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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MAY 10 1996

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Glufosinate-Ammonium (Ignite[R]; Hoe 039866): Review of metabolism studies

Caswell No.: 580I **PC Code:** 128850
EPA ID No.: 5F04578 **DP Barcode:** D219070
MRID No.: 43766913, 43766914, & 43778402

TO: D. McCall, Chemical Manager
RCAB/HED (7509C)

FROM: Whang Phang, Ph.D. 5/1/96
Pharmacologist
Tox. Branch II / HED (7509C)

THROUGH: James Rowe, Ph.D. James N. Rowe 5/1/96
Section Head
and
Stephanie Irene, Ph.D. 5/8/96
Acting Branch Chief
Tox. Branch II / HED (7509C)

The registrant, AgrEvo (A Co. of Hoechst and NOR-AM), submitted 2 metabolism studies in rats along with a petition for tolerance of glufosinate-ammonium on corn and soybeans. These two studies are presented in three reports; one report contains the procedures of the high-dose (500 mg/kg) study, and the other report presents the experimental procedures, results and discussions. A separate report presents a metabolism study with a low dose (2 mg/kg). The reports have been evaluated. The DER of each report is attached. The citation and conclusion for each study are presented below:

Lauck-Birkel, S. (1995). Hoe 039866-^{14}C: Metabolism in male and female rats following single oral administration of test substance at a dose level of 500 mg/kg body weight. Hoechst Schering AgroEvo GmbH, Germany; Study No.: CM93/071. July 12, 1995. Submitted to US EPA by AgrEvo™; MRID No. 43766914. Unpublished.

Maas, J. and Braun (1995) Hoe 039866-^{14}C: Metabolism in male and female rats following single oral administration of test substance at a dose level of 500 mg/kg body weight. Hoechst Schering AgroEvo GmbH, Germany; Study No.: CM93/071. Report No. A54450. May 23, 1995. Submitted to US EPA by AgrEvo™; MRID No. 43778402. Unpublished. (This report contains only the details of the Methods and Materials for the study)

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In a metabolism study (MRID No. 43766914), groups of Wistar rats (5/sex or 2/sex) received a single dose (500 mg/kg) of ^{14}C-Hoe 039866 (glufosinate ammonium) by gavage. Animals were sacrificed at various times (2 & 6, 24, and 96 hrs) after dosing. The majority of the radioactivity was eliminated during the first 24 to 48 hrs after dosing. The parent compound, Hoe 039866, accounted for the majority of the radioactivity eliminated in the excreta of both males (≈80% of the dose) and females (88% of the dose). This finding is consistent with the results of previous metabolism studies (MRID No. 40345638 and MRID No. 43766913). The metabolite, Hoe 061517, was consistently found in both urine (0.22-1.20% of the dose) and feces (0.44-1.36% of the dose) of both sexes. Hoe 099730 was found in feces (0.28-1.72% of the dose) of both male and female rats and barely above or at the level of the detection in the urine of both sexes (0.02-0.04% of the dose). Hoe 042231 was mainly found in the feces of both male and females (≈0.2-0.28% of the dose). Very little if any of administered Hoe 039866 was sequestered in any tissues examined.

This study is **acceptable**, and provided valuable information about the metabolism of glufosinate ammonium (Guideline No. 81-5).

Stumpf, K. (1993). Hoe 039866 -^{14}C: Metabolism in male and female rats following single oral administration of test substance at a dose level of 2 mg/kg body weight. Produktentewicklung GB-C, Okologie I and Radiochemisches Laboratorium, Hoechst Aktiengesellschaft, Germany; Study No.: A49981. April 26, 1993. Submitted to US EPA by AgrEvo™; MRID No. 43766913. Unpublished.

In a metabolism study, groups of Wistar rats (5/sex) received a single dose (2 mg/kg) of ^{14}C-Hoe 039866 (glufosinate ammonium) by gavage. The majority of the radioactivity (95-98% of the dose) was eliminated during the first 24 hrs after dosing. The parent compound, Hoe 039866, accounted for most of the eliminated radioactivity in the urine and feces of both males (80% of the dose) and females (73% of the dose). The metabolite, Hoe 061517, was consistently found in both urine and feces of both sexes. Hoe 099730 (7-8% of the dose) and Hoe 042231 (≈3% of the dose) were found in the feces of both male and female rats and none in the urine.

This study is **acceptable**, and provided valuable information about the metabolism of glufosinate ammonium (Guideline No. 81-5).

CC: E. Wilson / J. Miller, PM Team 23
Fungicide-Herbicide Branch
Registration Division (7505C)

EPA Reviewer: Whang Phang, Ph.D. 4/18/96
Review Section III
Toxicology Branch II/HED (7509C)

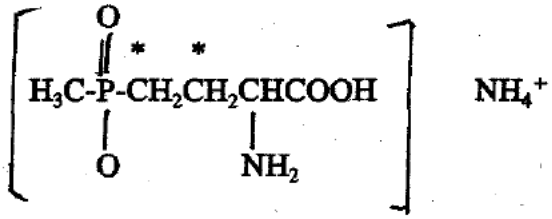
EPA Secondary Reviewer: Alberto Protzel, Ph.D. 4/18/96
Review Section III
Toxicology Branch II/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism study in rats (85-1)
(Single oral doseof 500 mg/kg)

DP BARCODE: D219070 **SUBMISSION CODE:** S493376
P.C. CODE: 128850 **TOX. CHEM. NO.:** 580I
MRID No.: 43766914 **ID No.:** 5F04578
43778402 (Information on Methods and Materials)

CHEMICAL: DL-homoalanin-4-yl(methyl)-phosphinic acid, ammonium salt



(*: location of ^{14}C-label)

SYNONYMS: Hoe 039866; DL-glufosinate ammonium

CITATION: Lauck-Birkel, S. (1995). Hoe 039866-^{14}C: Metabolism in male and female rats following single oral administration of test substance at a dose level of 500 mg/kg body weight. Hoechst Schering AgroEvo GmbH, Germany; Study No.: CM93/071. July 12, 1995. Submitted to US EPA by AgrEvo™; MRID No. 43766914. Unpublished.

Maas, J. and Braun (1995) Hoe 039866-^{14}C: Metabolism in male and female rats following single oral administration of test substance at a dose level of 500 mg/kg body weight. Hoechst Schering AgroEvo GmbH, Germany; Study No.: CM93/071. Report No. A54450. May 23, 1995. Submitted to US EPA by AgrEvo™; MRID No. 43778402. Unpublished. (This report contains only the details of the Methods and Materials for the study)

SPONSOR: Hoechst

EXECUTIVE SUMMARY: In a metabolism study (MRID No. 43766914), groups of Wistar rats (5/sex or 2/sex) received a single dose (500 mg/kg) of ^{14}C-Hoe 039866 (glufosinate ammonium) by gavage. Animals were sacrificed at various times (2 & 6, 24, and 96 hrs) after dosing. The majority of the radioactivity was eliminated during the first 24 to 48 hrs after dosing. The parent compound, Hoe 039866, accounted for the majority of the radioactivity eliminated in the excreta of both males (≈80% of the dose) and females (88% of the dose). This finding is consistent with the results of a previous metabolism studies (MRID No. 40345638 and MRID No. 43766913). The metabolite, Hoe 061517, was consistently found in both urine (0.22-1.20% of the dose) and feces (0.44-1.36% of the dose) of both sexes. Hoe 099730 was found in feces (0.28-1.72% of the dose) of both male and female rats and barely above or at the level of the detection in the urine of both sexes (0.02-0.04% of the dose). Hoe 042231 was mainly found in the feces of both male and females (≈0.2-0.28% of the dose). Very little if any of administered Hoe 039866 was sequestered in any tissues examined.

This study is **acceptable**, and provided valuable information about the metabolism of glufosinate ammonium (Guideline No. 81-5).

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

Information on the methods and materials is distributed between both reports (MRID No. 43766914 & MRID No. 43778402).

A. MATERIALS:

1. Test Material: ^{14}C-Hoe 039866

Description: kept as a free acid form in solution, converted to the ammonium form for testing

Batch #: 24037

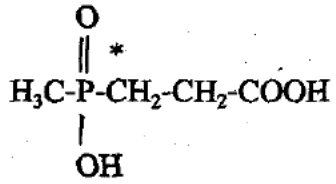
Purity: 99.9% (HPLC analysis)

CAS #: CA 77182-82-2

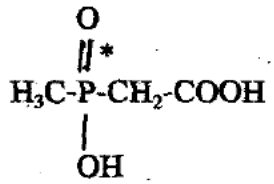
Specific radioactivity: 0.478 mCi/gm; 210 dpm/nmol

The reference compounds for identifying metabolites are presented below:
(* : ^{14}C-label; Hoe 065594 and Hoe 039046 were not labeled with ^{14}C).

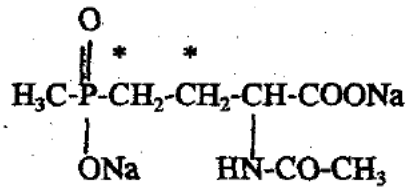
1. Hoe 061517 (3 methylphosphinico-propionic acid): >98% purity



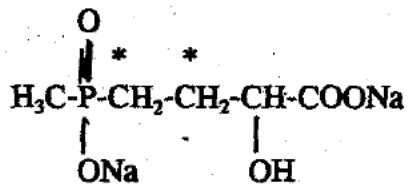
2. Hoe 064619 (2-methylphosphinico-acetic acid): >98% purity



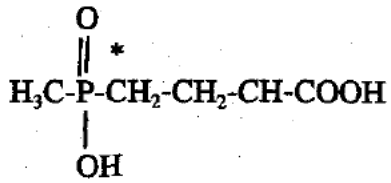
3. Hoe 099730 (disodium L-2-acetamido-4-methylphosphinato-butyrate)
Purity: 98%



4. Hoe 042231 (Disodium-2-hydroxy-4-methylphosphinico-butyrate); 91% purity



5. Hoe 039046 (4-methylphosphinico-butanoic acid):98% purity



3. Test animals:
   Species: Wistar rats
   Strain: Hoe WISKf(SPF71)
   Age and/or weight at dosing: approximate 6 to 9 weeks old and body weights ≈200 gm.
   Source: Hoechst AG, Kastenground, SPF breeding colony.
   Acclimation period: 1-2 days prior to dosing
   Diet: Altromin 1324/1321 rat diet; ad libitum
   Water: tap water; ad libitum
   Housing: The animals were housed 1 and 2 per cage (plastic metabolism box with device for separate collections of urine and feces) and in normal laboratory conditions

B. STUDY DESIGN and METHODS:

1. In life dates - Oct. 13, 1994 to July 12, 1995 (Including the analytical part of the study).

2. Dose groups: There were 3 groups per sex, and each group was sacrificed at a different time after dosing as shown in Table 1 below:

Table 1+: Dose groups, number of animals in each group, and time of sacrifice

SEX	Group	No. of Animals	Time of Sacrifice (Hours after dosing)
Males	G	5	24
	H	5	96
	L	2	2 and 6
Females	I	5	24
	K	5	96
	M	2	2 and 6

+: Data excerpted from the report, p. 17 (MRID No.43766914).

2. Dose preparation and administration - The test article was dissolved in saline (≈100 mg/g solution). A dose of 500 mg/kg body weight was administered (by gavage) to the above groups rats (5/sex or 2/sex). Each test rat received an approximate mean radioactive dose of 2.197 MBq/animal (≈132 x 10^6 dpm/animal). The details of the data on the dosing of the test animals are excerpted from the report and presented in Table 2.

Table 2+. Mean dose levels and the corresponding radioactivity in each mean dose

SEX / GROUP	RADIOACTIVE DOSE (dpm x 10^6 /rat)	DOSE LEVEL (mg a.i./kg)
M / H	125.53	568.7
F / K	104.47	518.0
M / G	136.21	560.4
F / I	115.96	568.9
M / L	136.62	572.1
F / M	117.57	568.1

+: Data excerpted from the report, p. 31 & 32 (MRID No. 43766914).

3. Sampling: Samples of urine and feces were collected at intervals of 0-24 hours (hrs) after dosing for Groups G & I, 0-24, 24-48, 48-72 and 72-96 hrs after dosing for groups H and K. The urine and fecal samples were collected in polyethylene bottles and glass dishes, respectively. To each sampling container, 1 ml of 0.1 M sodium azide solution was added prior to collection. Cage washes and tissue samples were not studied. Each cage was washed with ethanol:water (1:1) solution after each collection. The urine, feces, or cage wash samples were pooled for each group at each collection interval and were frozen until analysis.

For the tissue samples, spleen, kidney, liver, brain, and blood were collected from animals in Groups G, H, I, K, L and M. In addition, stomach and intestine including the contents were collected from animals in Groups L and M. Blood and organ/tissue samples from each group were pooled and were frozen until analysis

4. Sample processing for radioactivity measurements

Urine samples: The volume of the urine collected at any time interval was

measured and made up to a defined volume with water in a graduated cylinder containing a small amount of sodium azide. Appropriate amount of scintillation fluid was added, and 0.5 ml aliquots were directly measured for radioactivity with a Rotiszint Eco Plus scintillator.

Fecal samples: The fecal samples from each collection interval were mixed with ≈ 40-120 g of water containing sodium azide and homogenized by shaking. Then portions of 400-1000 mg fecal samples were placed onto porcelain boats, air dried, and combusted in a Biological Oxidizer (Model OX500, Zinsser Analytic). The $^{14}CO_2$ generated was absorbed in 12 ml of absorption mixture containing scintillator Oxysolve C-400 (Zinsser). The radioactivity was measured by a 1219 RackBeta liquid scintillation counter (Pharmacia LKB) or Tri-CARB 1900 CA (Canberra Parkard). Every measurement was corrected for the background signal of the instrument (ca 30 dpm).

Tissue/organ samples: Pooled tissue/organ samples from each group were mixed with ≈ 15-160 gm water containing sodium azide solution and were homogenized with an Ultra Turraz in an ice bath (≈ 4°C). Up to 8 aliquots of 0.5 - 1 ml were placed onto porcelain boats, air dried, and combusted in a sample oxidizer.

Plasma: The plasma samples from each group were pooled and made up to a defined volume in a graduated cylinder with water containing sodium azide. The radioactivity of the prepare plasma samples was measured directly after addition of scintillation cocktail to 3 aliquots of 0.5 ml prepared samples.

5. Metabolite study

Urine: The urine samples were filtered and analyzed directly with HPLC or TLC.

Feces: Fecal samples (≈ 5 gm) were extracted 5 times with 5 to 11 ml of water containing sodium azide. After 3 extractions, >90% of the radioactivity was found in the extract. The extracts from 1-3 and 4-5 extractions were combined and filtered. The filtrates were analyzed with both HPLC and TLC.

6. High performance liquid chromatography (HPLC): A Spectra Physics SP 8700 XR was used. The stationary phase consisted of Spherisorb SAX, 5 μm strong base anion exchanger. The eluent consists of A (aqueous potassium phosphate solution acidified with phosphoric acid (pH 1.9-2) and B (methanol) in various ratios as indicated in the Table 2. Different ratios of A and B were used in order to separate the reference substances or metabolites. The retention times of the reference compound are also listed in the Table 3.

Table 3+. Retention Time for the reference compounds and the Ratio of the eluent

Chemicals	Ratio 90:10 (A:B)++ Retention Time (minutes)	Ratio 50:50 (A:B) Retention Time (minutes)	Ratio 30:70 (A:B) Retention Time (minutes)
Hoe 039866	5.06 - 5.55	11.07	16.10
Hoe 039046*	6.59 - 7.32	9.42	9.52
Hoe 061517	8.55 - 9.46	14.41	16.10
Hoe 042231	9.41 - 10.54	16.21	20.34
Hoe 099730	14.29 - 17.07	22.47	29.16
Hoe 064619	16.41 - 18.18	31.51	38.40

+ : Table excerpted from the report, p. 24 (MRID No. 43766914).
++ : A:B = potassium phosphate solution : methanol
* : Hoe 039046 also present as an impurity at 0.23% in dosing solution. All the other impurities were less than 0.1%.

7. Thin layer chromatography (TLC): For the TLC analysis, silicagel, SIL G25 was used as a stationary phase, and a mobile phase consisting of isopropanol/water/acetic (2:1:1) was utilized. To evaluate the developed plates, a mixture of argon and methane (9:1) was used as the counting gas. The R_f-values of the reference compounds are as follows:

Compounds	R_f - Values
Hoe 039866	0.33
Hoe 039046	0.63
Hoe 061517	0.63
Hoe 042231	0.24
Hoe 099730	0.51
Hoe 064619	0.42

8. Calculations and statistical evaluation: The radioactivity concentrations and were expressed as mg a.i. equivalents/kg or L sample as in the following equation:

$$\frac{dpm}{g * dpm/\mu g} = \frac{mg}{kg} \quad \text{or} \quad \frac{dpm}{ml * dpm/\mu g} = \frac{mg}{L}$$

Where g = weight of the sample
ml = volume of the sample
dpm/μg = specific radioactivity of the test substance

Concentrations can also be converted to nmol by applying 1 μg = 5.046 nmol Hoe 039866. All radioactivity concentrations are presented on the basis of a.i. equivalents/kg (mg-equ/kg) using the specific radioactivity of Hoe 039866.

The limit of quantification (LOQ): This is calculated as follows:

LOQ = dpm of the control + 3 x S
Where S = standard deviation of the respective sample

The HPLC detection limit was approximately 0.02% of the administered dose radioactivity for urine sample and 0.07-0.19% of the dose for the fecal samples.

RESULTS

1. Stability of the test substance and reference compounds: The reference and test chemicals were found to be stable at 4-6°C for more than 6 months. The urine and fecal samples were also stable for a maximum of 8 months at -20°C. However, at room temperature, urine and fecal samples were found to be unstable with prolonged storage.

2. Elimination of radioactivity: Although there were several groups of rats employed in this study, the two groups which provided a fuller picture of all the radioactivity eliminated were Groups H (males) and K (females), both of which were sacrificed at 96 hrs after dosing (Table 4). The other groups were sacrificed at 24 hrs or earlier after dosing, and Table 5 presented data on Groups G and I, both of which were sacrificed at 24 hrs post dosing. For this reason, the discussion of the amount of radioactivity eliminated at various intervals will be focused on the results of Groups H and K.

The majority of the radioactivity was eliminated during the first 48 hrs post dosing and in the feces (Group H: 63.5% of the dose; Group K: 77.8%). Urinary elimination was rather minor, less than 6% in both groups. The total amount of radioactivity eliminated was 86.4% and 96.4% of the dose for Groups H and K, respectively (Table 4) (Figure 1).

Table 4+. Elimination of radioactivity following an oral dose of ^{14}C-Hoe 039866 (500 mg/kg) to Wistar rats of Groups H and K (% of administered dose)

TIME (hr)	GROUP H: MALES		GROUP K: FEMALES	
	Urine (% of dose)	Feces (% of dose)	Urine (% of Dose)	Feces (% of Dose)
0 - 24	3.4	38.4	2.8	51.1
24 - 48	2.3	25.1	1.3	26.7
48 - 72	1.1	8.3	0.7	8.2
72 - 96	0.9	3.4	0.4	2.6
Sum	7.7	75.2	5.2	88.6
Cage Wash	3.5		2.6	
Total ^{14}C-eliminated	86.4		96.4	

+; Data excerpted from the report, Table II, p. 33 (MRID No. 43766914).

Table 5+: ^{14}C- Eliminated in rats of Groups G and I. Both groups were sacrificed at 24 hrs post dosing.

TIME (Hrs)	GROUP G MALES		GROUP I FEMALES	
	URINE (% of dose)	Feces (% of dose)	URINE (% of dose)	FECES (% of dose)
0 - 24	2.9	71.2	3.3	34.6
Cage Wash	0.4		0.6	
Total ^{14}C-Eliminated	74.5		38.5	

+: Data excerpted from the report; p. 34 (MRID No. 43766914).

3. Radioactivity found in selected organs and tissues: In all organs or tissues examined less than 0.1% of the administered dose was found. If there was any radioactivity found in any organ or tissue, it was rapidly eliminated.

Sequestration of the radioactivity was not seen in any organ (Table 6).

Table 6+: ^{14}C- Found in various organs and tissues at different time post dosing.

Organs or Tissues	% OF ADMINISTERED DOSE							
	MALES				FEMALES			
	TIME (hrs) - GROUPS				TIME (hrs) / GROUPS			
	2 - L	6 - L	24 - G	96 - H	2 - M	6 - M	6 - I	96 - K
Spleen	0.005	0.003	0.002	0.002	0.019	0.005	0.003	0.002
Kidney	0.096	0.070	0.034	*	0.091	0.061	0.027	0.054
Liver	0.100	0.100	0.039	0.014	0.129	0.059	0.041	0.017
Brain	<0.001	0.001	<0.001	0.001	0.001	0.001	< LOQ	0.001
Platelets	0.002	0.001	0.001	0.001	0.003	0.001	0.001	< LOQ
Plasma	0.005	0.002	0.001	< 0.001	0.007	0.002	0.003	< LOQ

+: Data excerpted from the report; p.35 & 36 (MRID No. 43766914).

*: Not determined due to errors in processing the sample.

LOQ = Limit of quantitation.

4. Metabolites in urine and feces: The metabolites in urine and feces were determined using HPLC and TLC. The results of the HPLC analysis are excerpted from the report and presented in Table 7. The analytical results from TLC indicate that this method of identifying the metabolites is not as sensitive as the HPLC because many metabolites were not separated with TLC. Hence, the results of the TLC are not presented in this DER. The majority of the metabolites in the urine or feces were found in the samples of the first 48 hrs.

   Urinary metabolites: In the urine samples of Groups G/I (0-24 hrs) rats, Hoe 039866 (parent compound), Hoe 039046, and Hoe 061517 consisted of up to 2.7%, 0.19%, and 0.39% of the administered dose, respectively. In the samples of Groups H/K rats (0-96 hrs), Hoe 039866, Hoe 039046, and Hoe 061517 accounted for up to 5.92%, 0.22%, and 1.20% of the administered dose, respectively (Table 7). An unknown peak (M1) composed of approximately 0.2% was found in urine samples of Groups G/I and H/K.

   Compounds Hoe 039046 and Hoe 061517 are impurities of the test material. However, elimination of Hoe 061517 appears to be higher than that expected from its presence as an impurity, it is postulated that some Hoe 061517 is formed by metabolism.

In Groups G/I animals, Hoe 042231 and Hoe 099730 were found in the urine of males only, and the amount was barely above or at the limit of quantitation level (02-0.04% of the dose). In the Groups H/K, Hoe 042231 was not found in the urine. Hoe 064619 was below the detection limit (0.02% of the dose) in the urine of both male and female rats.

Table 7+: Metabolites in urine and feces of rats administered (by gavage) a single dose of 500 mg/kg ^{14}C-Hoe 039866.

METABO-LITES	% OF THE ADMINISTERED DOSE							
	GROUPS G / I (0-24 hrs)				GROUPS H / K (0-96 hrs)*			
	URINE		FECES		URINE		FECES	
	Males	Female	Males	Females	Males	Females	Males	Females
Hoe 039866	2.10	2.72	67.12	31.39	5.92	4.27	72.11	84.00
Hoe 039046	0.16	0.19	0.16	0.06	0.22	0.17	0.29	0.11
Hoe 061517	0.39	0.22	1.36	1.11	1.20	0.51	0.58	0.44
Hoe 042231	0.02 (LOQ)	<LOQ	0.17	0.20	<LOQ	<LOQ	0.26	0.28
Hoe 099730	0.04	0.03	0.66	0.28	0.04	0.02	1.23	1.74
Hoe 064619	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

+: Data excerpted from the report; p. 38-40 (MRID No. 43766914).

*: The data for Groups H & K were calculated by this reviewer from the data presented for the 4 sampling intervals (0-24, 24-48, 48-72, & 72-96 hrs post dosing (Table V; p.38 of the report).

LOQ = Limit of Quantitation

Fecal metabolites: In the feces of rats of Groups G/I and H/K, the parent compound, Hoe 039866, accounted for majority of the radioactivity up to 84.00% of the dose. Hoe 039046 was less than 0.3% of the dose. In Groups G/I animals, the metabolites, Hoe 061517 was found to be 1.36% in males and 1.11% in females while in Groups H/K animals this metabolite consisted of 0.58% and 0.44% of the dose, respectively. In Groups G/I, Hoe 042231 acounted 0.17 and 0.20% of the dose, and this metabolite was slight more in Groups H/K animals consisted of 0.26% and 0.28% of the dose. This is due to longer collection time (96 hrs vs. 24 hrs). Hoe 099730 was the third most abundant compound in the feces of Groups G/I rats consisted of 0.66% and 0.28% of the dose. In Groups H/K rats, Hoe 09973 was the second most abundant compound in the feces and the major fecal metabolite. Again, this was due to longer collection time. Hoe 064619 was below the limit of quantitation in dose groups (Table 7). The unknown peak (M1 was below the limit of quantitation.

DISCUSSION

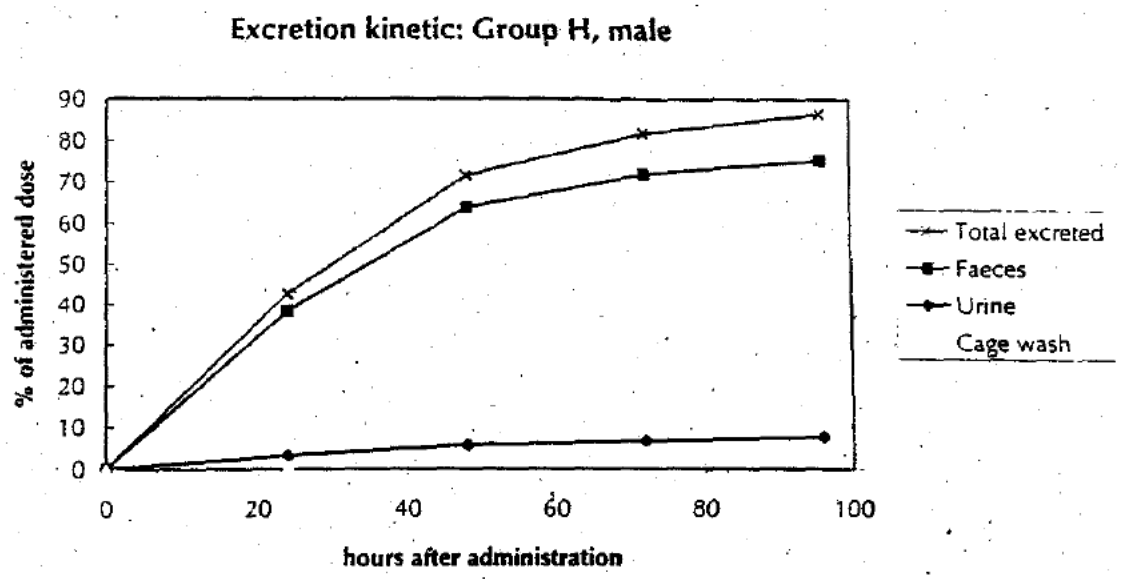
Groups of Wistar rats (5/sex or 2/sex) received a single dose (500 mg/kg) of ^{14}C-Hoe 039866 (glufosinate ammonium) by gavage. Animals were sacrificed at various times (2 & 6, 24, and 96 hrs) after dosing. With oral dosing, Hoe 039866 was eliminated rapidly; the majority of the radioactivity was eliminated in the feces during the first 24 to 48 hrs after dosing. The parent compound, Hoe 039866, accounted for the majority of the radioactivity eliminated in the excreta of both males (≈80% of the dose) and females (88% of the dose). This finding is consistent with the results of the previous metabolism studies (Tox. Doc. No. 006936 and MRID No. 43766913). The metabolite, Hoe 061517, was consistently found in both urine (0.22-1.20% of the dose) and feces (0.44-1.36% of the dose) of both sexes. Hoe 099730 was found in feces (0.28-1.72% of the dose) of both male and female rats and barely above or at the level of the detection in the urine of both sexes (0.02-0.04% of the dose). Hoe 042231 was mainly found in the feces of both male and females (≈0.2-0.28% of the dose).

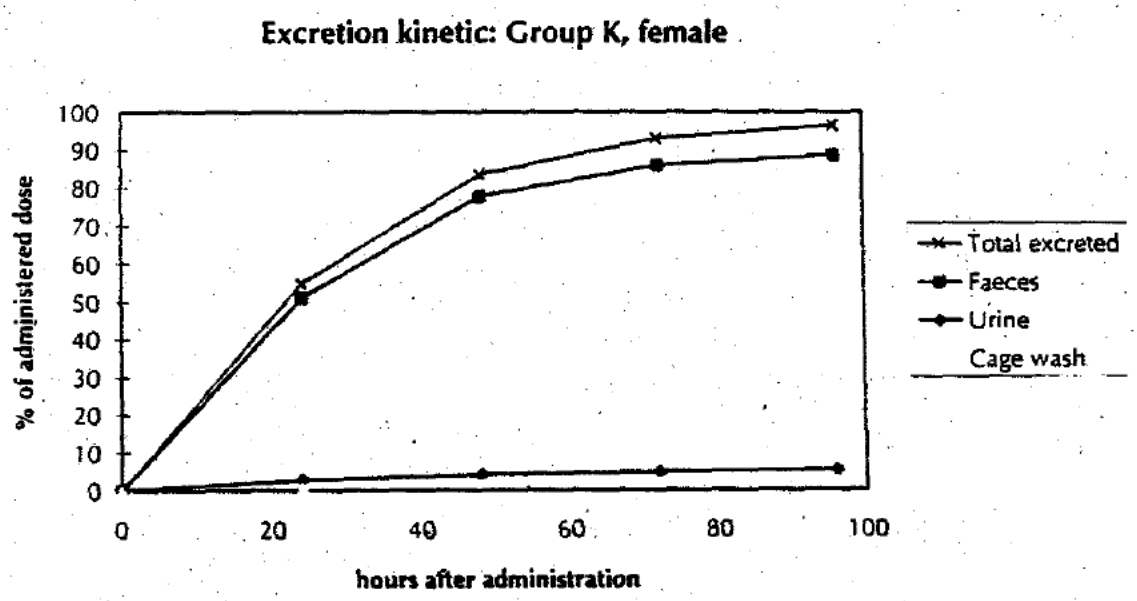
The tissue examinations indicate that at 2 hrs after dosing, the highest amount of radioactivity was found in the liver, and it was of 0.13% of the dose. At 96 hrs post dosing, the level dropped down to less than 0.02% of the dose. Therefore, very little Hoe 039866 was sequestered in any tissue.

Based upon these results a metabolism scheme was proposed for this compound (Figure 2). It appears that for fecal metabolites, Hoe 039866 underwent N-acetylation and deacetylation presumably by the intestinal bacterial, and this process was thought to be reversible. The urinary metabolites indicate that Hoe 039866 is predominantly metabolized via decarboxylation and beta-oxidation.

Based on all the metabolism data available on Hoe 039866, all the studies indicated that fecal elimination is the major route. In the absence of the data on biliary elimination, it is not clear how much of the test compound is actually absorbed by the G.I. tract.

Figure 1+: Excretion kinetics of Hoe 039866-^{14}C in male and female rats following single oral administration of 500 mg/kg bw





+: **Figure excerpted from the report, p. 44 (MRID No. 43766914).**

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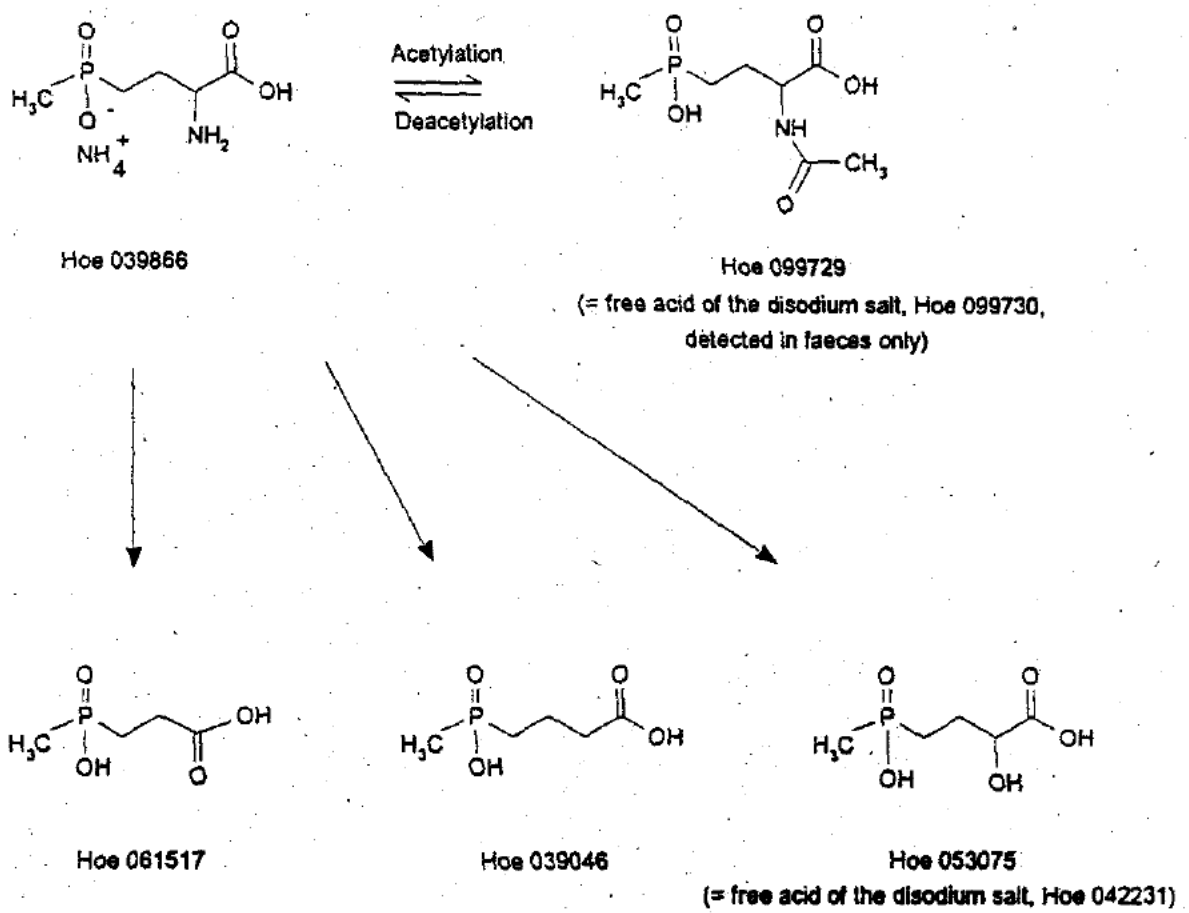
Figure 2+: Proposed metabolism of Hoe 039866 in rats



The arrows do not represent only one step of enzymatic reaction but mean complex metabolic transformations leading to the compounds shown.

+: Figure excerpted from the report, p. 57 (MRID No. 43766914).

SPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

EPA Reviewer: Whang Phang, Ph.D. 3/27/96
Review Section III
Toxicology Branch II/HED (7509C)

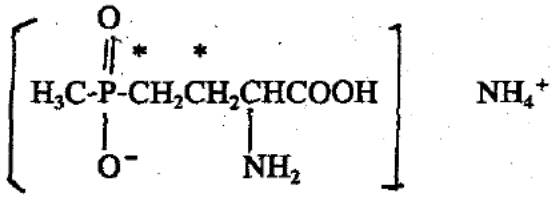
EPA Secondary Reviewer: Alberto Protzel, Ph.D. 3/29/96
Review Section III
Toxicology Branch II/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism study in rats (85-1)

DP BARCODE: D219070 **SUBMISSION CODE:** S493376
P.C. CODE: 128850 **TOX. CHEM. NO.:** 580I
MRID No.: 43766913 **ID No.:** 5F04578

CHEMICAL: DL-homoalanin-4-yl(methyl)-phosphinic acid, ammonium salt



(*: location of ^{14}C-label)

SYNONYMS: Hoe 039866; DL-glufosinate ammonium

CITATION: Stumpf, K. (1993). Hoe 039866 -^{14}C: Metabolism in male and female rats following single oral administration of test substance at a dose level of 2 mg/kg body weight. Produktentewicklung GB-C, Okologie I and Radiochemisches Laboratorium, Hoechst Aktiengesellschaft, Germany; Study No.: A49981. April 26, 1993. Submitted to US EPA by AgrEvo™; MRID No. 43766913. Unpublished.

SPONSOR: Hoechst

EXECUTIVE SUMMARY: In a metabolism study (85-1), groups of Wistar rats (5/sex) received a single dose (2 mg/kg) of ^{14}C-Hoe 039866 (glufosinate ammonium) by gavage. The majority of the radioactivity (95-98% of the dose) was eliminated during the first 24 hrs after dosing. The parent compound, Hoe 039866, accounted for most of the eliminated radioactivity in the urine and feces of both males (80% of the dose) and females (73% of the dose). The metabolite, Hoe 061517, was consistently found in both urine and feces of both sexes. Hoe 099730 (7-8% of the dose) and Hoe 042231 (≈3%

of the dose) were found in the feces of both male and female rats and none in the urine.

This study is **acceptable**, and provided valuable information about the metabolism of glufosinate ammonium (Guideline No. 81-5).

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

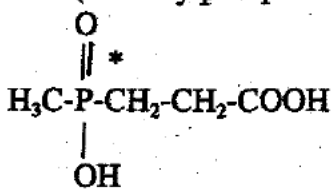
I. MATERIALS AND METHODS

A. MATERIALS:

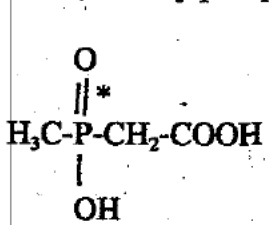
1. Test Material: ^{14}C-Hoe 039866
   Description: kept as a free acid form in solution, converted to the ammonium form for testing
   Batch #: 17069 IV
   Purity: 93-95% (HPLC analysis)
   CAS #: CA 77182-82-2
   Specific radioactivity: 69.45 mCi/gm; 23931 dpm/nmol

   The reference compounds for identifying metabolites are presented below:
   (* : ^{14}C-label; Hoe 065594 and Hoe 039046 were not labeled with ^{14}C).

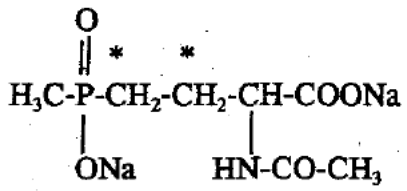
   1. Hoe 061517 (3 methylphosphinico-propionic acid): >90% purity



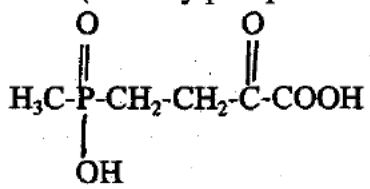
   2. Hoe 064619 (2-methylphosphinico-acetic acid): >90% purity



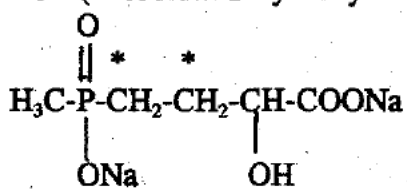
3. Hoe 099730 (disodium L-2-acetamido-4-methylphosphinato-butyrate)
   Purity: >90%



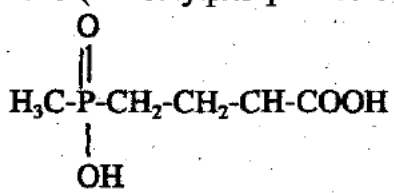
4. Hoe 065594 (4-methylphosphinico-2-oxo-butanoic acid): >90% purity



5. Hoe 042231 (Disodium-2-hydroxy-4-methylphosphinico-butyrate)



6. Hoe 039046 (4-methylphosphinico-butanoic acid): >90% purity



3. <u>Test animals</u>:
   Species: Wistar rats
   Strain: Hoe WISKf(SPF71)
   Age and/or weight at dosing: approximate 8 to 10 weeks old and body weights ranging from 200 to 210 gm.
   Source: Hoechst AG, Kastenground, SPF breeding colony.
   Acclimation period: At least 5 days

Diet: Altromin 1324 rat diet; ad libitum
Water: tap water; ad libitum
Housing: The animals were housed 1/cage and in normal laboratory conditions

B. STUDY DESIGN and METHODS:

1. In life dates - The report does not provide the duration of the study.

2. Dose preparation and administration - The test article was kept as a free acid and concentrated to the ammonium form, Hoe 039866, for testing. A dose of 2 mg/kg body weight was administered (by gavage) to a group of rats (5/sex). Each test rat received an approximate mean radioactive dose of $6x10^7$ dpm. The data on the dosing of the test animals are excerpted from the report and presented in Table 1 (p. 10).

3. Sampling: Samples of urine and feces were collected at intervals of 0-24, 24-48, 48-72 and 72-96 hours (hrs) after dosing. The urine and fecal samples were collected in polyethylene bottles and glass dishes, respectively. The urine or feces samples were pooled for each sex and were frozen until analysis. Cage washes and tissue samples were not studied.

4. Sample processing for radioactivity measurements

Urine samples: The volume of the urine collected at any time interval was measured and made up to a whole-number ml with water in a graduated cylinder containing a small amount of sodium azide. Appropriate amount of scintillation fluid was added, and 0.5 ml aliquots were directly measured for radioactivity with a Rotiszint Eco Plus scintillator.

Fecal samples: The fecal samples from each collection interval were mixed with 100 ml of water containing sodium azide and homogenized in an Ultra Turrax. Then portions of 500-1000 mg fecal samples were placed onto porcelain boats and combusted in a Biological Oxidizer (Model OX500, Zinsser Analytic). The $^{14}CO_2$ generated was absorbed in 20 ml of absorption mixture containing scintillator Oxysolve C-400 (Zinsser). The radioactivity was measured by a 1219 RackBeta liquid scintillation counter (Pharmacia LKB) or Tri-CARB 4530 (Parkard Instr.). Every measurement was corrected for the background signal of the instrument (ca 30 dpm).

5. Metabolite study

Urine: The urine samples from days 2 to 4 were pooled, concentrated, and analyzed directly with HPLC or TLC.

Feces: Fecal samples from days 1 & 2 were pooled and extracted 4 times with 5 to 8 ml of water. The extracts were combined and dialyzed with acetyl cellulose tubes. The aqueous dialysates were concentrated to approximately 25 ml and analyzed with both HPLC and TLC.

6. High performance liquid chromatography (HPLC): A Spectra Physics SP 8700 was used. The stationary phase consisted of Spherisorb SAX, 5 μm strong base anion exchanger. The eluent consists of A (aqueous potassium phosphate solution acidified with phosphoric acid (pH 1.9-2) and B (methanol) in various ratios as indicated in the Table 2. Different ratios of A and B were used in order to separate the reference substances or metabolites. The retention times of the reference compound are also listed in the Table 2.

Table 2+. Retention Time for the reference compounds and the Ratio of the eluent

Chemicals	Ratio 90:10 (A:B)++ Retention Time (minutes)	Ratio 70:30 (A:B) Retention Time (minutes)	Ratio 30:70 (A:B) Retention Time (minutes)
Impurity I*	2 - 3	2 - 3	3
Hoe 039866	4.6	5.9	12.5
Hoe 039046*	7 - 8	≈ 8	8 - 9
Hoe 061517*	9.4	9.9	15.2
Hoe 042231	9.9	10.9	18.9
Hoe 099730	14.4	15.2	25.2
Hoe 064619	18.0	20.3	37.4
Hoe 065594	30 - 50	40 - 60	>60

+ : Table excerpted from the report, p. 23 (MRID No. 43766913).
++ : A:B = potassium phosphate solution : methanol
* : Main impurity peaks in the test substance: Impurity I at 2.2%, Hoe 039046 at 1.8%, and Hoe 061517 at 0.8%.

7. Thin layer chromatography (TLC): For the TLC analysis, silicagel, SIL G25 was used as a stationary phase, and a mobile phase consisting of isopropanol/water/acetic (2:1:1) was utilized. To evaluate the developed plates, a mixture of argon and methane (9:1) was used as the counting gas. The R_f-values of the reference compounds are as follows:

Compounds	R_f - Values
Impurity I*	ca 0.15
Hoe 039866	0.19
Hoe 039046*	0.59
Hoe 061517*	0.61
Hoe 042231	0.15
Hoe 099730	0.46
Hoe 064619	0.28

* : Main impurity peaks in the test substance

8. Calculations and statistical evaluation: The radioactivity concentrations and were expressed as mg a.i. equivalents/kg or L sample as in the following equation:

$$\frac{\text{dpm}}{\text{g} * \text{dpm}/\mu\text{g}} = \frac{\text{mg}}{\text{kg}} \quad \text{or} \quad \frac{\text{dpm}}{\text{ml} * \text{dpm}/\mu\text{g}} = \frac{\text{mg}}{\text{L}}$$

Where g = weight of the sample
ml = volume of the sample
dpm/μg = specific radioactivity of the test substance

Concentrations can also be converted to nmol by applying 1 μg = 5.046 nmol Hoe 039866. All radioactivity concentrations are presented on the basis of a.i. equivalents/kg (mg-equ/kg) using the specific radioactivity of Hoe 039866.

The limit of quantification (LOQ): This is calculated as follows:

$$\text{LOQ} = \text{dpm of the control} + 3 \times S$$

Where S = standard deviation of the respective sample

The chromatographic detection limit was approximately 0.05% of the administered dose radioactivity.

RESULTS

1. Stability of the test substance and reference compounds: The reference and test chemicals were found to be stable after being frozen for one year. The urine and fecal samples were also stable for more than a year at -20°C. However, at room temperature, urine and fecal samples were found to be unstable with prolonged storage.

2. Elimination of radioactivity: The data on radioactivity eliminated in urine and feces are presented in Table 3. Majority of the radioactivity is eliminated in the feces and during the first 24 hr collection period in both males (87.6% of the dose) and females (90.7% of the dose). Renal elimination during the entire study duration consisted of 9.8% and 8.6% of the dose in males and females, respectively. By the end of 48 hrs less than 1% of the administered dose was found in either urine or feces of both sexes (Table 3). The total recovery of the administered radioactivity was over 100% for both males and females. The report stated that radioactivity in the cage wash and tissue was not determined, but the previous studies indicated the radioactivity in the cage wash and the tissue was less than 1% of the administered dose.

Table 2+. Elimination of radioactivity following an oral dose of ^{14}C-Hoe 039866 (2 mg/kg) to Wistar rats (% of administered dose)

TIME (hr)	MALES		FEMALES	
	Urine (% of dose)	Feces (% of dose)	Urine (% of Dose)	Feces (% of Dose)
24	8.1	87.6	7.7	90.7
48	1.0	2.7	0.6	4.4
72	0.5	0.3	0.2	0.1
96	0.2	0.1	0.1	0.1
Total	9.8	90.7	8.6	95.3
Recovery	100.5		103.9	

+: Data excerpted from the report, Table II, p. 35 (MRID No. 43766913).

3. Metabolites in urine and feces: The metabolites in urine and feces were determined using HPLC and TLC. The results are excerpted from the report and presented in Table 4 (HPLC) and 5 (TLC). The majority of the metabolites in the urine or feces were found in the samples of the first 24 hrs. The following discussion will focus on the samples of the first 24 hr interval.

A. HPLC Analysis (Table 4)

Urinary metabolites: In the urine of both male and female rats, Hoe 039866 (parent compound), Hoe 039046, and Hoe 061517 consisted of approximately 4%, 1%, and 2% of the administered dose, respectively. An unknown peak composed of approximately 1% was found also. Compounds Hoe 039046 and Hoe 061517 are impurities of the test material. However, elimination of Hoe 061517 appears to be higher than that expected from its presence as an impurity, it is postulated that some Hoe 061517 is formed by metabolism. A minor amount (0.1% of the dose) of Hoe 064619 (barely above the detection limit, 0.05% of the dose) was found in the urine of female rats only.

Fecal metabolites: In the feces of males, the parent compound consisted of 73.5% of the dose, and no Hoe 039046 was found. The metabolites, Hoe 061517, Hoe 042231, and Hoe 099730 accounted for 1.3%, 3.4%, and 7.1% of the dose, respectively. In female fecal samples, the parent compound consisted of 73.5% of the dose, and no Hoe 039046 was found. Hoe 061517, Hoe 042231, and Hoe 099730 consisted of 0.9%, 2.9%, and 8.1% of the dose, respectively. The unknown peak accounted for 0.6 and 0.3% of the dose in male and female feces respectively.

B. TLC Analysis (Table 5)

The TLC results are similar to those of HPLC in that the majority of the administered dose found in both urine and feces samples of males and females was the parent compound, Hoe 039866 (Table 5). In the urine of both sexes, the metabolites, Hoe 061517 and Hoe 099730, were found. Hoe 061517 accounted for approximately 2% of the dose in the urine samples of both sexes whereas only 0.6% of the dose was detected in male fecal samples and below the LOQ in female fecal samples. Hoe 099730 accounted for 5.4% and 5.7% of the dose in the fecal sample of males and females, respectively. In the urine sample, Hoe 099730 consisted of 0.1% and 0.2% of the dose in male and female, respectively.

DISCUSSION

Groups of Wistar rats (5/sex) received a single dose (2 mg/kg) of ^{14}C-Hoe 039866 (glufosinate ammonium) by gavage. The majority of the radioactivity was eliminated during the first 24 hrs after dosing. The parent compound, Hoe 039866, accounted for majority of the radioactivity eliminated in the excreta of both males (80% of the dose) and females (73% of the dose). This finding is consistent with the results of a previous metabolism study (MRID No. 40345638). The metabolite, Hoe 061517, was consistently

found in both urine and feces of both sexes. Hoe 099730 (7-8% of the dose) and Hoe 042231 (≈3% of the dose) were found in feces of both male and female rats and none in the urine.

Based upon these results a metabolism scheme was proposed for this compound (Figure 1). It appears that for fecal metabolites, Hoe 039866 underwent N-acetylation and deacetylation presumably by the intestinal bacterial, and this process was thought to be reversible. The urinary metabolites indicate that Hoe 039866 is predominantly metabolized via decarboxylation and beta-oxidation.

Table 1+: Single oral administration of Hoe 039866-14C (2 mg/kg) to male and female rats
Experimental data

Rat No	Sex	Animal weight (g)	Specific radioactivity (MBq/g)	Concentration of dose solution (mg ai/g)	Weight of sol. per animal (g/anim)	Weight of test compound (mg ai/anim)	Radioactive dose (dpm/anim)	Dose level (mg ai/kg b.w.)
1		210			1.1221	0.4455	$6.28 \cdot 10^7$	2.12
2		210			1.0800	0.4288	$6.04 \cdot 10^7$	2.04
3	male	200	2348.74	0.397	1.0372	0.4118	$5.80 \cdot 10^7$	2.06
4		200	(140924		1.0599	0.4208	$5.93 \cdot 10^7$	2.10
5		210	dpm/µg)		1.1531	0.4578	$6.45 \cdot 10^7$	2.18
mean		206			1.0905	0.4329	$6.10 \cdot 10^7$	2.10 ± 0.05
sum (100 %)		1030			5.4523	2.1646	$3.05 \cdot 10^8$	
1		210			1.0795	0.4286	$6.04 \cdot 10^7$	2.04
2		210			1.1235	0.4460	$6.29 \cdot 10^7$	2.12
3	fe-	200	2348.74	0.397	1.0514	0.4174	$5.88 \cdot 10^7$	2.09
4	male	200	(140924		1.0653	0.4229	$5.96 \cdot 10^7$	2.11
5		200	dpm/µg)		1.0572	0.4197	$5.91 \cdot 10^7$	2.10
mean		204			1.0754	0.4269	$6.02 \cdot 10^7$	2.09 ± 0.03
sum (100 %)		1020			5.3769	2.01346	$3.01 \cdot 10^8$	

+: Data excerpted from the report, p. 34 (MRID No. 43766913).

Table 4+: Nature of radioactivity in urine and faecal extracts of male and female rats following oral administration of Hoe 039866-14C (2 mg/kg) HPLC results (expressed as % of administered dose)

Sex	Urine 0 - 24	Urine 24 - 48	Urine 48 - 72	Urine 72 - 96	Faeces 0 - 24	Faeces 24 - 48 hour
% dose in sample male	8.1	1.0	0.5	0.2	87.6	2.7
extractable	-	-	-	-	90.5	2.7
non-extractable residues	-	-	-	-	1.5	0.08
recovery after dialysis	-	-	-	-	86.0	2.4
test substance and metabolites (impurity peaks[1]):						
Hoe 039866	4.1[3]	0.6	0.3	0.1	73.5	1.8
unknown	1.2	0.1	0.0	0.0	0.6	0.0
Hoe 039046	1.5	0.1	0.0	0.0	0.0	0.0
Hoe 061517	1.3	0.3	0.2	0.1	1.3	0.0
Hoe 042231	0.0	0.0	0.0	0.0	3.4	0.2
Hoe 099730	0.0	0.0	0.0	0.0	7.1	0.3
Hoe 064619	0.0	0.0	0.0	0.0	0.0	0.0
% dose in sample female	7.7	0.6	0.2	0.1	90.7	4.4
extractable	-	-	-	-	93.1	4.7
non-extractable residues	-	-	-	-	1.4	0.09
recovery after dialysis	-	-	-	-	78.1	4.0
test substance and metabolites (impurity peaks[1]):						
Hoe 039866	3.9	0.4	0.1	0.1	66.0	2.5
unknown	1.4	0.1	0.0	0.0	0.3	0.0
Hoe 039046	1.7	0.1	0.0	0.0	0.0	0.0
Hoe 061517	0.6	0.1	0.05	0.0	0.9	0.0
Hoe 042231	0.0	0.0	0.0	0.0	2.9	0.4
Hoe 099730	0.0	0.0	0.0	0.0	8.1	1.1
Hoe 064619	0.1	0.0	0.0	0.0	0.0	0.0

balance	urine male	urine female	faeces male	faeces female
sum of known metabolites	8.6	7.2	87.6	81.9
sum of unknown metabolites	1.3	1.5	0.6	0.3
non-extractable residues	-	-	0.8	0.8
recovery	9.9	8.7	89.0	83.0
recovery (Table II)	9.8	8.6	90.7	95.3
not investigated (faeces 48 - 96 h)	-	-	0.4	0.2
loss during clean-up[2]	-0.1	-0.1	1.7	12.3

1): Hoe 061517, Hoe 039046, and some unknown compounds were still present in the test substance as impurities, <1 - 2 % each, purity of Hoe 039866 94 %

2): the crude extract contained almost the total radioactivity, losses were during dialysis, chromatograms of crude extracts and dialysate were not different

3): 0.0 % means < detection limit, i.e. 0.05 % of dose or ca 1 % of sample radioactivity

+: Data excerpted from the report, p. 36 (MRID No. 43766913)

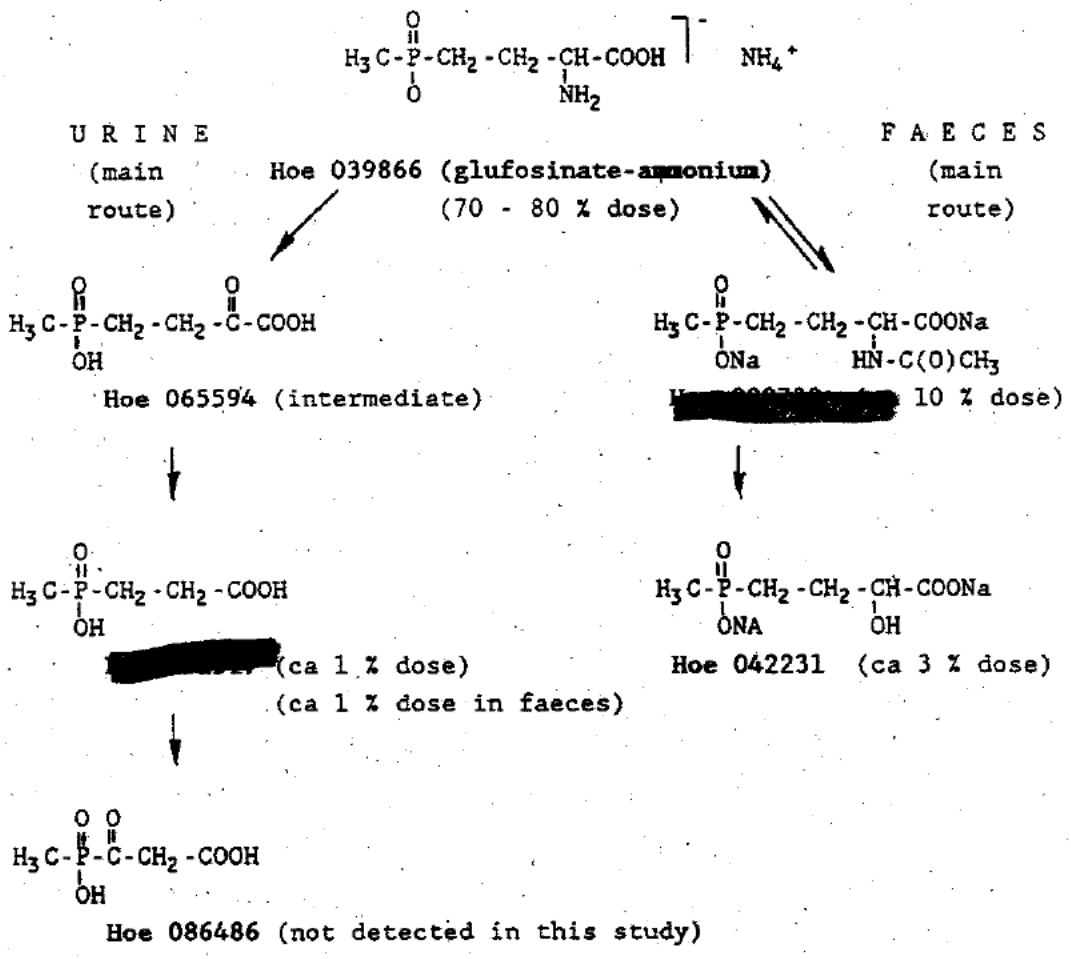
Table 5+: Nature of radioactivity in urine and faecal extracts of male and female rats following oral administration of Hoe 039866-14C (2 mg/kg) TLC results (expressed as % of administered dose)

Sex	Urine 0 - 24	Urine 24 - 48	Urine 48 - 72	Urine 72 - 96	Faeces 0 - 24	Faeces 24 - 48 hour
% dose in sample male	8.1	1.0	0.5	0.2	87.6	2.7
extractable	-	-	-	-	90.5	2.7
non-extractable residues	-	-	-	-	1.5	0.08
recovery after dialysis	-	-	-	-	86.0	2.4
test substance and metabolites (impurity peaks):						
Hoe 039866	5.6	nd	nd	nd	80.0	nd
unknown	-	nd	nd	nd	-	nd
Hoe 039046	-	nd	nd	nd	-	nd
Hoe 061517	2.4	nd	nd	nd	0.6	nd
Hoe 042231	-	nd	nd	nd	-	nd
Hoe 099730	0.1	nd	nd	nd	5.4	nd
Hoe 064619	0.0	nd	nd	nd	0.0	nd
% dose in sample female	7.7	0.6	0.2	0.1	90.7	4.4
extractable	-	-	-	-	93.1	4.7
non-extractable residues	-	-	-	-	1.4	0.09
recovery after dialysis	-	-	-	-	78.1	4.0
test substance and metabolites (impurity peaks):						
Hoe 039866	5.4	nd	nd	nd	72.4	nd
unknown	-	nd	nd	nd	-	nd
Hoe 039046	-	nd	nd	nd	-	nd
Hoe 061517	2.2	nd	nd	nd	0.0	nd
Hoe 042231	-	nd	nd	nd	-	nd
Hoe 099730	0.2	nd	nd	nd	5.7	nd
Hoe 064619	0.0	nd	nd	nd	0.0	nd

nd: not determined
Only the day one samples could be investigated by TLC. The substance pairs Hoe 039866/Hoe 042231 and Hoe 061517/Hoe 039046 could not be separated with the TLC system used see also remarks in Table III

+: Data excerpted from the report, p. 37 (MRID No. 43766913)

Figure [redacted]: Metabolism of Hoe 039866-^{14}C in rat after single oral administration of 2 mg/kg
Proposed major metabolite pathways



Remarks:

The presence of Hoe 099730 and Hoe 042231 in faeces was established in this study for the first time. Hoe 086486 is a metabolite found in high dose metabolism studies of Hoe 039866.

+: Figure excerpted from the report, p. 48 (MRID No. 43766913).

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Chemical:	**Glufosinate-ammonium**
PC Code:	**128850**
HED File Code	**13000 Tox Reviews**
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