Microfiche

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

DATE August 6, 1992

009686

MEMORANDUM AUG 19 1992

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT 2-ETHYLHEXYL ESTER OF 2,4-D: Generic Data Submission As Required in the Registrations Standard [Guideline 85-1].

FROM: Jess Rowland, Toxicologist Jess Rowland 8/6/92
Section II, Toxicology Branch II
Health Effects Division (H7509C)

TO: W. Waldrop / J. Coombs
Product Manager (71)
Reregistration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 8/6/92
Section II, Toxicology Branch II
Health Effects Division (H7509C)
and
for Marcia van Gemert, Ph.D., Chief James N. Rowe 8/10/92
Toxicology Branch II
Health Effects Division (H7509C)

TASK IDENTIFICATIONS: Submission: S416536 BARCODE: D177262

Caswell No. 315 AS PCCODE: 030063

Registrant: Industry Task Force II on 2,4-D Research data

ACTION REQUESTED: Review of a general metabolism study [85-1] for the 2-Ethylhexyl ester of 2,4-D [2,4-D-EHE].

SUMMARY: Following a single oral administration to male rats, ^{14}C-2,4-D-EHE was rapidly absorbed with a peak plasma concentration of 1.03 µg/g of plasma occurring 4 hr post-dosing and then decreasing with a half-life of 9 hr. Once absorbed, ^{14}C-2,4-D-EHE was extensively metabolized and eliminated in the urine, feces and as expired $^{14}CO_2$. It was evident that 2,4-D-2-EHE was rapidly hydrolyzed to 2,4-D and ^{14}C-2-ethylhexanol since no 2,4-D-2-EHE was found in the blood, urine or feces. The principal route of excretion was the urine [62-66%] which was followed by feces [14 to 21%] and expired $^{14}CO_2$ [9 to 12%].

Printed on Recycled Paper

Metabolites found both in the urine and feces were: 2-ethylhexanol, 2-ethylhexanoic acid, 2-ethyl-1,6-hexanedioic acid, and 2,4-D. Metabolites found in the urine but not in the feces were: 2-ethyl-5-ketohexanoic acid, 2-ethyl-5-hydroxy hexanoic acid, 2-heptanone and 4-heptanone. These metabolites have been previously reported as the metabolites of ^{14}C-2-ethylhexanol.

This study demonstrated that orally administered ^{14}C-2,4-D-EHE was rapidly absorbed and hydrolyzed to 2,4-D and ^{14}C-2-ethylhexanol. 2,4-D was rapidly excreted unchanged in the urine. ^{14}C-2-ethylhexanol was further metabolized to a variety of metabolites and then rapidly eliminated.

In a protocol for a metabolism study on 2,4-D submitted to the Agency, the registrant proposed conducting a single dose study with 2,4-D-EHE which will show that "the disposition of radioactivity following administration of ^{14}C-2,4-D-EHE is expected to be similar to the reported disposition of radioactivity following administration of ^{14}C-2-ethylhexanol and will provide evidence that the fate of the ester moiety was not affected by the presence of 2,4-D"[see attached Memo from B.Backus, HED to C. Grubbs, Rd, 01/29/90]].

In its review of the protocol, the Agency concurred with this approach "if the study findings indicate that the fate of the 2-ethylhexanol is not affected by the presence of 2,4-D, then this will be sufficient to satisfy the metabolism data requirement on 2,4-D-EHE" [see attached Memo from B.Backus, HED to C. Grubbs, Rd, 01/29/90]].

However, the data obtained from this study failed to show that the fate of the alcohol moiety is not affected by the presence of 2,4-D as an ester. Urinary metabolites observed in this study were qualitatively similar to those observed in urine of rats given a single oral dose of 2-ethylhexanol [Albro, 1975]. Although qualitatively similar, their relative proportions were markedly different. Although many factors may have contributed to these differences, the present data do not support the concept that biotransformation of 2-ethylhexanol given alone is the same as the biotransformation of 2-ethylhexanol given as the 2,4-D ester.

CORE CLASSIFICATION: UNACCEPTABLE; this study is classified as unacceptable since the results do not show that the disposition of the 2-ethylhexanol given as 2,4-D ester is the same as when given alone as 2-ethylhexanol. Therefore, this study does not satisfy guideline requirement 85-1 for a general metabolism study. A full metabolism study is required to satisfy guideline 85-1.

009686

PRIMARY REVIEWER: Jess Rowland, Toxicologist Jess Rowland 8/5/92
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head K. Clark Swentzel 8/6/92
Section II, Toxicology Branch II

DATA EVALUATION REPORT

STUDY TYPE: General Metabolism **GUIDELINE:** 85-1

CASWELL NO. 315 AS MRID No. 422618-01 BARCODE: D177262

TEST MATERIAL: 2-ETHYLHEXYL ESTER of 2,4-D [2,4-D-EHE]

REGISTRANT: Industry Task Force II on 2,4-D Research Data.

TESTING LABORATORY: The Toxicology Research Laboratory, Michigan

STUDY IDENTIFICATION: K-020054-009

TITLE OF REPORT: *2,4-DICHLOROPHENOXYACETATE 2-ETHYLHEXYL ESTER: METABOLISM IN FISHER 344 RATS.*

AUTHOR(S): M.D. Dryzga, K. A. Brzak and R.J. Nolan

REPORT DATE: March 6, 1992

SUMMARY: Following a single oral administration to male rats, ^{14}C-2,4-D-EHE was rapidly absorbed with a peak plasma concentration of 1.03 µg/g of plasma occurring 4 hr post-dosing and then decreasing with a half-life of 9 hr. Once absorbed, ^{14}C-2,4-D-EHE was extensively metabolized and eliminated in the urine, feces and as expired $^{14}CO_2$. It was evident that 2,4-D-2-EHE was rapidly hydrolyzed to 2,4-D and ^{14}C-2-ethylhexanol since no 2,4-D-2-EHE was found in the blood, urine or feces. The principal route of excretion was the urine [62-66%] which was followed by feces [14 to 21%] and expired $^{14}CO_2$ [9 to 12%]. Metabolites found both in the urine and feces were: 2-ethylhexanol, 2-ethylhexanoic acid, 2-ethyl-1,6-hexanedioic acid, and 2,4-D. Metabolites found in the urine but not in the feces were: 2-ethyl-5-ketohexanoic acid, 2-ethyl-5-hydroxy hexanoic acid, 2-heptanone and 4-heptanone. These metabolites have been previously reported as the metabolites of ^{14}C-2-ethylhexanol. The data also indicated that the biotransformation pattern of the 2-ethylhexanol moiety of the ester is different from the biotransformation pattern of 2-ethylhexanol administered alone.

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a general metabolism study.

II. MATERIALS AND METHODS

1. Test Material

Common Name: 2-Ethylhexyl ester of 2,4-D

Radioactive: 2-ethylhexyl-1-^{14}C-ester of 2,4-D
Radiochemical purity: 98.4%
Specific activity: 7.88 mCi/mmol
Lot No. GHD 1345-78a

Nonradioactive: 2-ethylhexyl ester of 2,4-D
99.5% pure
Lot No. AGR #236832

2. Test Animals

Species: Rats
Strain: Fisher 344
Sex: Male
Age: Approximately 7 weeks
Weight: 194 - 203 g
Identification: Ear tags
Acclimation: 1-week
Housing: Individual Roth-type metabolism cages
Food: Certified rodent chow #5002 ad libitum
Water: Tap water ad libitum
Environment: Temperature, 19-23°C; Humidity, 40-60%; Photocycle, 12 hr.

3. Study Design:

This study was designed to demonstrate that the metabolism and excretion of 2-ethylhexanol, a hydrolysis product of 2,4-D-EHE, is similar regardless of wheather the compound in given as the 2,4-D ester or alone. The expired air was collected to determine whether significant amounts of $^{14}CO_2$ would be eliminated in the expired air of treated animals. The amount of ^{14}C-2,4-D-EHE and radioactivity administered to rats are summarized in Table 1.

4. Test Material Formulation

A stock solution in acetone was prepared by adding 0.987 mCi of ^{14}C-2-EHE [7.88 mCi/mmol] to 1.7 mL of acetone. The dosing solution was prepared by adding 0.0331 g of non-radiolabeled 2-EHE into a vial and then spiking with 216 µL of the stock solution. The acetone was removed by evaporating under a N_2 stream and 5 mL of corn oil was added. The 2,4-D EHE concentration in the dose solution was 8.21 mg/g and the specific activity of the dose solution was 6943 dpm/µg 2,4-D-2-EHE.

5. Treatment

The rats were weighed and based on their body weight a measured volume of the dose solution was administered by gavage. The quantity of the dose solution was determined by weighing the syringe prior to and following dosing [see Table 1]. Administration of the dose solution at the rate of 2 mL/kg resulted in a targeted dose of 15 mg ^{14}C-2-EHE/kg

6. Experimental Procedures

Blood was collected from each rat at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 hours post-dosing, and plasma was analyzed for radioactivity

Urine was collected at 6, 12, 24 and 48 hours post-dosing, and was analyzed for radioactivity. The urine and cage rinse radioactivity was combined for each collection interval and expressed as radioactivity excreted in the urine. Total urinary ^{14}C-activity for each interval represented the combination of the urine and cage rinse for that interval.

Feces were collected at 24-hour intervals in dry-ice chilled containers, and analyzed for radioactivity.

Expired $^{14}CO_2$ collected at 6, 12, 24 and 48 hours was trapped in a solution of monoethanolamine: 1-methoxy-2-propanol and the radioactivity was quantified.

Tissue samples were not analyzed.

^{14}C radioactivity was quantified with a Beckman LS3801 liquid scintillation counter.

Metabolite characterization studies were performed with pooled urine [0-6 and 6-12 hr] and fecal [0-24 hr] samples. Gas chromatography/mass spectrometry analysis was employed for characterization of metabolites. Analysis of unchanged 2,4-D-EHE in pooled urine and fecal extracts was done by HPLC.

7. Regulatory Compliance

A signed Statement of No Confidentiality Claim was provided that was dated February 28, 1992.

A signed Statement of Compliance with EPA's GLP was provided that was dated March 6, 1992.

A flagging statement per 40 CFR. 158.34 indicated that the criteria for flagging studies does not apply to this study; this was dated February 28, 1992.

A signed Quality Assurance Statement was dated March 6, 1992. This date conforms to the review of the study phases and the draft and the final reports.

III. RESULTS

1. Adequacy of Dosing

The amount of ^{14}C-2-EHE, 2,4-D and radioactivity each animal received are summarized in Table 1. The average amounts of ^{14}C-2-EHE [14.9 mg/kg] administered was within 1% of the targeted dose of 15 mg/kg. The average amount of radioactivity given [46.5 µCi/kg] was 93% of the targeted 50 µCi/kg.

2. Absorption

Concentrations of ^{14}C-2-EHE in the plasma at various intervals are summarized in Table 2. Orally administered ^{14}C-2-EHE was readily and extensively absorbed by the gastro-intestinal tract. The rapid absorption was evident since the highest concentration of radioactivity in the plasma peaked at 4 hr after dosing at 1.03 µg eq ^{14}C-2-ethylhexanol/g plasma and then decreased with an apparent half-life of approximately 9 hours. The amount of radioactivity detected in the urine, cage rinse, and eliminated as expired $^{14}CO_2$ indicate that a minimum of 75% of the administered ^{14}C-2-EHE was absorbed. No unchanged 2,4-D-2-EHE was found in the blood.

3. Distribution

The distribution of radioactivity recovered 48 hours post-dosing is summarized in Table 3. Approximately 93% of the radioactivity was recovered: 64% in the urine, 18% in the feces, 10% as $^{14}CO_2$, and 0.65% in the final cage wash.

4. Metabolism

The proposed metabolic pathway of 2,4-D-EHE is shown in Figure 1. The metabolites identified and quantified in the urine are presented in Table 4. The urinary metabolites identified were: 2-ethylhexanol, 2-ethylhexanoic acid, 2-ethyl-5-ketohexanoic acid, 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-1,6-hexanedioic acid, 2-heptanone, 4-heptanone, and 2,4-D. No parent 2,4-D-2-EHE was detected in the urine.

Of the radiolabelled urinary metabolites, 2-ethyl-1,6-hexanedioic acid was the major metabolite and accounted for approximately 59% of the radioactivity in the 0-6 hr urine sample and 41% in the 6-12 hr urine samples. The 2-Ethyl-5-hydroxyhexanoic acid represented about 11% of the urinary radioactivity in the 0-6 and 6-12 hr samples.

The other radiolabelled metabolites accounted for 10% of the urinary radioactivity: 2-ethylhexanol [0.5%], 2-ethylhexanoic acid [3.7%], and 2-ethyl-5-ketohexanoic acid [5.9%] in the 0-6 and 6-12 hr urine samples. The remaining [approximately 25-41%] of the urinary radioactivity was not identified. Of the non-radiolabeled metabolites, 2- and 4-heptanone accounted for less than 4% of the dose while 71.8% of the administered dose was excreted unchanged as 2,4-D acid.

The metabolites identified and quantified in the 0-24 hr fecal samples are presented in Table 5. Fecal metabolites identified were: 2-ethylhexanol, 2-ethylhexanoic acid, 2-ethyl-1,6-hexanedioic acid, and 2,4-D. Neither the parent 2,4-D-2-EHE nor the other urinary metabolites [2-ethyl-5-ketohexanoic acid, 2-ethyl-5-hydroxyhexanoic acid, 2- and 4-heptanone] were detected in the feces.

Of the radiolabeled fecal metabolites, 2-ethyl-1,6-hexanedioic acid was the major fecal metabolite and accounted for approximately 72% of radioactivity in feces followed by 2-ethylhexanoic acid [19%] and 2-ethylhexanol [8%]. A total of 99.2% of the radioactivity in feces was identified. Of the non-radiolabeled metabolites only 2.5% of the administered dose was excreted as the 2,4-D acid.

The proportion of 2,4-D excreted in the urine during the first 12 hr post-dosing [i.e.,71.8% of the dose] was almost identical to the proportion of 2,4-D excreted by male rats given a single 1 mg/kg oral dose of ^{14}C-2,4-D acid [69.3%; MRID No. 417373-02; HED Document No. 008561].

5. Excretion

Percentages of radioactive dose excreted in the urine, feces and as $^{14}CO_2$ are presented in Tables 6, 7, and 8, respectively. Following rapid absorption and extensive biotransformation, radioactivity from ^{14}C-2-EHE is rapidly eliminated in the urine, feces and as expired $^{14}CO_2$. After 48 hr, between 91 and 95% of the administered radioactivity was recovered in the urine, feces, $^{14}CO_2$ and final cage wash.

The principal route of excretion is the urine, which contained 62 to 66% of the radioactive dose [Table 6]. The urinary excretion rate peaked at approximately 5.4% dose/hr during the 6-12 hr period and then declined with a half-life of about 4 hr. Greater than 60% of the dose was excreted in the urine by 24 hr post-dosing.

Fecal excretion accounted for approximately 18% of the dose [Table 7]. As with the urine, most [17% of administered dose] of the radioactivity was eliminated during the first 24-hr post-dosing.

Elimination via the expired $^{14}CO_2$ accounted for a total of 10.4% of administered radioactivity with 9.25% eliminated within 12 hr post-dosing and the reminder [1.1%] being eliminated by 48 hr. The final cage wash contained less than 1% of the administered radioactivity [table 8].

6. Metabolite Characterization

Metabolites characterized in the urine and feces are listed below:

Metabolites	Urine	Feces
2-ethylhexanol	Yes	Yes
2-ethylhexanoic acid	Yes	Yes
2-ethyl-5-ketohexanoic acid	Yes	No
2-ethyl-5-hydroxyhexanoic acid	Yes	No
2-ethyl-1,6-hexanedioic acid	Yes	Yes
2-heptanone	Yes	No
4-heptanone	Yes	No
2,4-D	Yes	Yes

The metabolites identified in the urine and feces in this study are similar to the metabolites identified in the urine and feces of rats given ^{14}C-2-ethyl hexanol by Albro 1975 [Table 4]. Although qualitatively the metabolites are similar, their relative proportions are very different as those found in this study. For example, Albro reported that 2-ethylhexanoic acid comprises 45.8% of the urinary radioactivity, whereas the presnet study finds that it comprises only 1.8 to 1.9% of the urinary radioactivity. In addition, 2-ethyl-1,6-hexanedioic acid comprises 18.8% of the urinary radioactivity in the Albro study, whereas in the present study a 3-fold higher volume of up to 59.2% is found [Table 4]. These differences do not support the concept that biotransformation patterns of 2-ethylhexanol given as the 2,4-D ester are the same as those of 2-ethylhexanol given alone.

III. DISCUSSION

Following a single oral administration to male rats, ^{14}C-2-EHE is rapidly absorbed and hydrolyzed to 2,4-D and ^{14}C-2-ethylhexanol. The concentration of radioactivity in the plasma peaked 4 hr post-dosing and then decreased in with a half-life of 9 hr. No 2,4-D-EHE was found in the blood, urine or feces indicating that 2,4-D-EHE was rapidly hydrolyzed. Once absorbed, ^{14}C-2-EHE is extensively metabolized and eliminated in the urine, feces and as expired $^{14}CO_2$.

The major route of excretion was the urine [62-66%] while the feces contained between 14 and 21% of the dose. Expired $^{14}CO_2$ accounted for 9 to 12% of the dose and the final cage wash contained less than 1% of the administered radioactivity. The majority of the 2,4-D is rapidly eliminated unchanged in the urine, and the excretion of 2,4-D in the urine was similar to that previously reported in male rats following oral administration of 1 mg/kg of ^{14}C-2,4-D [MRID No. 417373-02; HED Document No. 008561]. Metabolites found both in the urine and feces were: 2-ethylhexanol, 2-ethylhexanoic acid, 2-ethyl-1,6-hexanedioic acid, and 2,4-D. Metabolites found in the urine but not in the feces were: 2-ethyl-5-ketohexanoic acid, 2-ethyl-5-hydroxy hexanoic acid, 2-heptanone and 4-heptanone.

In a protocol for a metabolism study on 2,4-D submitted to the Agency, the registrant proposed conducting a single dose study with 2,4-D-EHE which will show that "the disposition of radioactivity following administration of ^{14}C-2,4-D-EHE is expected to be similar to the reported disposition of radioactivity following administration of ^{14}C-2-ethylhexanol and will provide evidence that the fate of the ester moiety was not affected by the presence of 2,4-D."

In its review of the protocol, the Agency concurred with this approach "if the study findings indicate that the fate of the 2-ethylhexanol is not affected by the presence of 2,4-D, then this will be sufficient to satisfy the metabolism data requirement on 2,4-D-EHE".

Albro [1975] reported the same metabolites in urine of rats given a single oral dose of ^{14}C-2-ethylhexanol as those observed in this study. Although qualitatively the metabolites were similar, their relative proportions are very different between the two studies. Consequently, the data from this study, failed to show that the fate of the alcholol moiety is not affected by the presence of 2,4-D as an ester. These differences do not support the concept that biotransformation patterns of 2-ethylhexanol given as the 2,4-D ester are the same as those of 2-ethylhexanol given alone.

IV. CONCLUSION

This study demonstrates that orally administered ^{14}C-2,4-D-EHE is rapidly absorbed and hydrolyzed to 2,4-D and ^{14}C-2-ethylhexanol; 2,4-D is rapidly excreted unchanged in the urine. ^{14}C-2-ethylhexanol is further metabolized to a variety of metabolites and then rapidly eliminated. The study did not show that the biotransformation of 2-ethylhexanol given alone is the same as the biotransformation of 2-ethylhexanol given as the 2,4-D ester.

V. CORE CLASSIFICATION

UNACCEPTABLE; this study is classified as unacceptable since the results does not show that the disposition of the 2-ethylhexanol given as 2,4-D ester is the same as when given alone as 2-ethylhexanol. Therefore, this study does not satisfy guideline requirement 85-1 for a general metabolism study. A full metabolism study is required to satisfy guideline 85-1.

Table 1. Amount of Radioactivity and 2,4-D-2EHE Administered as a Single Oral Dose to Male Rats.

Animal No.	Body Weight [kg]	Syringe Weight Full [g]	Syringe Weight Empty [g]	Net [g]	Dose Administered[a]		
					dpm	mg	mg/kg
90A-2268	0.203	9.7382	9.3620	0.3762	21444821	3.089	15.2
90A-2269	0.199	9.7252	9.3667	0.3585	20435854	2.943	14.8
90A-2272	0.194	9.7187	9.3654	0.3533	20139435	2.901	15.0
90A-2273	0.202	9.7293	9.3672	0.3621	20641068	2.973	14.7
Mean	**0.200**			**0.3625**	**20665295**	**2.977**	**14.9**
S.D	**0.004**			**0.0098**	**558993**	**0.081**	**0.2**

a) dpm/gram dose solution = 57,003,778 and there was 8.21 mg 2,4-D-2EHE/g dose solution.

Specific activity = 6,943 dpm/ µg 2,4-D-2-EHE.

00096386

Table 2. Concentration of Radioactivity [as µg eq 2-ethylhexanol/g of plasma] in the Plasma of Rats Following A Single Oral Dose of 15 mg ^{14}C-2,4-D-2-EHE/kg

Sampling Time [hr]	90A-2268	90A-2269	90A-2272	90A-2273	Mean	S.D
0.5	0.1757	0.3060	0.3590	0.0170	0.2144	0.1525
1.0	0.5861	0.4082	0.4883	0.0796	0.3906	0.2197
1.5	0.8439	0.6817	1.0029	0.2463	0.6937	0.3258
2.0	0.6614	1.1499	1.3359	0.5145	0.9154	0.3903
4.0	1.1344	1.0083	1.1015	0.8757	1.0300	0.1159
6.0	0.8959	0.8785	0.9194	0.8027	0.8741	0.0505
8.0	0.7818	0.6166	0.7054	0.6692	0.6932	0.0694
12.0	0.5136	0.4469	0.04944	0.4591	0.4785	0.0309
18.0	0.4045	0.2938	0.3071	0.3085	0.3285	0.0511
24.0	0.2565	0.1818	0.2149	0.1823	0.2089	0.0353

* µg equivalent ^{14}C-2-ethylhexanol/g plasma based on 17,803 dpm/µg of 2-ethylhexanol.

009686

Table 3. Distribution of Radioactivity Recovered in Male Rats 48 hours Following A Single Oral Dose of 15 mg ^{14}C-2,4-D-2EHE/kg.

Percent of Administered Dose

	90A-2268	90A-2269	90A-2272	90A-2273	Mean	S.D
Urine	65.28	62.04	64.38	65.63	64.33	1.62
Feces	17.06	20.88	14.78	18.39	17.78	2.55
$^{14}CO_2$	11.09	9.08	11.52	10.08	10.44	1.09
Final Cage Wash	0.99	0.50	0.87	0.24	0.65	0.34
Total	94.42	92.50	91.55	94.34	93.20	1.41

009686

Table 4. Metabolites in the Urine of Male Rats Given A Single Oral Dose of 15 mg ^{14}C-2,4-D-2-EHE/kg.

Radiolabeled Metabolites	Percent of Urinary Radioactivity		
	0-6 hr Urine	6-12 hr Urine	Albro [1975]* 0-18 hr Urine
2-Ethylhexanol	0.3	0.2	2.8
2-Ethylhexanoic acid	1.9	1.8	45.8
2-Ethyl-5-ketohexanoic acid	1.4	4.5	<1
2-Ethyl-5-hydroxyhexanoic acid	11.7	11.4	10.1
2-Ethyl-1,6-hexanedioic acid	59.2	41.1	18.8
Total Identified	**74.5%**	**59.0%**	**77.5%**
Total Unidentified	**25.5%**	**41.0%**	**22.5%**
	Percent of Dose		
Non-Radiolabeled Metabolites	**0 - 6 hr Urine**	**6-12 hr Urine**	**0-18 hr Urine**
2-Heptanone	0.7	1.8	7.5% [2-and 4-heptanone
4-Heptanone	0.6	0.6	
2,4-D acid	21.1	50.7	NA

* Comparative data cited from Albro [Xenobiotica 5;625-636, 1975] by the Registrant. Metabolites of 2-ethylhexanol in urine of CD rats after a single dose of 2-ethyl [1-^{14}C] hexanol.

009638

Table 5. Metabolites in the Feces of Male Rats Given A Single Oral Dose of 15 mg ^{14}C-2,4-D-2-EHE/kg.

Percent of Fecal Radioactivity

Radiolabeled Metabolites	0 - 24 hr Feces
2-Ethylhexanol	8.0
2-Ethylhexanoic acid	19.3
2-Ethyl-5-ketohexanoic acid	Not Detected
2-Ethyl-5-hydroxyhexanoic acid	Not Detected
2-Ethyl-1,6-hexanedioic acid	71.9
TOTAL	**99.2%**
Non-Radiolabeled Metabolites	**% of Dose 0 - 24 hr Feces**
2-Heptanone	Not Detected
4-Heptanone	Not Detected
2,4-D acid	2.5

009688

16

Table 6. Radioactivity Excreted in the Urine of Rats Administered A Single Oral Dose of 15 mg ^{14}C-2,4-D-2EHE/kg.

Percent Administered Dose

Collection Interval	90A-2268	90A-2269	90A-2272	90A-2273	Mean	S.D
0-6 hr Urine	13.32	17.98	25.56	14.59		
0-6 hr Cage Rinse	1.55	5.14	2.55	3.22		
Urine + Rinse	**14.87**	**23.12**	**28.11**	**17.81**	**20.98**	5.85
6-12 hr Urine	35.31	28.49	26.72	33.39		
6-12 hr Cage Rinse	1.31	1.74	1.48	1.19		
Urine + Rinse	**36.62**	**30.23**	**28.20**	**34.58**	**32.41**	3.87
12-24 hr Urine	11.09	6.08	6.71	11.26		
12-24 hr Cage Rinse	0.26	0.70	0.25	0.71		
Urine + Rinse	**11.35**	**6.78**	**6.96**	**11.97**	**9.27**	2.78
24-48 hr Urine	2.35	1.74	0.97	1.19		
24-48 hr Cage Rinse	0.08	0.17	0.14	0.09		
Urine + Rinse	**2.43**	**1.91**	**1.11**	**1.28**	**1.68**	0.61
TOTAL	**65.27**	**62.04**	**64.38**	**65.64**	**64.33**	1.62

009686

Table 7. Radioactivity Excreted in the Feces of Rats Administered A Single Oral Dose of 15mg ^{14}C-2,4-D-2-EHE/kg

Percent Administered Dose

Collection Interval	90A-2268	90A-2269	90A-2272	90A-2273	Mean	S.D
0 - 24 hr	15.39	19.62	14.07	18.08	16.79	2.52
24 - 48 hr	1.67	1.26	0.71	0.31	0.99	0.60
TOTAL	17.06	20.88	14.78	18.39	17.78	2.55

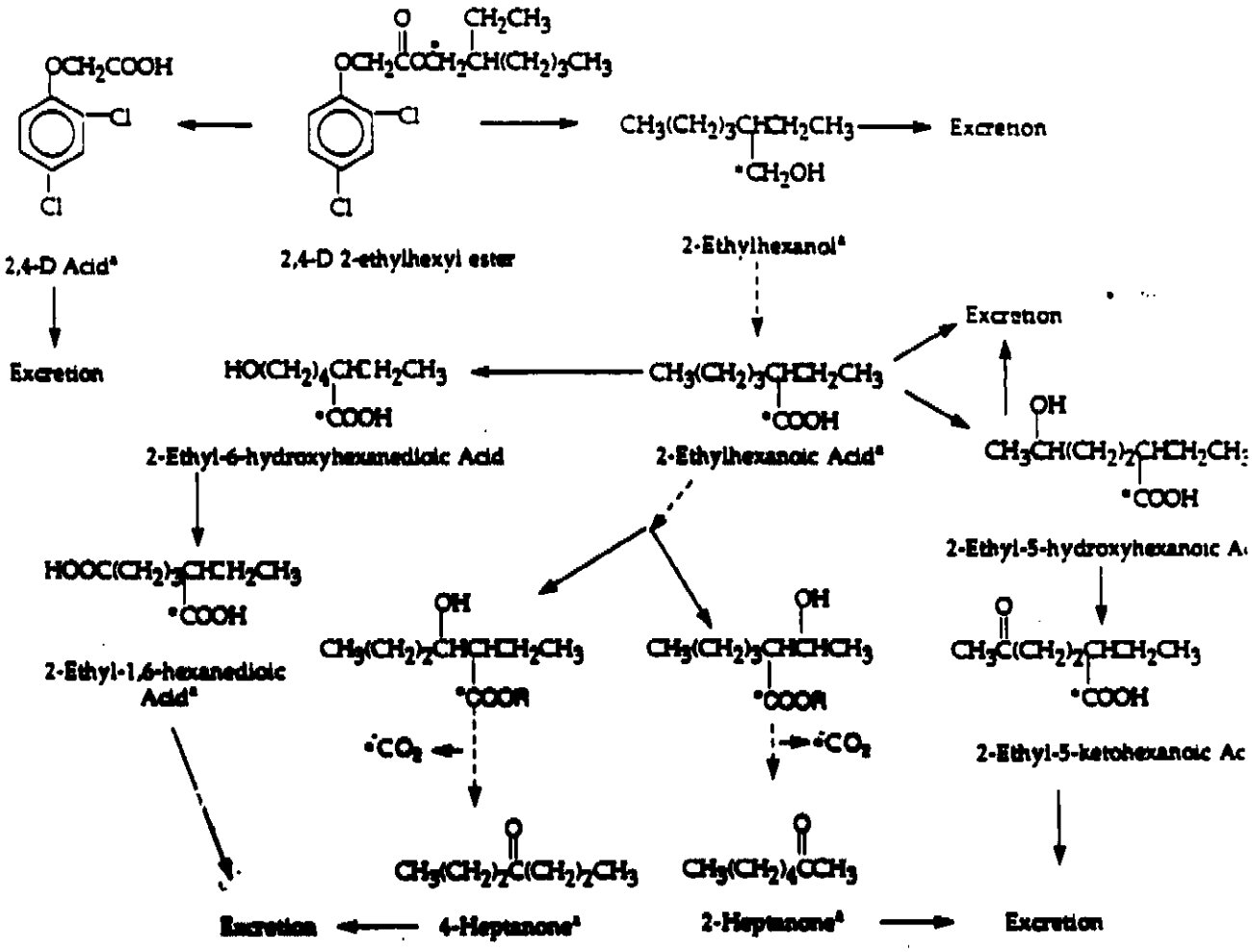
Table 8. Radioactivity Excreted As $^{14}CO_2$ in Rats Administered A Single Oral Dose of 15 mg ^{14}C-2,4-D-2EHE/kg.

Percent Administered Dose

Collection Interval	90A-2268	90A-2269	90A-2272	90A-2273	Mean	S.D
0 - 12 hr	5.73	4.96	5.93	4.33	5.24	0.74
6 - 12 hr	4.03	3.11	4.29	4.61	4.01	0.65
12 - 24 hr	0.77	0.57	0.66	0.59	0.65	0.09
24 - 48 hr	0.56	0.44	0.64	0.55	0.55	0.08
TOTAL	11.09	9.08	11.52	10.08	10.44	1.09

009698

Figure 1. Proposed Metabolic Pathway of 2,4-D EHE in Male Rats



Note: * designates 14C and [a] designates compounds actually observed as metabolites.

IOE METOD

CASWELL FILE

009686

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

JAN 29 1990

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Protocol for 2,4-D Metabolism Study

TO: Ms. Clare Grubbs, PM 74
Registration Division (H7508C)

FROM: Byron T. Backus, Ph.D. Byron T. Backus 01/22/90
Toxicologist
Fungicide/Herbicide/Antimicrobial Toxicology Branch
HED (H7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 1/23/90
Section Head, Review Section II
Fungicide/Herbicide/Antimicrobial Toxicology Branch
HED (H7509C)

and

Marcia van Gemert, Ph.D. Marcia Gemert 1/24/90
Branch Chief
Fungicide/Herbicide/Antimicrobial Toxicology Branch
HED (H7509C)

EPA Record No. 252821

Project No. 9-2213

EPA Reg. No.

Tox. Chem. 315

Action Requested:

Review a protocol for a metabolism study on 2,4-D,

Comments and Recommendations:

1. On page 4 of the protocol for the 2,4-D metabolism study it is specified that: "the first and second groups will be given single oral doses of 1 or 100 mg 2,4-^{14}C-D/kg body weight. The third group will be given a single intravenous (iv) dose of 1 mg 2,4-^{14}C-D/kg body weight. The fourth group will be given 14 daily single oral doses of non-radiolabelled 2,4-D at

the low dose level (3 mg/kg) followed by a single oral dose of 2,4-^{14}C-D on day 15." It is recommended that the low dose - whether 1 or 3 mg 2,4-^{14}C-D/kg body weight - be consistent for the first and fourth (as well as possibly the third) groups.

2. On page 5 it is stated that the 2,4-^{14}C-D will be "uniformly labelled." It is uncertain whether this should be "uniformly ^{14}C-ring labelled 2,4-D" or whether labeling would include the carbons of the acetyl group. The Section F Guidelines state that labeling should be "in positions that may be expected to follow the "core" of the molecule or significant portions thereof." However, this is a fairly minor point as the information provided indicates that 2,4-D is not metabolized to any appreciable extent.

3. The reporting of the proposed studies should include findings from individual animals, as well as group means ± S.D. The information presented should also include detection limits.

4. The cover letter states that Dow Chemical is proposing to submit data that will show the DMA salt of 2,4-D rapidly dissociates in water to DMA (dimethylamine) and 2,4-D. It also states that Dow Chemical believes that there is already sufficient information in the literature on DMA that no additional studies are needed to define its metabolic fate. The presentation (p. 4) on the DMA salt of 2,4-D notes that dimethylamine is formed endogenously and is a normal constituent of human urine, with a normal individual excretion rate of 17 mg/kg/day. After examining this information, we concur with this proposed approach, and with the position of the registrant that no additional studies on DMA would be necessary if it is demonstrated that 2,4-D-DMA rapidly hydrolyzes.

5. For the isooctyl ester of 2,4-D, Dow is proposing a metabolism study involving four cannulated male rats which would each be given a single oral dose of 2,4-D-IOE (^{14}C-labeled in the ester moiety) at 15 mg/kg in a corn oil vehicle. According to the protocol (p. 5): "The absence of significant amounts of 2,4-D-IOE in the blood and excreta will be taken as confirmation of earlier reports that 2,4-D-IOE is rapidly hydrolyzed <u>in vivo</u>... The disposition of radioactivity following administration of 2,4-D-^{14}C-IOE is expected to be similar to the reported disposition of radioactivity following administration of 1^{4}C-labeled 2-ethylhexanol and will provide evidence that the fate of the ester moiety was not affected by the presence of 2,4-D." We concur with this proposed approach. If the study findings indicate that the fate of the 2-ethylhexanol (with 2,4-D, one of the expected hydrolysis products of 2,4-D-IOE) is not affected by the presence of 2,4-D, then this will be sufficient to satisfy the metabolism data requirement on 2,4-D-IOE.

6. For the butoxyethyl ester of 2,4-D (2,4-D-BEE) Dow Chemical is proposing a study that will be divided into two phases. Phase I will involve assays of esterase activity from rat liver, stomach, and duodenal homogenates, as well as blood serum utilizing 2,4-D-BEE as a substrate. This will allow calculations of the in vitro half-life of 2,4-D-BEE under the assay conditions. Subsequently, four male rats will be cannulated, and then, after 24 hours, will be orally dosed with 13.6 mg/kg of 2,4-D-^{14}C-BEE. Blood and urine samples, as well as expired CO_2, will be collected from these rats over the next 48 hours and will be analyzed for radioactivity.

   If these studies demonstrate that 2,4-D-BEE is rapidly hydrolyzed, and that the excretion of radioactivity from the cannulated male rats following administration of 2,4-D-^{14}C-BEE (labeled in the ester moiety) is essentially the same as that observed in previous studies involving 2-butoxyethanol (one of the expected hydrolysis products of 2,4-D-BEE, the other being 2,4-D), then this will be sufficient to satisfy the metabolism study requirement for 2,4-D-BEE.

Tox Chem No. 315 AS File Last Updated _____ Current Date _____

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
85-1 General Metab Species:Rat Tox.Res.Lab K-020054-009 03/06/92	^{14}C-2,4-D- EHE; 98.4% pure 7.88 mCi/mmol Nonradioactive 99.5% tech.	422618-01	Male Fischer 344 rats were given a single oral doses of 15 mg ^{14}C-2,4-D-EHE/kg. ^{14}C-2,4-D-EHE was rapidly absorbed and hydrolyzed to 2,4-D and ^{14}C-2-ethylhexanol 2,4-D was rapidly excreted unchanged in the urine. ^{14}C-2-ethylhexanol was further metabolized to a variety of metabolites and then rapidly eliminated. The results does not show that the disposition of the 2-ethylhexanol given as 2,4-D ester is the same as when given alone as 2-ethylhexanol. Therefore, this study does not satisfy guideline requirement 85-1 for a general metabolism study. A full metabolism study is required to satisfy guideline 85-1.	NA	Unacceptable 009686

NEW INPUT
JR
8/5/92

009686

23

END