UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 27 1987

005915

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Kelthane: Evaluation of a rat metabolism study of dicofol

FROM: Whang Phang, Ph.D. 5/26/87 Caswell #93
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Toxicology Branch/HED (769c)

TO: D. Edwards, PM (12)
Registration Division (767c)

THROUGH: Marcia van Gemert, Ph.D. 5/26/87
Head, Section III
and
Theodore M. Farber, Ph.D.
Chief
Toxicology Branch/HED (769c)

The registrant, Rohm & Haas Co., has submitted a rat metabolism study of p,p'-dicofol, an active ingredient of Kelthane. This study have been evaluated by Dynamac Corp. and is approved by Toxicology Branch. The DER is attached.

Sinlge dose (50 mg/kg) of ^{14}C-dicofol was orally administered to groups of rats (4/sex). After 168 hrs of dosing, the major route of excretion was found to be through feces for both male and females. The highest tissue residue level was in adipose tissue (30% and 80.5% of the dose in males and females, repectively), after 48 hrs of dosing.

Dicofol was found to be metabolized via several pathways. The major pathway appeared to be dechlorination of a non-ring chlorine atom to yield FW-152 and followed by oxidation to form dichlorobenzophenone (DCBP) and dichlorobenzoic acid (DCBA). In this study very little DDE was found. Apparently, the metabolism of dicofol is different from that of DDT which is metabolized to DDE.

This study is acceptable as a study to demonstrate the metabolism of dicofol.

005915

EPA: 68-02-4225
DYNAMAC No. 266-A
May 26, 1987

DATA EVALUATION RECORD

KELTHANE

Metabolism in Rats

STUDY IDENTIFICATION: Tillman, A. M., Mazza, L. S., and Williams, M. Part I: Absorption and excretion of ^{14}C-p,p'-dicofol in male and female rats. Part II: A metabolism study of ^{14}C-p,p'-dicofol in male and female rats. (Unpublished study No. 31L-86-02 prepared by Rohm and Haas, Spring House, PA, and Analytical Bio-Chemistry, Columbia, MO, for Rohm and Haas, Spring House, PA; dated December 30, 1986.) Accession No. 400420.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 5-26-87

1. CHEMICAL: Kelthane; p,p'-dicofol; dicofol; 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol.

2. TEST MATERIAL: 1,1-Bis(4-[U-^{14}C]chlorophenyl)-2,2,2-trichloroethanol ([^{14}C]dicofol) with a specific activity of 26.4 mCi/mg and a radiochemical purity of 98 percent.

3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Tillman, A. M., Mazza, L. S., and Williams, M. Part I: Absorption and excretion of ^{14}C-p,p'-dicofol in male and female rats. Part II: A metabolism study of ^{14}C-p,p'-dicofol in male and female rats. (Unpublished study No. 31L-86-02 prepared by Rohm and Haas, Spring House, PA, and Analytical Bio-Chemistry, Columbia, MO, for Rohm and Haas, Spring House, PA; dated December 30, 1986.) Accession No. 400420.

5. REVIEWED BY:

Charles E. Rothwell, Ph.D.
Principal Reviewer
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Signature: Charles E. Rothwell
Date: 5-26-87

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6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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EPA Reviewer

Signature: W. Phang
Date: 5/26/87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Groups of four male and four female Sprague-Dawley rats were administered single oral doses of [^{14}C]dicofol at 50 mg/kg and sacrificed 48 or 168 hours later. The major route of excretion was through the feces for all groups, accounting for 61.7 and 32.2 percent of the administered dose in males and females, respectively, after 168 hours. In addition, approximately 15 percent of the dose was excreted in the urine of both males and females after 168 hours. The highest residue levels of [^{14}C]dicofol equivalents were observed in adipose tissue at 48 hours: 200 ppm, or 30.6 percent of the dose, in males and 547 ppm, or 80.5 percent of the dose, in females. [^{14}C] residues in fat, liver, and blood decreased with time for both sexes, but the decrease was faster in males than in females.

Urine, feces, fat, liver, and plasma were analyzed for dicofol metabolites. Dicofol is metabolized via several pathways to form a large number of compounds (Figure 1). The major pathway appears to be one whose mechanisms involve replacement of a nonring chlorine atom with hydrogen (dechlorination) to yield FW-152 and subsequent oxidation to form dichlorobenzophenone (DCBP) and dichlorobenzoic acid (DCBA). Further metabolism yields dichlorobenzil (DCBH) and various hydroxy or conjugated secondary metabolites. Cleavage also occurs to yield p-chlorobenzoic acid (CBA), which is subsequently conjugated with glycine to yield p-chlorohippuric acid (CHA). Apparently, the hydroxyl group of the trichloroethanol moiety (lone oxygen atom) of dicofol is not cleaved and, therefore, the metabolism of dicofol is totally different from that of DDT (Figure 2). Specific analysis of these tissues revealed that, at most, 0.2 percent of the [^{14}C] residues were DDE. Although this study indicates that little, if any, formation of DDE from pure dicofol occurs, technical Kelthane may contain larger amounts of DDE and/or DDT, which should accumulate in fat as DDE.

B. This metabolism study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, [^{14}C]dicofol, was purchased from ICI, England. It had a specific activity of 26.4 mCi/g and a radiochemical purity of 98 percent. Cochromatographic analysis with pure standards by high-performance liquid chromatography (HPLC) and subsequent radioanalysis by

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[1]Only items appropriate to this DER have been included.

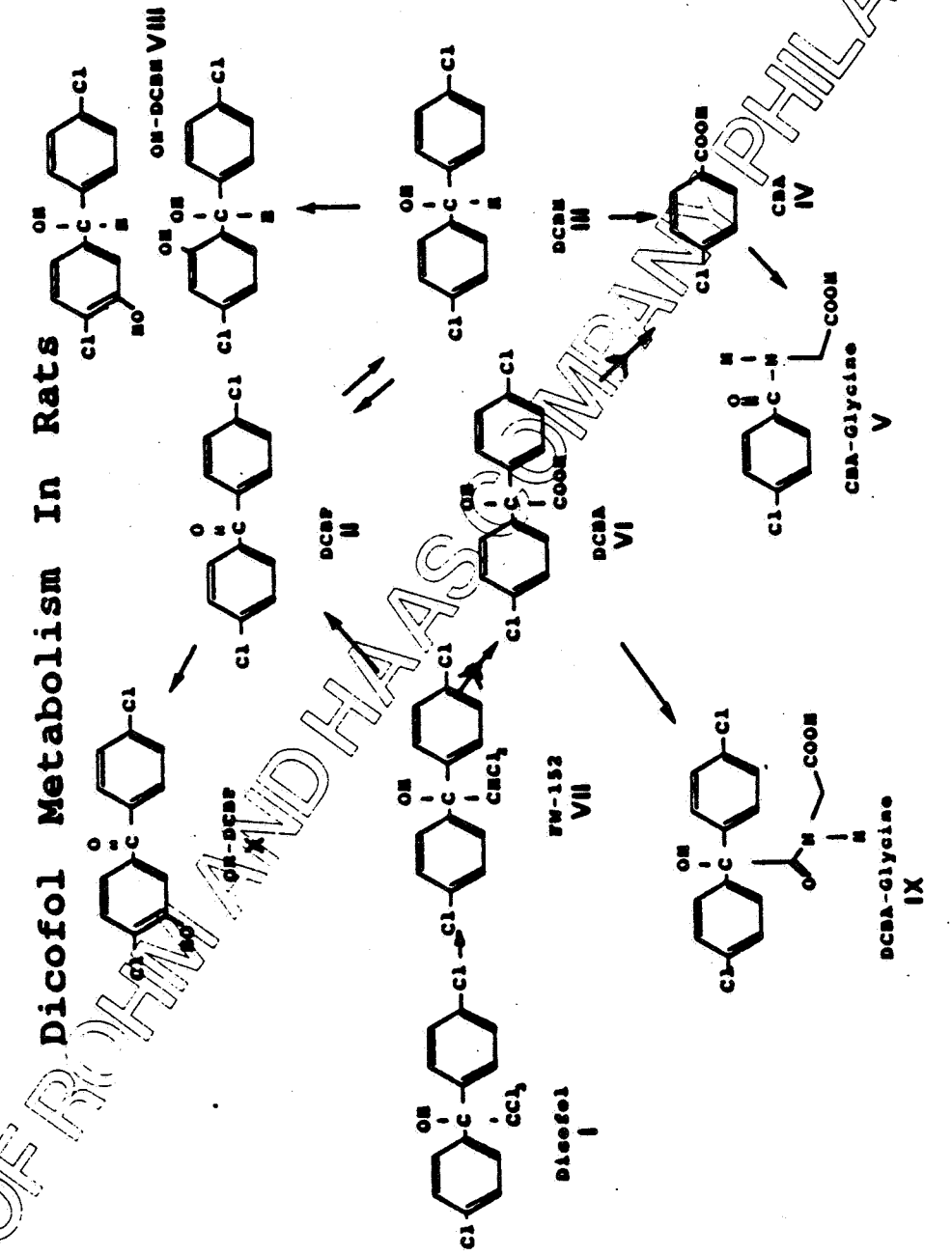
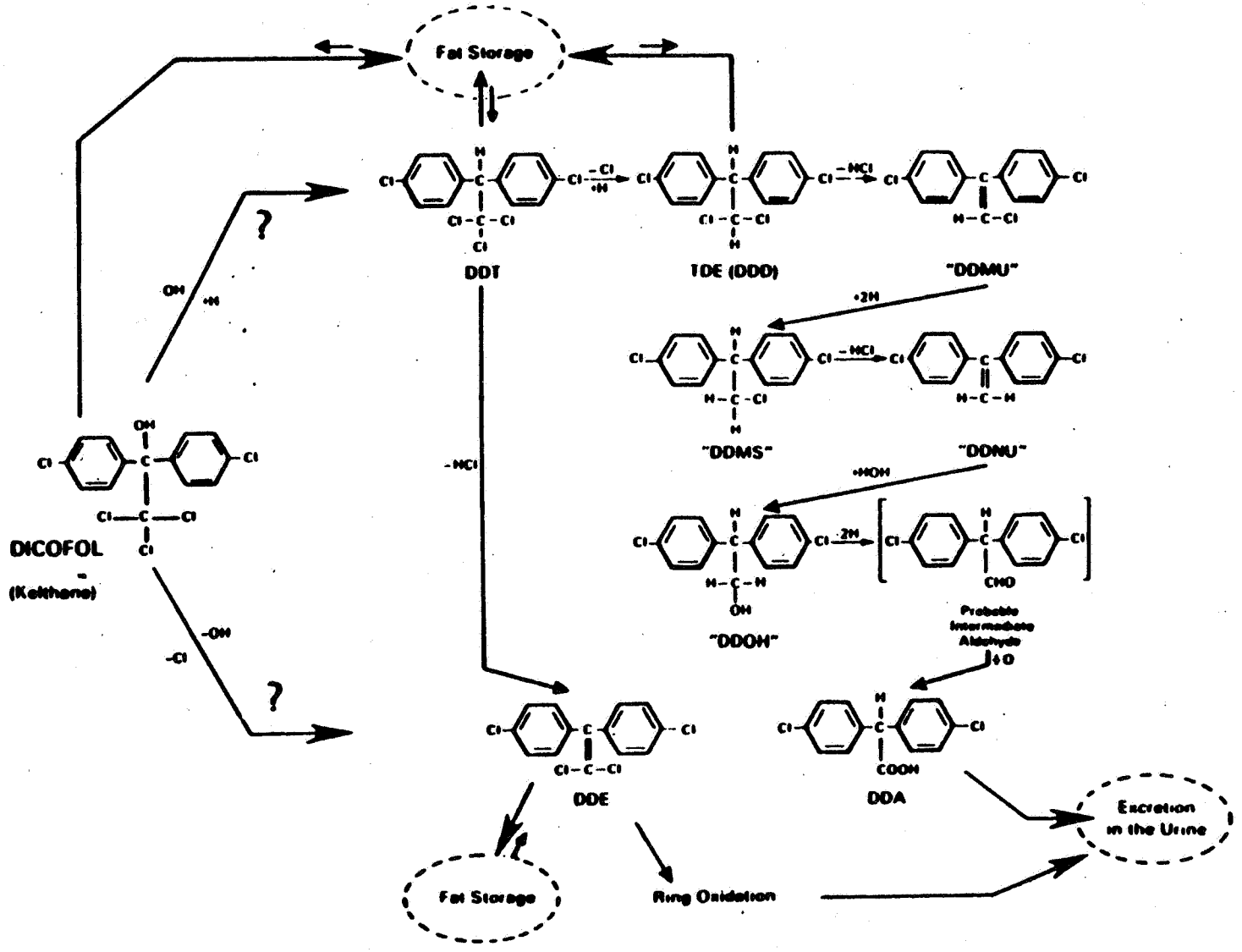


Figure 1.



Source: The assessment of the carcinogenicity of dicofol (Kelthane), DDT, DDE, and DDD (TDE). U.S. EPA, Carcinogen Assessment Group (1986).

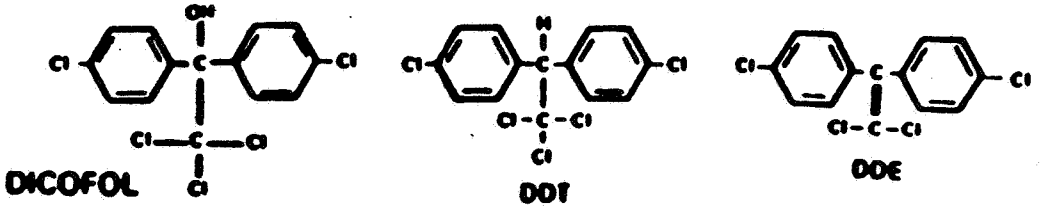
Figure 2. Theoretical metabolism of dicofol, and known metabolism of DDT, DDE, and DDD.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

liquid scintillation counting (LSC) showed that the test material contained the following compounds:

98.0 percent	p,p'-dicofol
0.4 percent	o,p'-dicofol
[redacted]	

The chemical structures of dicofol, DDT, and DDE are shown below:



2. Eight male and eight female Sprague-Dawley rats, 6 to 8 weeks old, were purchased from Charles River Kingston (Stoneridge, NY). The animals were provided Purina Rodent Lab Chow and water ad libitum. After a 1-week quarantine, the animals were acclimated to their individual metabolism cages for 3 days prior to dosing. The rats were assigned to the following schedule of sacrifice postdosing:

Rat No.	Sex	Wt. (g)	Scheduled Sacrifice	Rat No.	Sex	Wt. (g)	Scheduled Sacrifice
1	Male	191	48 hrs	9	Male	235	168 hrs
2	Male	265	48 hrs	10	Male	175	168 hrs
3	Male	255	48 hrs	11	Male	175	168 hrs
4	Male	200	48 hrs	12	Male	185	168 hrs
5	Female	155	48 hrs	13	Female	155	168 hrs
6	Female	150	48 hrs	14	Female	155	168 hrs
7	Female	170	48 hrs	15	Female	178	168 hrs
8	Female	167	48 hrs	16	Female	170	168 hrs

3. The test material was diluted with unlabeled dicofol (98 percent pure) to 10 μCi/mg and subsequently dissolved in corn oil. Each rat was administered a single oral dose of [^{14}C]dicofol at 50 mg/kg body weight (76-132 μCi/rat) and returned to its metabolism cage. One rat (No. 10) died 48 hours after dosing and, therefore, no samples were collected from this animal.

4. Urine and feces were collected at 24, 48, 72, 96, and 168 hours postadministration when possible. Expired air was not collected. The liver and samples of adipose tissue were removed and weighed from all dosed rats after sacrifice.

Additionally, the kidneys were removed from rats sacrificed at 168 hours postadministration. Blood samples were collected from all 15 rats just before sacrifice. The urine collection funnels and cages were washed with water and the washes were saved for radioassay. All samples were stored frozen at -15°C until required for analysis.

5. Aliquots of urine, plasma, cage washes, and washes from the urine collection funnel were radioassayed by LSC. Samples of liver, kidney, adipose tissue, whole blood, and feces were radioassayed by combustion and subsequently by LSC. The following assumptions were made for the conversion of dpm per sample to percent dose recovery: 1) whole blood and plasma both have a specific gravity of 1 g/mL, 2) the rat contains 65 mL of whole blood/kg body weight, and 3) total body fat weight is 7 percent of the rat's body weight at sacrifice.

6. For metabolite identification, all samples were extracted with organic solvents to remove unbound radioactivity. Feces were extracted twice with methanol in a Soxhlet apparatus; the extracts were concentrated and subsequently purified by Sep-Pak chromatography. Urine samples were acidified with hydrochloric acid (pH 2) and extracted three times with ethyl acetate; the ethyl acetate extracts were combined and concentrated. Liver samples from rat Nos. 2, 4, 6, 8, 12, 14, and 16 were extracted twice (combined) by homogenization in methanol-sodium sulfate and once by homogenization in methanol acidified with hydrochloric acid. The methanol extracts were concentrated separately and extracted three times with petroleum ether and then three times with ethyl acetate. Samples of adipose tissue from rat Nos. 2, 4, 6, 8, 12, 14, and 16 were extracted three times with petroleum ether by sonication; the extracts were combined and concentrated. Plasma from rat Nos. 1, 3, 7, 8, 11, 12, 15, and 16 was mixed with 1 to 2 mL of 10 percent sodium chloride, acidified with hydrogen chloride, and extracted three times with n-butanol; the butanol extracts were combined and concentrated. Tissues and excreta from each rat were extracted separately. Aliquots of each extract and residue were radioassayed to determine recoveries of [^{14}C].

7. Radioactive metabolites were separated from the various extracts by preparative TLC and by HPLC. Several metabolites were identified by cochromatography with analytical standards using TLC and HPLC and by isotope dilution experiments. Structural identification of other metabolites was accomplished by mass spectrometry (MS) or gas chromatography/mass spectrometry (GC/MS). DDE was specifically looked for in an aliquot of the methanolic fecal extract from rat No. 6 by comparison of HPLC chromatograms of the sample and a DDE standard.

8. Liver and adipose tissue samples from rat Nos. 1, 3, 5, 7, 9, 11, 13, and 15 were sent to Analytical Bio-Chemistry, Columbia, MO, to quantitate dicofol, DDE, and FW-152 (a major dicofol metabolite) residues. The methodology employed by Analytical Bio-Chemistry was slightly different from that used by Rohm and Haas, but basically involved the same steps. The tissues were extracted with an organic solvent and the extracts were purified (by gel-permeation chromatography) before metabolite identification by HPLC cochromatography with analytical standards. A copy of Analytical Bio-Chemistry's methodology is included in this report as Appendix A.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Recovery of Excreted Radioactivity: Approximately 85 percent of the [^{14}C]-dicofol dose was recovered in the tissues and excreta of male rats sacrificed at 48 and 168 hours and in female rats sacrificed 168 hours postadministration (Table 1). The balance of the dose for these groups may have been in the carcasses, which were not radioassayed. However, total recoveries of [^{14}C] in females sacrificed at 48 hours averaged 124 percent of the administered dose; [^{14}C] residues in the carcasses were not radioassayed.

B. Elimination: The major route of [^{14}C] excretion for all groups was the feces (Table 1). Male rats excreted a larger percentage of the dose in the feces than did females. Urinary excretion, however, was similar for males and females at 48 hours (means of 5.6 and 5.7 percent, respectively) and at 168 hours (means of 16.3 and 13.3 percent, respectively). Total excretion of [^{14}C] averaged 51.5 and 40.5 percent in males and females, respectively, at 48 hours postdosing and 80.0 and 54.6 percent in males and females, respectively, at 168 hours.

C. Tissue Distribution: [^{14}C] residues found in selected tissues at 48 and 168 hours postadministration are presented in Tables 1 and 2. [^{14}C] residues were expressed as percent of administered dose (Table 1) or ppm (Table 2). The highest [^{14}C] residues were found in fat. [^{14}C] residues in fat accounted for 30.6 and 80.5 percent of the dose in males and females, respectively, 48 hours postadministration (Table 1). Mean concentrations of [^{14}C]dicofol equivalents were 200 and 547 ppm, respectively (Table 2). Levels of [^{14}C] in liver and blood were similar in males and females at 48 hours and were much lower than those in fat. In male rats, tissue levels of [^{14}C] at 168 hours were 80 to 90 percent lower than at 48 hours. Tissue concentrations of [^{14}C] declined more slowly in female rats; levels at 168 hours were 60 to 70 percent of those at 48 hours. In male and female

TABLE 1. Mean Recoveries of Radioactivity 48 and 168 Hours after Administration of [^{14}C]Dicofol to Rats at 50 mg/kg[a]

Sample	Recovery of [^{14}C] (Percent of administered dose) after: 48 hours[b] Males	48 hours[b] Females	168 hours[c] Males	168 hours[c] Females
Feces	44.35±3.69	31.96±9.25	61.70±12.85	32.21±4.12
Urine[d]	5.59±2.19	5.72±1.46	16.33±9.72	13.33±3.02
Cage Wash	1.57±0.32	2.85±0.57	2.00±1.63	7.07±2.64
	51.49±5.12	40.53±8.73	80.02±8.99	54.62±6.95
Fat	30.6±12.1	80.5±22.7	3.20±4.54	28.37±5.33
Liver	2.83±0.94	2.03±0.30	0.28±0.11	0.82±0.10
Kidney	No sample	No sample	0.02±0.01	0.16±0.08
Blood	0.77±0.38	0.71±0.29	0.11±0.04	0.34±0.10
	34.2±12.2	83.3±23.0	3.62±4.59	29.69±5.27
Total	85.73±13.20	123.8±14.9	83.63±8.21	84.30±1.69

[a]All values were recalculated from individual animal data by our reviewers.

[b]Values are mean ± S.D. of four animals.

[c]Values are mean ± S.D. of three animals; male rat No. 10 died and female rat No. 14 was excluded from these calculations as an outlier.

[d]Includes urinary funnel wash.

TABLE 2. Tissue Concentrations (ppm) of [^{14}C]Dicofol Equivalents 48 and 168 Hours after Administration of [^{14}C]Dicofol to Rats at 50 mg/kg

Tissue	48 hours[a]		168 hours[b]	
	Males	Females	Males	Females
Fat	200.0±77.0	546.7±202.4	20.59±30.01	195.4±32.3
Liver	24.66±7.39	22.55±5.35	2.48±0.90	9.99±1.06
Kidney	No sample	No sample	1.14±0.20	9.81±4.43
Blood	5.34±2.44	5.19±2.49	0.66±0.23	2.54±0.81

[a]Values are mean ± S.D. of four animals.

[b]Values are mean ± S.D. of three animals.

rats sacrificed 168 hours after dosing, mean levels of [^{14}C]dicofol equivalents in fat were 29- and 83-fold higher than the corresponding blood levels, respectively.

D. Fecal Metabolites: Methanol extraction of fecal samples removed an average of 92.4±17.6 percent of the sample radioactivity. Of that amount, approximately 91.1 percent was recovered after Sep-Pak purification. Dicofol and six metabolites were identified in the fecal extracts. The structures of all identified dicofol metabolites are presented in Figure 1. The distribution of radioactive metabolites in the feces is shown in Table 3. Quantities of metabolites varied greatly for individual samples, as is reflected by the large standard deviations.

Parent compound accounted for only 2 to 5 percent of the radioactivity in purified fecal extracts of all four groups. Metabolite FW-152 was the major metabolite found in the purified extracts of 0- to 48-hour feces from males and females and the 48- to 168-hour feces of female rats, accounting for 27 to 51 percent of the radioactivity in those samples. DCBH accounted for 30 and 35 percent of the [^{14}C] in the 0- to 48-hour and 48- to 168-hour samples from males, respectively, but only 6 and 13 percent of the respective samples from female rats. Other identified metabolites, DCBH, OH-DCBP, OH-DCBH, and DCBA-glycine, were present in smaller amounts.

A zone of radioactivity that cochromatographed with analytical DDE on TLC accounted for approximately 1 to 3 percent of the fecal radioactivity. However, HPLC analysis of this zone from the 0- to 48-hour fecal extract from male rats showed that only 0.22 percent of the extract's [^{14}C] could come from DDE; the bulk of the zone's [^{14}C] was not identified. Unidentified fecal metabolites accounted for approximately 13 to 24 percent of the total radioactivity. In general, nonpolar metabolites (dicofol, DCBP, FW-152, DCBH, and the "DDE" zone) accounted for a larger percentage of the fecal radioactivity at 0 to 48 hours than at 48 to 168 hours.

E. Urinary Metabolites: Ethyl acetate extraction removed means of 80 to 82 percent and 95 to 99 percent of the radioactivity present in urine from male and female rats, respectively (Table 4). Dicofol and DDE were not identified in urine. The only nonpolar metabolite, DCBP, accounted for less than 2 percent of the urinary [^{14}C] in each group. Most of the urinary [^{14}C], therefore, was associated with polar metabolites (DCBH was shown by other techniques to be a minor component of the OH-DCBP/DCBH zone). One major metabolite and numerous minor metabolites in urine from each group could not be identified. Together, these unidentified metabolites accounted for 45 to 57 percent of the extractable urinary radioactivity. These unknowns were more polar than the most polar identified metabolite, CHA. As with fecal metabolites, there was a large individual variation

Table 3.

Distribution of Metabolites in Feces Extracts from Male and Female Rats After a Single Oral Dose of ^{14}C-Dicofol

	Male Rats		Female Rats	
	0-48 h[b]	48-168 h[c]	0-48 h[d]	48-168 h[e]
Average % of ^{14}C Dose[a]:	44.35 ± 3.69	61.70 ± 12.9	31.96 ± 9.25	31.95 ± 5.64
Average % of ^{14}C in Methanol Extract:	92.7 ± 18.8	93.1 ± 16.8	96.8 ± 18.7	86.3 ± 17.0
Average % Recovery Sep-Pak Purification:	95.8 ± 7.49	91.8 ± 9.71	86.6 ± 8.87	86.0 ± 16.6
Average % of ^{14}C in Sep-Pak Eluent[a]:				
Dicofol	4.87 ± 7.23[f]	2.07 ± 0.90[f]	4.74 ± 4.63	2.62 ± 1.44[g]
DCBP	2.97 ± 0.72[h]	2.13 ± 0.62	2.89 ± 1.26	2.54 ± 1.49[h]
FW-152	36.6 ± 25.7	16.6 ± 20.4	51.0 ± 25.0	27.1 ± 11.3
DCBH	29.54 ± 20.6	34.5 ± 20.3	5.87 ± 4.68	12.9 ± 3.23
OH-DCBP	5.07 ± 2.68[i]	9.91 ± 3.86	10.1 ± 9.81	22.8 ± 6.53
OH-DCBH/DCBA-glycine	5.91 ± 6.03	10.4 ± 7.61	4.08 ± 3.27	6.70 ± 3.39
"DDE" zone[k]	2.63 ± 2.16[j]	0.88 ± 0.38	1.01 ± 0.64	1.27 ± 1.26
unidentified (includes baseline + 3-10 minor zones)	12.8 ± 9.50	21.1 ± 17.9	19.1 ± 17.6	23.6 ± 13.9

[a] Mean ± standard deviation
[b] Total of 10 samples from 5 animals chosen as representative samples
[c] Total of 8 samples from 3 animals chosen as representative samples
[d] Total of 10 samples from 5 animals chosen as representative samples
[e] Total of 9 samples from 3 animals chosen as representative samples
[f] Some DCBP and DDD residues are included in this data
[g] Some DCBP residues are included in this data
[h] Some Dicofol residues are included in this data
[i] Some DCBH residues are included in this data (no separation from OH-DCBP on tlc)
[j] Some DDT and DDD residues are included in this data
[k] Data are from a zone which cochromatographed with DDE but was later shown not to be DDE. See text for details.

Table 4

Distribution of Metabolites in Urine Extracts from Male and Female Rats After a Single Oral Dose of ^{14}C-Dicofol

	Male Rats		Female Rats	
	0-48 h[b]	48-168 h[c]	0-48 h[d]	48-168 h[e]
% of ^{14}C Dose:[a]	5.59 ± 2.19	16.33 ± 9.72	5.72 ± 1.46	19.40 ± 12.50
% of ^{14}C in Ethyl Acetate Extract:	80.3 ± 13.0	81.9 ± 13.8	98.9 ± 32.8	95.0 ± 26.6
% of ^{14}C Extract as[a]:				
DCBP	1.62 ± 0.92	1.66 ± 0.30	1.21 ± 0.46	1.28 ± 0.62
OH-DCBP/DCBH[f]	24.4 ± 6.4	30.8 ± 9.66	23.3 ± 5.94	22.1 ± 9.04
OH-DCBH/CBA[f]	5.34 ± 2.14	5.60 ± 2.61	3.46 ± 1.88	2.46 ± 1.50
DCBA	4.00 ± 8.01	3.78 ± 4.63	2.26 ± 0.96	1.37 ± 0.81
DCBA glycine/CHA[f]	24.7	7.32 ± 3.69	22.1 ± 9.47	10.0 ± 6.24
CHA[g]	5.11 ± 3.22	6.43 ± 2.60	6.05 ± 1.91	5.65 ± 0.07
DCBA-glycine[g]	13.9 ± 7.81	10.5 ± 5.96	19.75 ± 1.34	17.7 ± 3.96
unknown metabolite	12.2 ± 10.5	17.2 ± 15.9	9.83 ± 4.98	17.2 ± 11.8
unidentified (baseline + 5-10 minor zones)	33.2 ± 13.8	29.4 ± 12.8	38.1 ± 10.3	40.2 ± 15.6

[a] Mean ± Standard deviation
[b] Total of 9 samples analyzed from 5 animals taken as representative subsamples
[c] Total of 9 samples analyzed from 3 animals taken as representative subsamples
[d] Total of 9 samples analyzed from 5 animals taken as representative subsamples
[e] Total of 9 samples analyzed from 3 animals taken as representative subsamples
[f] These metabolites were inseparable on most tlc plates; % reported represents the sum of both compounds.
[g] These data were generated from tlc analyses in which CHA and DCBA-glycine were separable.

in the quantities of metabolites; urine samples from rat Nos. 11, 12, and 14 were consistently high in unidentified metabolites.

F. Metabolites in Fat: Petroleum ether extraction of adipose tissue removed approximately 109±36.5 (93 to 143) percent of the sample radioactivity. The majority of the radioactivity in fat was associated with the parent compound; 83 percent in males and 89 percent in females (Table 5). FW-152 was the major dicofol metabolite in fat, accounting for 7.8 and 3.8 percent of the extractable radioactivity in males and females, respectively. Only 3.5 and 2.5 percent of the extractable [^{14}C] in males and females, respectively, was associated with unidentified metabolites.

Similar results were obtained by Analytical Bio-Chemistry on fat samples from the odd-numbered animals. Recoveries of extractable radioactivity ranged from 71.3 to 284 percent for individual samples; the study authors felt the high recoveries for some samples were due to desiccation. Purification of the extracts resulted in almost no loss of radioactivity; recoveries averaged 96 percent. Dicofol accounted for 92.8±3.9 and 92.3±2.9 percent of the adipose [^{14}C] in males and females, respectively. FW-152 accounted for 3.06±2.1 and 2.07±1.0 percent in males and females, respectively. DDE was detected in only one of seven samples, from male rat No. 3, at 0.2 percent of the extractable [^{14}C]. Analytical Bio-Chemistry estimated elimination half lives for dicofol and FW-152 in fat to be 30 and 24 hours, respectively.

G. Metabolites in Liver: Results are presented in Table 6. Average recoveries of hepatic [^{14}C] were 87.2 and 67.3 percent after methanol extraction in males and females, respectively. The bulk of the extracted [^{14}C] (81-91 percent) partitioned into petroleum ether. Only 13 to 19 percent partitioned into ethyl acetate. Dicofol was present at less than 5 percent of extractable hepatic radioactivity. FW-152 was the major hepatic residue, accounting for approximately 65.3 and 74.5 percent of the total extractable [^{14}C] in males and females, respectively. The "DDE" zone from the petroleum ether extract was used in an isotope dilution experiment. After a single purification step, 85 percent of the zone's [^{14}C] did not cochromatograph with analytical DDE.

Analytical Bio-Chemistry reported similar results. Dicofol accounted for 3.98±3.7 and 3.07±1.7 percent of the extractable hepatic radioactivity in males and females, respectively. FW-152 accounted for 81.5±18.2 and 82.7±1.2 percent in males and females, respectively. DDE was detected in the livers of two female rats (Nos. 13 and 15) at 0.34 and 0.25 percent of the extractable [^{14}C], respectively.

Table 5

Distribution of Metabolites in Fat Extracts from Male and Female Rats After a Single Oral Dose of ^{14}C-Dicofol

	Males[b]		Females[c]	
	0-48 h	48-168 h	0-48 h	48-168 h
Average % of ^{14}C Dose:	34.2 ± 12.2	3.62 ± 4.59	83.3 ± 23.0	22.6 ± 14.9
Average % ^{14}C in Pet Ether Extract:	143 ± 66.9	94.6	93.4 ± 7.50	101.7 ± 17.5
Average % of ^{14}C Extract as[a]:				
Dicofol	82.9 ± 9.58		89.2 ± 4.32	
DCBP	2.67 ± 0.06		2.80 ± 0.68	
FW-152	7.77 ± 2.74		3.83 ± 2.46	
DCBH	0.73 ± 0.75		0.18 ± 0.15	
OH-DCBP	0.43 ± 0.35		0.30 ± 0.29	
OH-DCBH	1.37 ± 1.95		0.93 ± 1.09	
"DDE" zone[d]	0.63 ± 1.01		0.33 ± 0.59	
unidentified	3.53 ± 3.46		2.45 ± 1.42	

[a] Total of 3 samples analyzed (2 from 0-48 h group and 1 from 48-168 h group)

[b] Total of 4 samples analyzed (2 from each group)

[c] Mean ± Standard Deviation

[d] Data was generated from a zone which cochromatographed with DDE but was shown not to be DDE. See text for details

Table 6

Distribution of Metabolites in Liver Extracts from Male and Female Rats After a Single Oral Dose of ^{14}C-Dicofol

	Male Rats[b]		Female Rats[c]	
	0-48 h	48-168 h	0-48 h	48-168 h
Average % of ^{14}C Dose:	2.83 ± 0.94	0.28 ± 0.11	2.03 ± 0.30	0.69 ± 0.28
Average % ^{14}C in MeOH Extract:	86.1 ± 27.8	90.8	73.6 ± 25.1	60.9 ± 16.8
Average % ^{14}C in Pet Ether Partition:	83.6 ± 7.85	88.4	90.5 ± 0.07	81.3 ± 9.26
Average % ^{14}C Pet Ether Extract as[a]:				
dicofol	2.97 ± 1.00[d]		3.70 ± 2.30[d]	
DCBP	2.00 ± 0.57		2.27 ± 1.40	
FW-152	72.8 ± 8.19[e]		77.7 ± 4.26	
DCBH	6.45 ± 2.47		4.43 ± 0.61	
OH-DCBP	2.37 ± 0.96		4.33 ± 2.48	
OH-DCBH	4.10 ± 1.08		1.45 ± 0.73	
DCBA-glycine[i]	3.06 ± 1.32		1.55 ± 1.91	
"DDE" zone	0.77 ± 0.55		0.43 ± 0.29	
unidentified	5.47 ± 3.50		4.68 ± 1.56	
Average % ^{14}C in EtOAc Partition	13.5 ± 3.89	10.9	12.5 ± 2.19	16.9 ± 1.98
Average % ^{14}C EtOAc Partition as:				
dicofol	0.95 ± 0.35[d]		3.80[d]	
DCBP	0.60 ± 0.30		1.23 ± 0.73	
FW-152	21.9 ± 10.2[f]		48.2 ± 11.9[f]	
	16.6 ± 1.13[g]		52.9[g]	
DCBH	2.85 ± 0.07		3.00 ± 1.16	
OH-DCBP	2.0 ± 0.0[f]		3.0 ± 1.82[f]	
	4.9 ± 4.10[g]		3.10[g]	
DCBA-glycin	5.47 ± 5.67		3.78 ± 6.78	
CBA/OH-DCBH[h]	5.37 ± 5.85		1.57 ± 0.50	
DCBA	2.77 ± 0.70		3.85 ± 1.58	
CMA	3.60 ± 3.22		2.48 ± 2.70	
unknown	24.8 ± 10.3		13.5 ± 5.91	
unidentified (baseline + 4-11 minor bands)	42.0 ± 12.0		26.6 ± 7.73	

[a] Mean ± Standard Deviation
[b] Total of 3 samples analyzed (2 from 0-48 h group and 1 from 48-168 h group) as representative subsample
[c] Total of 4 samples analyzed (2 from each group) as representative subsample
[d] DCBP residues are included in data (inseparable from dicofol on tlc)
[e] One set of data includes DCBH residues (inseparable from FW-152 on tlc)
[f] Dicofol residues included in data (inseparable from FW-152 on tlc)
[g] This data was generated from separate analyses under less polar tlc conditions which separated FW-152 and dicofol or DCBH and OH-DCBP
[h] CBA and OH-DCBH residues are reported together since they were inseparable under the tlc conditions used
[i] Data is from a zone which cochromatographed with DDE but was shown not to be DDE. See text for details.

Source: CBI p.70.

H. Metabolites in Plasma: Results are shown in Table 7. Extraction of plasma [^{14}C] varied considerably among groups. Less radioactivity was extractable from 48- to 168-hour samples than from 0- to 48-hour samples. Extractable [^{14}C] was associated mainly with polar metabolites, most of which remained unidentified.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Following a single oral dose of [^{14}C]dicofol at 50 mg/kg to male and female rats, 85.7±13.2 and 102.5±25.2 percent of the dose was accounted for up to 168 hours postadministration in males and females, respectively. Feces was the major route of excretion. Levels of [^{14}C] were highest in tissues after 48 hours and decreased by 168 hours after dosing. Adipose tissue contained the highest levels of radioactivity, with concentrations in females approximately 2.5 to 4.5 times those in males. Concentrations of [^{14}C] in liver and whole blood were similar for males and females at 48 hours postdosing.

In spite of its close structural similarity to DDT, dicofol is metabolized in rats by a unique pathway (Figure 1), quite distinct from DDT metabolism (Figure 2). Dicofol is not converted to DDE. The small amounts of radioactivity associated with the "DDE" zone are most likely an artifact of the chromatographic methods used or from the 0.01 percent DDE present in the test material. Dicofol is converted to a number of polar metabolites such as FW-152, DCBH, OH-DCBP, CHA, and DCBA-glycine, which are excreted through the feces and urine. The unidentified metabolites were most likely conjugates or glucuronides of dicofol metabolites. [^{14}C]dicofol is eliminated from rats two to ten times faster than is [^{14}C]DDT.

B. A signed quality assurance statement dated March 24, 1986 was included for the work performed by Analytical Bio-Chemistry. No statement was present for the work performed by Rohm and Haas.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study was designed to produce samples of urine, feces, liver, fat, and blood that contained sufficient radioactivity for metabolite analysis. Therefore, it does not meet guideline requirements for excretion or tissue distribution studies. However, it is acceptable as a study to demonstrate the metabolism of dicofol.

The data on excretion and tissue distribution of [^{14}C] showed large individual variation, with some standard deviations exceeding the corresponding means. Female rat No. 14, in particular, deviated from the other animals, showing relatively high [^{14}C] levels in urine and low [^{14}C] levels in feces. Additionally, [^{14}C] levels in tissues, especially fat and blood, were extremely low. The study

Table 7

Distribution of Metabolites in Blood Plasma from Male and Female Rats After a Single Oral Dose of ^{14}C-Dicofol

	Males[a]		Females[a]	
	0-48 h	48-168 h	0-48 h	48-168 h
Average % of ^{14}C Dose:	0.77 ± 0.38	0.11 ± 0.04	0.71 ± 0.29	0.29 ± 0.13
Average % of ^{14}C in Extract:	75.1 ± 2.26	33.8 ± 8.77	75.1 ± 18.9	55.9 ± 12.4
Average % of ^{14}C Extract as[b]:				
DCBP/Dicofol/FW-152[c]	11.8 ± 9.55	7.50 ± 1.41	14.3 ± 1.06	18.3 ± 0.35
DCBH	4.50 ± 3.82[d]	1.55 ± 0.35	3.55 ± 0.21	2.45 ± 0.92
OH-DCBP	2.80	1.95 ± 0.35	7.50 ± 0.99	3.55 ± 1.48
OH-DCBH/CBA[e]	4.55 ± 0.64	3.80 ± 2.69	4.45 ± 1.78	3.00 ± 0.85
DCBA	7.20 ± 5.52[f]	5.30 ± 0.57	0.75 ± 0.35	0.75 ± 0.21
DCBA-glycine	9.20	4.65 ± 2.19	2.45 ± 0.49	2.25 ± 1.63
unknown	31.9	4.4	12.6 ± 1.70	9.90
unidentified (includes baseline + 4-6 minor zones)	29.4 ± 17.7	77.3 ± 8.3	54.5 ± 5.44	64.8 ± 7.35

[a] Total of 4 samples analyzed, 2 from each group

[b] Mean ± Standard Deviation

[c] This data represents total residues of DCBP, dicofol and FW-152 (inseparable by tlc)

[d] Some OH-DCBP residues are included (inseparable from DCBH by tlc)

[e] These metabolites were inseparable under the conditions used

[f] Includes residues of unidentified metabolites (inseparable from DCBA)

authors did not discuss this point. One possible explanation could be that the animals had diarrhea associated with corn oil dosing, causing a decrease in the amount of [^{14}C]dicofol absorbed and of the mixture of urine and feces in the collection funnel. Another less likely explanation could be that this rat metabolized the absorbed dicofol much faster than the other rats, resulting in higher urinary versus fecal excretion of polar metabolites and lower tissue storage of less polar compounds. This reasoning is also supported by the results indicating that tissues and excreta from rat No. 14 were consistently higher in unidentified polar metabolites than the other animals. Consequently, results from this animal were not included in the mean values.

The authors used sufficient analytical methods to demonstrate the structure of the identified metabolites. Results from Rohm and Haas' laboratory agreed with those of Analytical Bio-Chemistry. The identified metabolites all had the original dicofol oxygen intact, strongly supporting the authors' conclusion that dicofol metabolism is totally different from DDT metabolism. Analyses by both laboratories of samples for residues of DDE, particularly in fat, indicated that traces of this compound were present in some of the samples. However, the amounts detected were extremely low and may have been due to the DDE present in the [^{14}C]dicofol or an artifact of the chromatography. The formation of DDE could theoretically occur by dehydration, i.e., removal of H_2O from FW-152. However, for this reaction to occur, it would require the removal of a positive hydroxyl radical, an unlikely process in biological systems. Therefore, we agree with the authors that despite the structural similarity between dicofol and DDT, they are metabolized by different pathways; dicofol metabolism does not produce significant amounts of DDE. However, it should be pointed out that these experiments were conducted with very pure dicofol. The concern therefore would be that technical Kelthane may contain larger amounts of DDE and/or DDT as impurities; DDT is readily metabolized to DDE, which then accumulates in fat.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Analysis of Liver and Fat: Analytical Bio-Chemistry's Methodology, CBI pp. 401-408; Appendix B, Study Protocol, CBI pp. 108-113.

APPENDIX A

Analysis of Liver and Fat:
Analytical Bio-Chemistry's Methodology
(CBI pp. 401-408)