrier to endrin penetration at the organismal level. S/R ratios of tissue radioactivity following 8-h exposure to 10.2 ppb [^{14}C]endrin indicative of tissue membrane barriers in the R fish included: brain, 45.9; liver, 7.7; gall bladder, 9.7; spleen, 20.9; intestine, 4.8; and ova, 4.1. The barrier to endrin penetration into the brain appeared to be most effective. The gall bladder/liver ratios suggested that the R fish could effectively excrete endrin over a wider range of exposure concentrations than S fish.

An organochlorine insecticide-resistant population of mosquitofish (*Gambusia affinis*) is the result of natural selection pressures from chronic exposure to agricultural chemical run-off in Mississippi. This unique population has provided a basis for the studies of vertebrate insecticide resistance (Boyd and Ferguson, 1964; Vinson et al., 1963; Culley and Ferguson, 1969). Similar to the investigations of insecticide resistance in insects, these studies have shown that vertebrate insecticide resistance is a multifactorial phenomenon involving: uptake, disposition, metabolism, and possible insensitivity at the target site (Wells and Yarbrough, 1972; Wells et al., 1973; Scales and Yarbrough, 1975; Watkins and Yarbrough, 1975; Fabacher and Chambers, 1976).

The purpose of this present study was to investigate the uptake and disposition of endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*,*endo*-1,4:5,8-dimethanonaphthalene) within the major organ and tissue compartments of insecticide-resistant (R) mosquitofish and -susceptible (S) mosquitofish. Endrin is an extremely toxic compound to freshwater fishes (Ferguson et al., 1966; Grant and Mehrle, 1973). Culley and Ferguson (1969) reported a 500-fold difference in the 48-h LC_{50} values for endrin between the R and S mosquitofish, which represents the greatest degree of resistance for any of the insecticides studied.

MATERIALS AND METHODS

Insecticide-resistant mosquitofish used in these experiments were collected from drainage ditches in Humphreys County, Mississippi, and susceptible mosquitofish from ponds located in Oktibbeha County, Mississippi. The fish were maintained in the laboratory for at least 1 week prior to use. Fish were treated with a 1% potassium permanganate solution to prevent fungal infections; however, no fish were used for experimentation for at least 24 h after permanganate treatments. All fish were fed daily Tetramin staple food diet.

Stock solutions of [1,2,3,4,10-^{14}C]endrin (California Bionuclear Corporation; sp act., 5.2 mCi/mM; 98% pure) were prepared in acetone. Nonradioactive endrin (recrystallized, Shell) stock solutions were also prepared in acetone. Treatment concentrations of 0.6, 10.2, and 314 ppb were selected. The 10.2-ppb treatment served as an equal concentration for direct comparisons between the R and S fish, while the 0.6 ppb treatment to S fish and the 314 ppb treatment to R fish provided equivalent toxicity concentration comparisons (based on 48-h LC_{50} values.

Sixteen adult female fish from each population were selected at random and were placed in an 8-L glass aquaria for 12-h equilibration periods before the insecticide was added. The endrin stock solutions were thoroughly mixed into the aquarium water. Aquaria were not aerated. Three fish from each tank were taken at 0.5, 2, 4, 6, and 8 h of endrin exposure. All fish were rinsed with acetone and blotted dry after removal from the aquaria.

Brain, liver, gill, spleen, gall bladder, intestine, ova, and kidney were removed from three fish, pooled, weighed, and homogenized in 1 mL of cold, glass-distilled, deionized water in a TenBroeck glass tissue grinder. The homogenates were digested in chlorox and added to a scintillation mixture consisting of: 0.05 g of 2,2′-p-phenylenebis(5-phenyloxazole), 4.0 g of 2,5-dipenyloxazole, 40.0 g of Cabosil M-5, 500 mL of scintillation grade toluene, and 500 mL of triton X-100. Radioactivity was counted in a Beckman "CPM-100" liquid scintillation counter. Data are expressed as nanograms endrin equivalents per milligram tissue wet weight. No differentiation was made

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