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Metabolism of *o,p'*-DDT in Chickens

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The following metabolites were identified in chicken excreta after dosing with *o,p'*-DDT-^{14}C: *o,p'*-DDD; *o,p'*-DDT; 3-hydroxy-2,4'-DDD; 3-hydroxy-2,4'-DDT; 4-hydroxy-3-methoxy-2,4'-DDD; 4-hydroxy-3-methoxy-2,4'-DDT; *o,p'*-DDA; 3-hydroxy-2,4'-DDA; 4-hydroxy-3-methoxy-2,4'-DDA; *o,p'*-DDE; 3-hydroxy-2,4'-DDE; 4-hydroxy-3-methoxy-2,4'-DDE; 4-hydroxy-2,4'-DDE; the methyl ester of *o,p'*-DDA; the methyl ester of a methoxy-2,4'-DDA (probably the 3 isomer); and 4-hydroxy-2,4'-DDD. A significant species difference was found in the metabolism of *o,p'*-DDT; chickens metabolized *o,p'*-DDT to four DDE-type compounds, whereas rats did not.

Our study of the metabolism of *o,p'*-DDT in rats revealed an extensive metabolic breakdown, with the formation of at least 13 metabolites (Feil et al., 1973); however, *o,p'*-DDE-type compounds were, surprisingly, not formed. Dehydrochlorination and hydroxylation at the vacant para position to yield 4-hydroxy-2,4'-DDE were postulated as an explanation for the greater estrogenicity of the ortho, para' isomer. Because responses of different species to technical DDT have been observed to vary with respect to several biological activities (Sell and Davison, 1973; Davison and Sell, 1974), we studied the metabolism of *o,p'*-DDT in chickens. The isolation and characterization of 16 metabolites are presented in this report.

EXPERIMENTAL SECTION

Apparatus. Some of the mass spectra were taken with a Varian CH-5DF mass spectrometer. Other equipment used was as previously described (Feil et al., 1973).

***o,p'*-DDT-*ring*-U-^{14}C.** Crude 1,1,1-trichloro-2-(*o*-chlorophenyl-U-^{14}C)-2-(*p*-chlorophenyl-U-^{14}C)ethane was obtained from Amersham/Searle Corp., Arlington Heights, Ill., and purified as previously reported (Feil et al., 1973).

Animal Experiments. Two mature Leghorn hens, surgically modified to facilitate collection of urine and feces (Paulson, 1969), were given a single oral dose of *o,p'*-DDT-*ring*-U-^{14}C (0.3 μCi/mg) corresponding to 150 mg/kg. Urine and feces were collected for 4 days. The hens had free access to water and to a commercial 16% protein laying mash for several weeks prior to and throughout the experiment.

A 2.1-kg Leghorn hen was given 10 mg (3 μCi) of *o,p'*-DDT-*ring*-U-^{14}C per day for 25 days. Excreta were collected for 31 days.

Purification of Urinary Metabolites. Urine was extracted, first with hexane, then with ethyl acetate, to yield three fractions (hexane soluble, ethyl acetate soluble, and water soluble) from which metabolites were isolated.

Metabolites **15** and **19** were isolated from the hexane-soluble fraction by gas chromatography without preliminary cleanup (6 ft, 3% OV-1, Gas-Chrom Q, 150–250° at 5°/min).

Ethyl acetate extracts were chromatographed on LH-20 with methanol to effect a partial separation of radioactive compounds. A large radioactive peak was eluted first, followed by a broad intermediate peak, then a small peak. After treatment with diazomethane, the material in the large peak was chromatographed on LH-20 with methanol and then on silica gel with ethyl acetate–ethanol. The methyl ester of metabolite **7** was purified finally by gas chromatography (6 ft, 3% OV-1, Gas-Chrom Q, 170–270° at 5°/min). Several other radioactive compounds were also present, but these could not be obtained in sufficient purity for identification. In like manner, the methyl ester of metabolite **7** was also isolated from the intermediate peak, again with evidence of other metabolites that could not be purified adequately for identification. No identifications were made on the activity in the small peak.

The water-soluble fraction was hydrolyzed by refluxing with either 2.5 N hydrochloric acid or 2.5 N sodium hydroxide for 24 hr. After acidification of the basic hydrolysis, the work-up procedures were similar for the two hydrolysates. The hydrolysates were extracted with ether, and the ether extracts in turn were extracted with sodium bicarbonate. The bicarbonate extracts were acidified, extracted with ether, and chromatographed on LH-20 with methanol. The radioactive fraction was then derivatized with diazomethane and purified by gas chromatography [6 ft, 3% OV-1, Gas-Chrom Q, 170–270° at 5°/min (retention times, 5.8 and 10.6 min)]; the methyl ester of metabolite **7** and the methyl ether–methyl ester of metabolite **9** were obtained in this manner after both acid and base hydrolyses.

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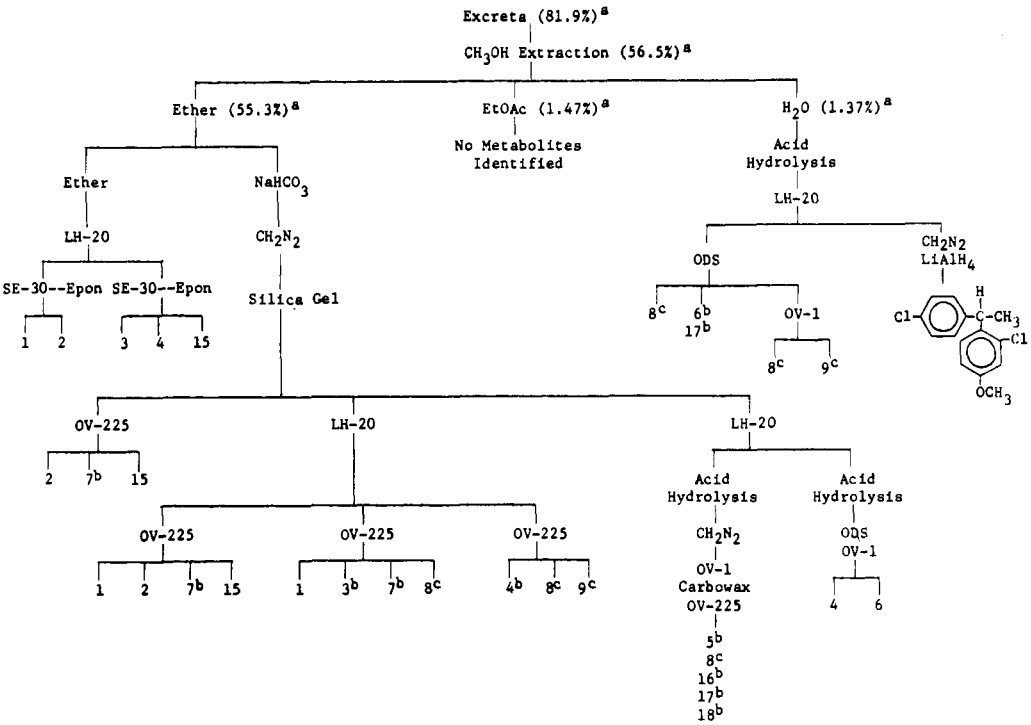


Figure 1. Isolation of metabolites from chicken excreta: superscript a, percentage of original dose; superscript b, isolated as the methyl derivative; superscript c, isolated as the dimethyl derivative.

The ether extracts obtained from the acid hydrolysis were chromatographed on silica gel with ethyl acetate–ethanol to yield two fractions. One fraction was allowed to react with diazomethane to yield the methyl ester of metabolite **7**, which was purified by gas chromatography. The other fraction was chromatographed on LH-20 with methanol to yield the ethyl ester of metabolite **7** (probably resulting from ester interchange with ethyl acetate on silica gel) and metabolite **16**.

The ether extracts from the base hydrolysis were chromatographed on LH-20 with methanol, and the two resulting fractions were allowed to react with diazomethane. They were purified by gas chromatography and identified as the methyl ester of metabolite **7** and the methyl ether of metabolite **16**.

Purification of Fecal Metabolites. Feces collected during the third and fourth days after dosing were extracted with isopropyl alcohol or methanol, and the extracts were chromatographed on LH-20 with methanol. The ^{14}C activity eluted as two broad overlapping peaks. Attempts at further purification were made by using the following procedures: LH-20, methanol; LH-20, benzene; silica gel, benzene–acetone; Porapak Q, water–methanol; LH-20, benzene–methanol; and HPLC ODS, acetonitrile–water. Some of the procedures were used several times. Although considerable purification was effected with these methods, few compounds were isolated in sufficient purity for identification.

The only compound in peak 1 that could be purified sufficiently for identification was *o,p'*-DDT (6 ft, 3% OV-1, Gas-Chrom Q, 150–280° at 5°/min).

Mass spectral evidence on impure fractions isolated from peak 2 suggested the presence of metabolites **3**, **5**, and **20**; the spectral evidence was such that these compounds can only be considered as metabolites because of additional evidence found in other sections.

The ^{14}C material that eluted between the peaks was separated into three peaks by gas chromatography [4 ft, 2% SE-30, 0.2% Epon 1001, Gas-Chrom Q, 150–200° at 3°/min (retention times 9.5, 13.5, and 14.5 min)]. Mass spectrometry suggested that peak 1 consisted of metabolites **15** and **20**, apparently contaminated with a five-chlorine PCB. Peak 2 was *o,p'*-DDT and peak 3 was metabolite **20** contaminated with a six-chlorine PCB.

Chemical Modification of Fecal Metabolites. Fecal metabolites, and presumably many of the impurities, were chemically modified with the hope of altering relative chromatographic characteristics of the compounds present. Methanol or isopropyl alcohol extracts of feces were partially purified by chromatography on LH-20 with methanol. All of the fractions containing ^{14}C activity were combined and, after the solvent was removed, allowed to react with sodium hydride in tetrahydrofuran. Sodium hydride was added in small portions until the evolution of hydrogen ceased; then an additional amount of sodium hydride was added to assure that an excess was present, and the mixture was stirred at room temperature for 30 min. Subsequently, an excess of dimethyl sulfate was added slowly and the mixture was refluxed for 15 hr. The solvent was removed and the brown residue was chromatographed, in sequence, on LH-20 with methanol, silica gel with ethyl acetate, and alumina with hexane–chloroform. Although each chromatographic procedure yielded a single radioactive peak, much nonradioactive material was removed. Five compounds were obtained by gas chromatography (4 ft, 2% SE-30, 0.2% Epon 1001, Chromosorb W, 150–200° at 3°/min) with the following retention times: (1) 17 min, a methoxy-1-(2-chlorophenyl)-1-(4-chlorophenyl)-2-chloroethylene, probably formed by dehydrochlorination of a methoxy-DDD; (2) 18.2 min, overlapping with peak 3, a methoxy-DDE, probably the methyl derivative of metabolite **16**; (3) 18.5 min, overlapping with peak 2, probably metabolite **20**, but position of methoxy group not rigorously established; (4) 21 min, methyl ether of metabolite **21**; and (5) 21.5 min, the methyl derivative of metabolite **3**.

Isolation of Metabolites from Excreta. Excreta, day 5 through day 25, from an unmodified chicken being fed 10 mg (3 μCi) of *o,p'*-DDT-*ring-U*-^{14}C per day for 25 days were extracted by vigorous stirring with methanol. The mixture was filtered through diatomaceous earth and the filtrate was evaporated to dryness. Water was added and

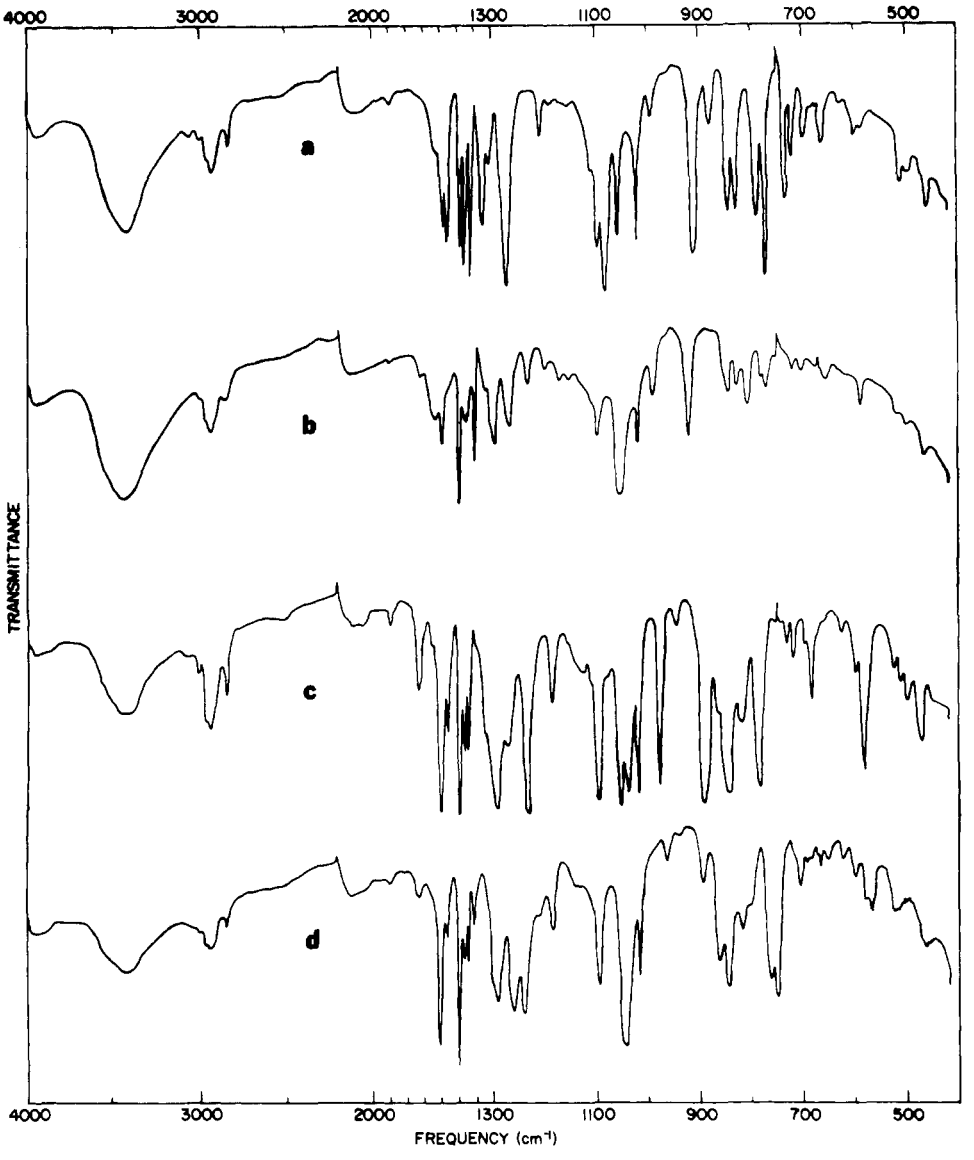


Figure 2. Infrared spectra of some of the compounds used in identification of metabolites: (a) metabolite **16** methyl ether; (b) metabolite **17** methyl ether; (c) metabolite **18** methyl ether; (d) metabolite **21** methyl ether.

the mixture was extracted first with ether and then with ethyl acetate to give the three (ether, ethyl acetate, and water) fractions in Figure 1. The isolation of metabolites or methyl derivatives of metabolites is outlined in Figure 1. The outline has been simplified by showing only the isolations that resulted in successful characterizations. Thus, a number of fractions contained metabolites in addition to those shown, but these could not be sufficiently purified for identification. The acid hydrolyses were done by refluxing for 24 hr with 2.5 *N* hydrochloric acid.

The following chromatographic conditions were used: (1) silica gel, J. T. Baker Chemical Co., columns were packed in methylene chloride; initial eluting solvent was methylene chloride, followed by methanol; (2) ODS, "Permaphase", E. I. DuPont de Nemours & Co., columns, 1 m × 8 mm, were used for high-pressure liquid chromatography with acetonitrile–water; solvent ratios were critical for effective separation and generally were in the range of 1:1 to 1:3; (3) Sephadex LH-20 columns were packed and eluted with methanol; (4) SE-30–Epon, 4 ft, 2% SE-30, 0.2% Epon 1001, Chromosorb W, 150–270° at 3°/min; (5) OV-1, 3 ft, 2%, Gas-Chrom Q, 150–270° at 5°/min; (6) OV-225, 6 ft, 3%, Gas-Chrom Q, 200–250° at 2°/min; (7) Carbowax, 6 ft, 2%, Chromosorb W, 150–250° at 5°/min.

The isolation of the methyl derivatives of metabolites **5**, **8**, **16**, **17**, and **18** (lower right-hand corner of Figure 1) required the use of three gas chromatography procedures to obtain each of the metabolites in sufficient purity for characterization. For example, considerable purification was realized when metabolites were trapped from an OV-1 column and rechromatographed on a Carbowax column.

1-(2-Chloro-4-methoxyphenyl)-1-(4-chlorophenyl)ethane was isolated after reduction with lithium aluminum hydride. This compound was important because it supported the presence of 4-hydroxy-2,4′-DDD, a compound for which evidence was obtained in feces (see Chemical Modification of Fecal Metabolites).

Synthesis of Compounds Used in Identifications. Synthesis procedures and physical data of many of the compounds used in metabolite identifications have been reported (Feil et al., 1973). Infrared spectra of some compounds used in identifications are shown in Figure 2. Normalizations of mass spectra were based on the ^{35}Cl isotope peak.

1,1-Dichloro-2-(2-chloro-3-methoxyphenyl)-2-(4-chlorophenyl)ethylene (Metabolite 16 Methyl Ether). 1,1,1-Trichloro-2-(2-chloro-3-methoxyphenyl)-2-(4-chlorophenyl)ethane (Feil et al., 1973), 250 mg, was refluxed for 6 hr with 600 mg of 57% sodium hydride dispersion (washed with hexane to remove the mineral oil) in 100 ml

of tetrahydrofuran. The solvent was removed by distillation. Ether and water were added, and the ether layer was removed and dried over magnesium sulfate. The solvent was removed and the resulting oil purified by gas chromatography [3 ft, 2% OV-1, Gas-Chrom Q, 150–250° at 5°/min (retention time, 7.5 min)]: mass spectrum *m/e* 346 (4 Cl, 21.1%, $M^{\cdot+}$), 311 (3 Cl, 29.3%, M − Cl), 296 (3 Cl, 17.7%, M − Cl, CH_3), 276 (2 Cl, 100%, M − Cl_2), 261 (2 Cl, 24.4%, M − Cl_2, CH_3), 233 (2 Cl, 61.4%).

1,1-Dichloro-2-(2-chloro-3,4-dimethoxyphenyl)-2-(4-chlorophenyl)ethylene (Metabolite 17 Methyl Ether). 1,1,1-Trichloro-2-(2-chloro-3,4-dimethoxyphenyl)-2-(4-chlorophenyl)ethane (Feil et al., 1973) was allowed to react with sodium hydride as in the preparation of metabolite 16 methyl ether. The product was purified by gas chromatography [3 ft, 2% OV-1, Gas-Chrom Q, 150–200° at 5°/min (retention time, 8 min)]: mass spectrum *m/e* 376 (4 Cl, 100%, $M^{\cdot+}$), 360 (4 Cl, 5.6%), 341 (3 Cl, 49%, M − Cl), 326 (3 Cl, 50%, M − Cl, CH_3), 306 (2 Cl, 46%, M − Cl_2).

1,1-Dichloro-2-(2-chloro-4-methoxyphenyl)-2-(4-chlorophenyl)ethylene (Metabolite 18 Methyl Ether). 2,2,2-Trichloro-1-(2-chloro-4-methoxyphenyl)ethanol was prepared from 2-chloro-4-methoxybenzaldehyde (Feil et al., 1973) by the procedure used in the synthesis of the 3-methoxy isomer (Feil et al., 1973), and was purified by distillation (137–142° at 0.1 Torr). To a solution of 2.5 g of this carbinol in 20 ml of chlorobenzene was added 20 ml of anhydrous hydrogen fluoride in two or three portions. The reaction was stirred at room temperature for 5 hr; water and ether were then added, and the ether layer was removed and washed with sodium bicarbonate. After drying the organic layer with magnesium sulfate, the ether and volatile impurities were removed by distillation at reduced pressure; the final temperature was 135° at 0.1 Torr. Gas chromatography [6 ft, 3% OV-1, Gas-Chrom Q, 150–250° at 10°/min (retention time, 13 min)] indicated the residue to be 90 to 95% 1,1,1-trichloro-2-(2-chloro-4-methoxyphenyl)-2-(4-chlorophenyl)ethane. Impurities isolated at this stage of the synthesis or after the dehydrochlorination could be explained by: (1) formation of the butylcarbinol instead of the trichloromethylcarbinol, (2) conversion of the carbinol to the benzyl fluoride, and (3) self-condensation of the carbinols.

The 1,1,1-trichloro-2-(2-chloro-4-methoxyphenyl)-2-(4-chlorophenyl)ethane was allowed to react with sodium hydride as in the preparation of metabolite 16 methyl ether. The product was purified by gas chromatography [6 ft, 3% OV-1, Gas-Chrom Q, 150–250° at 10°/min (retention time, 11 min)]: mass spectrum *m/e* 346 (4 Cl, 40%, $M^{\cdot+}$), 311 (3 Cl, 27%, M − Cl), 276 (2 Cl, 100%, M − Cl_2), 261 (2 Cl, 27%, M − Cl_2, CH_3), 233 (2 Cl, 42%).

1,1-Dichloro-2-(2-chloro-4-methoxyphenyl)-2-(4-chlorophenyl)ethane (Metabolite 21 Methyl Ether). An aqueous ethanol solution of crude 1,1,1-trichloro-2-(2-chloro-4-methoxyphenyl)-2-(4-chlorophenyl)ethane, used in the preparation of metabolite 18 methyl ether, was refluxed with aluminum amalgam for 5 hr (Inoi et al., 1962). Most of the ethanol was removed, and the remaining solution was extracted with ether. The ether layer was dried over magnesium sulfate, and the solvent was removed. Partial purification was obtained by "dry column" chromatography (Burger, 1967; Loev and Goodman, 1967) with carbon tetrachloride on silica gel. The desired compound was concentrated at 40 to 50 cm from the origin. Further purification was done by gas chromatography [6 ft, 10% OV-1, Gas-Chrom Q, 200–250° at 5°/min (retention time 6.5 min)].

RESULTS AND DISCUSSION

Purification of metabolites from chicken excreta was much more difficult than purification of metabolites from rat excreta (Feil et al., 1973), probably because the metabolites were present in lower concentrations in chicken excreta. When two surgically modified chickens were given single doses of *o,p'*-DDT-^{14}C the distributions of radioactivity (as percentages of dose) after 4 days were as follows: urine, 3.5, 3.7; feces, 61.7, 61.7; carcass, 17.3, 33.8; total, 82.5, 99.2. Approximately 60% of the fecal activity could be extracted; 72% of this activity was *o,p'*-DDT and 8% was *o,p'*-DDD. Thus, all other extractable metabolites accounted for only about 7.5% of the dose. At least ten *o,p'*-DDT metabolites were identified from feces extracts; however, the actual number present may have been several times greater. Because most of the metabolites were present in amounts substantially less than 1% of the original dose, some of the identifications had to be based on mass spectral data only. Comments in Table I reflect the rigor to which the identifications were subjected.

The lower concentrations of metabolites in chicken excreta were accompanied by an *o,p'*-DDT concentration nearly twice that found in rat excreta (Feil et al., 1973); this lower concentration suggests a lower rate of absorption. A chicken was given 10 mg of ^{14}C-labeled *o,p'*-DDT daily for 25 days to improve absorption and, hopefully, to reduce isolation difficulties. After 31 days the distribution of radioactivity (as percentages of the total dose) was: excreta, 81.9; eggs, 1.0; carcass, 7.3; total, 90.2. Methanol extracted 56.5% of the ^{14}C activity from the excreta. This dosing procedure may also stimulate microsomal enzyme activity (Sell and Davison, 1973). Conversion of *o,p'*-DDT to metabolites by increased absorption and/or metabolism improved to the extent that the ratio of metabolites to parent *o,p'*-DDT approximated the ratio found in the feces of rats given a single dose of *o,p'*-DDT (Feil et al., 1973); however, the ease of isolation was not improved because the absolute concentrations of metabolites in excreta were lower than those in the single-dose experiment.

The results of the present study and those of the previous rat study (Feil et al., 1973) are compared in Table I. Several differences are apparent in the metabolism of *o,p'*-DDT in rats and chickens.

(1) Chickens formed four *o,p'*-DDE-type compounds, whereas rats formed none of this type. Although hydroxylated DDE compounds could possibly have been overlooked in the rat because of low concentrations and conjugation, the probability of overlooking a compound which can be isolated and characterized as easily as *o,p'*-DDE is not likely.

(2) Glycine and serine conjugates of *o,p'*-DDA were isolated from rat excreta but were not isolated from chicken excreta. Evidence for the presence of this type of conjugate in chicken excreta was obtained by isolation of *o,p'*-DDA and 3-hydroxy-2,4'-DDA from extracts that did not contain these acids until they were subjected to hydrolysis. Possible explanations for the failure to isolate these conjugates from chicken excreta are low concentrations, different or additional amino acid conjugates, and different interfering impurities.

(3) The methyl ester of *o,p'*-DDA and the methyl ester of a methoxy-2,4'-DDA (probably the 3-methoxy isomer) were isolated from chicken excreta, but not from rat excreta. Some evidence was obtained indicating that these methyl derivatives were not formed by isolation procedures, but no evidence was obtained on possible microbial formation.

(4) The 4- and 5-hydroxy derivatives of *o,p'*-DDA were not isolated in chicken excreta. They were present at near our threshold of identification level in rat feces.

(5) *o,p'*-Dichlorobenzhydrol was isolated from rat urine but not from chicken excreta.

Some of the metabolites may have been present only as conjugates because they were isolated only after hydrolysis. Alternatively, the hydrolysis may have altered some of the impurities sufficiently to render purification possible.

Table I. Comparison of *o,p'*-DDT Metabolism in Rats and Chickens[a]

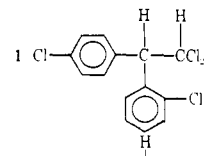
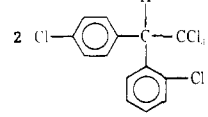
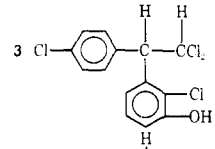
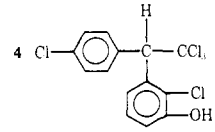
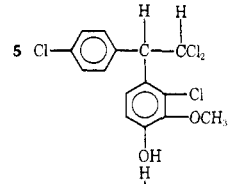
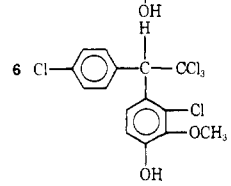
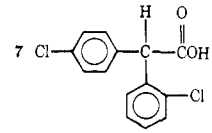
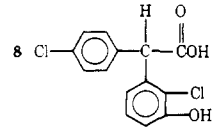
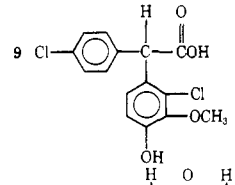
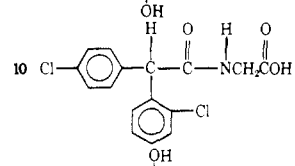
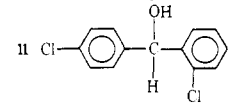
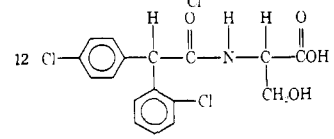
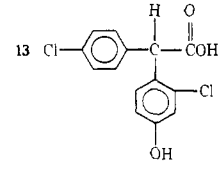
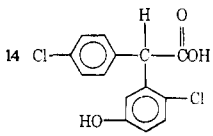
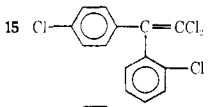
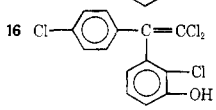
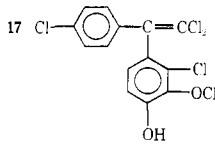
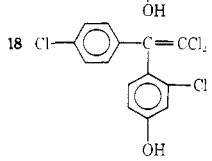
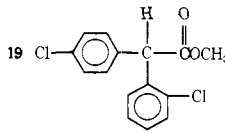
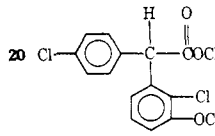
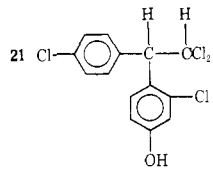
Metabolite	Rat[b]	Chicken	Comments[c]
1 	F	F, Ex	Feces, GC, MS; excreta, GC, MS, ir
2 	F	F, Ex	Feces, GC, MS, ir; excreta, GC, MS
3 	F	F, Ex	Feces, MS on impure sample and MS, ir on methyl derivative; excreta, GC, MS, ir
4 	F	F, Ex	Feces, MS on crude sample; excreta, GC, MS, ir
5 	F	Ex	MS on methyl derivative; isolated only after hydrolysis
6 	F	Ex	MS, ir on impure sample; GC, MS on methyl derivative; isolated only after hydrolysis
7 	F	F, U, Ex	GC, MS, ir on methyl derivative
8 	F	F, U, Ex	MS, ir on methyl derivative
9 	F	Ex	MS, ir on methyl derivative
10 	F		Not isolated in sufficient purity for identification; hydrolysis of samples that should have contained it yielded *o, p'*-DDA
11 	U	ND	
12 	F		See comments for metabolite 10
13 	F	ND	

Table I *(Continued)*

Metabolite	Rat[b]	Chicken	Comments[c]
14 	F	ND	
15 	ND	F, U, Ex	Feces, MS; urine, MS; excreta, MS, ir
16 	ND	U, Ex	Urine, MS, ir on methyl derivative; excreta, MS on methyl derivative; isolated only after hydrolysis
17 	ND	Ex	MS, ir on impure sample of methyl derivative; isolated only after hydrolysis
18 	ND	Ex	MS, ir on methyl derivative
19 	ND	F, U	Feces, MS; urine, MS, ir
20 	ND	F	MS; the 4-methoxy isomer was rejected on the basis of mass spectrometry. The 3 and 5 isomers gave identical mass spectra; the 3 isomer was proposed because metabolite **8** was found but metabolite **14** was not
21 	ND	F	Isolated as the methyl derivative after reaction with sodium hydride and dimethyl sulfate; metabolite may have been conjugated; MS, ir; 1-(2-chloro-4-methoxyphenyl)-1-(4-chlorophenyl)ethane was isolated from excreta after reduction with $LiAlH_4$ supporting this compd

[a] Abbreviations used are: F, feces; U, urine; Ex, excreta from an unmodified chicken; MS, mass spectrum; ir, infrared spectrum; GC, gas chromatography; ND, not detected. [b] Isolation of metabolites from rat excreta was reported in Feil et al. (1973). [c] Comments pertain only to results obtained with chickens. Spectral comparisons were made with authentic samples; synthesis reported in present paper or in Feil et al. (1973).

Comments in Table I designate which metabolites may have been excreted as conjugates, but the structures given are those of the isolated products.

Chemical modification of metabolites and impurities made the isolation of radioactive compounds possible from several otherwise intractable fractions. Diazomethane was frequently useful in the isolation of metabolites. Two other modifications were of value in predicting metabolite structures. Reaction of a water-soluble fraction from excreta (Figure 1), first with diazomethane, then with lithium aluminum hydride, yielded 1-(2-chloro-4-methoxyphenyl)-1-(4-chlorophenyl)ethane. Reaction of some extracts of feces (see Chemical Modifications of Fecal Metabolites) with sodium hydride and dimethyl sulfate resulted in the isolation of five compounds, including 4-methoxy-2,4′-DDD and a methoxy-1-(2-chlorophenyl)-1-(4-chlorophenyl)-2-chloroethylene. The isolation of these three compounds suggests, but does not prove, that 1,1-dichloro-2-(2-chloro-4-hydroxyphenyl)-2-(4-chlorophenyl)ethane (metabolite **21**), or a conjugate of it, was a metabolite.

These studies have shown that there is a significant species difference in the metabolism of o,p′-DDT. Chickens metabolized o,p′-DDT to several DDE-type compounds, whereas rats did not (Feil et al., 1973). Metabolic differences such as this may, in part, account for the varied biological responses of different species to technical DDT. p,p′-DDE has been proposed as the causative agent for the eggshell thinning caused by technical DDT in mallard ducks (Haegele and Tucker, 1974). o,p′-DDE has also been shown to cause eggshell thinning in mallard ducks (Davison, 1974). These compounds, however, do not cause eggshell thinning in chickens. One possible explanation for this difference might be different metabolism of the DDE's. If susceptible species such as ducks do not hydroxylate and conjugate o,p′-DDE as do chickens, the metabolic buildup of o,p′-DDE in the body may be sufficient to cause eggshell thinning.

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Metabolism of 2-Chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine (Cyprazine) in the Rat

Gerald L. Larsen* and Jerome E. Bakke

Rats given single oral doses of ring-^{14}C-labeled cyprazine (I) excreted 97.6% of the dose within 72 hr (urine, 72.7%; feces, 24.9%). Less than 0.1% of the radioactivity was detected as $^{14}CO_2$ in the expired air. The rat carcasses contained 7.5% of the radioactivity at sacrifice (72 hr after dosing). Four urinary metabolites were identified by comparison of their mass spectra with those of authentic compounds. These compounds and the percentages of the urinary radioactivity that they represented were: 2-hydroxy-4,6-diamino-*s*-triazine (II, 3.0%); 2-chloro-4,6-diamino-*s*-triazine (III, 13.9%); 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (IV, 5.3%); and 2-chloro-4-amino-6-isopropylamino-*s*-triazine (VII, 2.1%). Two fecal metabolites were identified as II and IV. Four additional urinary metabolites were characterized by mass spectrometry as 2-chloro-4-amino-6-[(2-hydroxy-1-methylethyl)-amino]-*s*-triazine (V, 5.9%); 2-chloro-4-amino-6-cyclopropylamino-*s*-triazine (VI, 7.7%); [2-chloro-4-cyclopropylamino-*s*-triazinyl(6)]alanine (VIII, 9.9%); and 2-chloro-4-cyclopropylamino-6-[(2-hydroxy-1-methylethyl)amino]-*s*-triazine (IX, 1.6%).

Bohme and Barr (1967) identified rat and rabbit urinary metabolites from the 2-chloro-*s*-triazines: simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine]; atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine); and propazine [2-chloro-4,6-bis(isopropylamino)-*s*-triazine]. The metabolites of these 2-chloro-*s*-triazines were all 2-chloro analogs of their respective parent molecules. Mono- and di-N-dealkylation and oxidation of the *N*-alkyl groups to acids were major routes of metabolism for these 2-chloro-*s*-triazines.

Bakke et al. (1972) identified four and characterized two metabolites from atrazine in rat urine. As isolated, these urinary metabolites were identified as 2-hydroxy analogs of atrazine. However, these metabolites were isolated by ion-exchange chromatography at elevated temperatures, and subsequent investigations indicated that these metabolites may have been artifacts. For instance, when atrazine and 2-chloro-4,6-diamino-*s*-triazine were placed on the ion-exchange column used in their isolation procedure, both compounds were quantitatively recovered from the ion-exchange column as their 2-hydroxy analogs. Bakke et al. (1972) concluded that any 2-chloro-*s*-triazine metabolites present in the urine were quantitatively converted to 2-hydroxy analogs. However, the presence of 2-hydroxy-*s*- triazine metabolites in the urine could not be ruled out.

This report describes the metabolism of the 2-chloro-*s*-triazine cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine, I) by the rat. The urinary metabolites were isolated by using paper chromatography to minimize hydrolysis of the 2-chloro group that occurs with the use of ion-exchange chromatography.

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EXPERIMENTAL SECTION

Chemicals. 2-Chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine-^{14}C, uniformly labeled in the ring (9.4 μCi/mg), and unlabeled cyprazine were provided by Gulf Research and Development Co., Merriam, Kan. Radiopurity of the radioactive chemical was greater than 99.0% as determined by thin-layer chromatography (TLC). Authentic standard compounds were obtained from Geigy Chemical Corporation.

Animal Treatment. All rats were dosed by stomach tube with ring-^{14}C-labeled cyprazine dissolved in 0.5 ml of ethanol. Urine and feces were collected at 24-hr intervals.

Two rats (each weighing 310–360 g) were each given 0.5 mg of cyprazine containing 0.93 μCi of ^{14}C as a single oral dose. These rats were housed in all-glass metabolism cages, and CO_2 was collected as previously reported (Bakke et al., 1967). After 3 days, the rats were sacrificed, and total carcass radioactivity was determined.

Twelve rats (each weighing 300–420 g) were each given 5.0 mg of cyprazine containing 0.51 μCi of ^{14}C as a single oral dose. These rats were housed in stainless steel cages, and urine was collected daily and pooled. Feces were also collected daily and pooled. The pooled urine and pooled fecal samples were assayed for radioactivity and used for metabolite isolation. After 3 days, the rats were sacrificed.

Instrumentation and Quantitation of Radioactivity. All column eluates were monitored with a Packard Model 320E liquid scintillation flow system. With aqueous eluates, anthracene was used as the scintillator in the flow cell, and with organic eluates, cerium-activated lithium glass beads were used as the scintillator in the flow cell. Radioactive components on paper chromatograms and