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

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MAR 11 1992

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

Subject: **EPA ID # 80801-04: Ametryn. Review of Metabolism Studies in the Rat**

Tox. Chem No. 471
PC No. 80801
EPA No. 263
HED Project No. 0-1249

From: **Myron S. Ottley, Ph. D.**
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I. CONCLUSIONS

The Toxicology Branch I has reviewed the battery of (three) Pharmacokinetics and Metabolism Studies submitted by Ciba Geigy Corporation. DER is attached.

CLASSIFICATION: Acceptable. These studies satisfy the guideline requirements (85-1) for Pharmacokinetics and Metabolism Study.

II. ACTION REQUESTED

Three studies were submitted for review to determine if they satisfy the

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requirements of Guideline 85-1. They are:

1. (XBL RPT0007) Metabolism of ^{14}C-Ametryn in Rats - Phase I Preliminary Study
2. (XBL RPT0022) Analysis, Quantitation, and Structure Elucidation of Metabolites in Urine and Feces from the Rat Dosed with ^{14}C-Ametryn
3. (BTC P01744) Absorption, Distribution and Excretion Studies of ^{14}C-Ametryn in the Rat

One Data Evaluation Report (DER) was prepared for these studies.

III. RESULTS AND DISCUSSION

Ametryn is readily absorbed by rats after a single or multiple oral dose(s) of 0.5 or 200 mg/kg. It is widely distributed, being found in all tissues and organs tested. It is metabolized into several polar products, 13 of which were identified. It is excreted mainly through the urine (50 - 61%) within 48 hours, with the feces being the other major route (30 - 42%). No significant differences in pharmacokinetic parameters were seen among dosing groups (sing oral high & low, multiple low, single i.v.) or between sexes.

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DATA EVALUATION REPORT

STUDY TYPE: **Metabolism -- RAT** (85-1)

TOX. CHEM. NO: 471
PC NO.: 80801
MRID NOs.: 1. 414633-01
2. 414633-02
3. 414633-03

TEST MATERIAL: **Ametryn**

SYNONYMS: None provided

REPORT NUMBERs: 1. XBL RPT0007
2. XBL RPT0022
3. BTC P01744

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITIES: 1. Xenobiotic Laboratories, Inc., Princeton, NJ
2. Xenobiotic Laboratories, Inc., Princeton, NJ
3. Biological Test Center, Irvine, CA

TITLEs OF REPORTs: 1. Metabolism of ^{14}C-Ametryn in Rats - Phase I Preliminary Study
2. Analysis, Quantitation, and Structure Elucidation of Metabolites in Urine and Feces from the Rat Dosed with ^{14}C-Ametryn
3. Absorption, Distribution and Excretion Studies of ^{14}C-Ametryn in the Rat

AUTHOR: 1. John L. Reynolds
2. Diana Wu, Ph. D.
3. Richard Braun, Ph.D.

REPORTs ISSUED: 1. March 8, 1990
2. April 9, 1990
3. February 9, 1990

CONCLUSIONS:

Ametryn is readily absorbed by rats after a single or multiple oral dose(s) of 0.5 or 200 mg/kg. It is widely distributed, being found in all tissues and organs tested. It is metabolized into several polar products, 13 of which were identified. It is excreted mainly through the urine (50 - 61%) within 48 hours, with the feces being the other major route (30 - 42%). No significant differences in pharmacokinetic parameters were seen among dosing groups (single, oral high & low, multiple low, single i.v.) or between sexes.

CLASSIFICATION: **Acceptable.** This study satisfies the guideline requirements (85-1) for a Metabolism Study.

MATERIALS

1. **Test Compounds**
   Description:
   a. Non-radiolabeled Ametryn
      Lot No. S82-0017 (P01744[1]); Purity: 98.0%
   b. Radiolabeled Ametryn
      Reference No. CL-XVIII-35 (RPT0022, P01744)
      Radiochemical purity: 99.0%
      Specific Activity: 19.6 μCi/mg
      Reference No. RAF-VIII-14 (P01744)
      Radiochemical purity: 94.3 - 95.3%
      Specific Activity: 93.9 μCi/mg
      Reference No. RAF-VIII-14 (RPT0007, RPT0022)
      Radiochemical purity: 98.2%
      Specific Activity: 93.9 μCi/mg
      Reference No. RAF-VIII-19 (RPT0007)
      Radiochemical purity: 98.2%
      Specific Activity: 0.5 μCi/mg

2. **Test Animals:** Species: Rat; Strain(s): Crl:CD(SD) BR (RPT0007), Sprague-Dawley (RPT0022, P01744)
   Age: 5½ - 8 wks; Weight: 91 - 240 GM;
   Source: Charles River Breeding Labs., MA and NY

3. **Environment:** Rats were housed individually in metal cages. Temperature: 61 - 70°F; Humidity: 50 ± 20%; Photoperiod: 12 hours light/dark; Food: Purina Rat Chow *ad libitum* except for pre- and post-exposure fasts; Water: tap *ad libitum*.

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[1]*Study number where used*

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METHODS

After health examination and acclimatization, rats were assigned to groups and dosed as outlined on the next page. Corn oil was the vehicle for non-radiolabeled ametryn. Radiolabeled ametryn was administered in 0.9% saline. Oral dosing was administered by gavage, while i.v. dosing was directly into the jugular vein of anesthetized animals.

Group	Route	Frequency of Dosing	Dose level (mg/kg)	Group Size ♂/♀
Control	Oral	Single	(Corn oil only)	2/2
SOLD	Oral	Single	0.5	5/5
MOLD	Oral	14 daily	0.5[§]	5/5
SOHD	Oral	Single	200.0	5/5
SIVD	Intravenous	Single	0.5	5/5

SOLD = Single Oral Low Dose
MOLD = Multiple Oral Low Dose
SOHD = Single Oral High Dose
SID = Single Intravenous Dose
[§] Non-radioactive ametryn was given daily for 14 days, followed by a single ^{14}C dose.

Single Oral Doses

Ten rats (5 male, 5 female) were fasted for 18 hr before oral administration of a single dose of 0.5 mg/kg or 200 mg/kg of radiolabeled ametryn (radioactivity: approx. 10 - 20 μCi/rat; approx. 5 ml dosing solution/kg). Immediately after dosing, animals were transferred to their individual metabolic cages equipped with a urine/feces separator.

Multiple Low Doses

Ten rats (5 male, 5 female) were fasted for 18 hr before administration of 0.5 mg/kg of non-radiolabeled ametryn. Six hr following dosing, normal *ad libitum* food was resumed. The animals were dosed once daily for 14 days without interruption of food. Doses were adjusted on days 8 and 15 to correspond with changing body weight. On day 15 animals were fasted for 18 hr and then administered a single dose of 0.5 mg/kg of radiolabeled ametryn (radioactivity: approx. 10 - 20 μCi/rat; approx. 5 ml dosing solution/kg). Immediately after dosing, animals were transferred to their individual metabolic cages. Six hr after dosing *ad libitum* food was resumed.

Single Intravenous Dose

Ten rats (5 male, 5 female) were fasted for 18 hr before administration of 0.5 mg/kg of non-radiolabeled ametryn. Following anesthetization with ether and incision was made to expose the jugular vein. A single dose of 0.5 mg/kg in normal saline was given by direct injection in the jugular, and the incision closed with wound clips. Animals were transferred to metabolic cages, and treated as described above.

Controls

Four rats (two male, two female) were treated with a single oral dose of corn oil, excreta collected and tissues collected to be used as radioassay and oxidizer background samples. Urine and feces samples were collected as described below for the animals receiving ametryn.

Sample Collection

Based on results from the Preliminary Study (RPT0007), it was determined that there was no detectable elimination of radioactivity as $^{14}CO_2$ following SOLD and SOHD exposures. Therefore, no $^{14}CO_2$ measurements were made in RPT0022 and P01744. What follows in this DER are the combined procedures and results from the three studies.

Urine and feces samples were collected at regular intervals during the seven- day post dosing period. Animals were sacrificed on day seven, and blood and tissue samples were taken and processed for analysis. Details are as follows:

Urine and Feces, as well as the urine/feces separator rinse, were collected at the following time intervals: 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours. All samples were promptly frozen and stored at -15°C until analysis. Upon collection the urine, cage wash and fecal sample weights were determined and recorded. At the end of the study, the cages were rinsed with water and the weight of water determined. A further rinse with methanol:water (1:1) was weighed.

Tissues and Organs: Upon sacrifice, blood samples were taken, a portion of which was for radioactivity determination. The remainder was centrifuged to separate plasma from red blood cells, and stored at -15°C. The following tissues were removed, individually weighed and stored at -15°C for radioassay: heart, kidney, liver, lung, bone, brain, fat, testes, ovaries, uterus, muscle (thigh), spleen, pancreas, skin (nape of neck, washed and shaved), and residual carcass.

Sample Processing for Radioassay

All urine, feces, blood and tissue samples were prepared as described below, and radioactive measurements were performed using a Beckman liquid scintillation spectrophotometer. Samples from controls were used to determine respective background radioactivity.

Feces were homogenized in distilled water (ca ¼ w/v) and duplicate aliquots representing about 100 mg feces combusted in a Harvey sample oxidizer The efficiency of the Harvey sample oxidizer was >95%. The combusted samples were trapped in Carbon 14 Cocktail in liquid scintillation counting vials, and radioactive measurements were performed.

Urine and Cage Washing samples were thawed (if frozen) and mixed, and duplicate aliquots were transferred to liquid scintillation counting vial and assayed for radioactivity as described above.

Tissues samples of 50 - 200 mg spleen, pancreas, fat, bone, prostate, seminal vesicles, ovary and uterus were dissected and analyzed. In addition, brain testes, heart, kidney, liver, lungs, muscle, skin and a 5 gm sample of residual carcass homogenate were homogenized together in distilled water 1/5 (w/v) and duplicate aliquots representing 100 mg of tissue were combusted in a Harvey sample oxidizer. The combusted material was collected in liquid scintillation vials and assayed for radioactivity.

Blood and Plasma. 100 μL aliquots of blood were directly transferred to combustion cones and combusted in a Harvey sample oxidizer. The combusted material was collected in liquid scintillation vials and assayed for radioactivity. 100 μL aliquots of plasma were transferred to liquid scintillation vials and the amount of radioactivity determined.

Analyses of Metabolites in Urine and Feces

In a separate, preliminary study, metabolites in rat urine and feces were isolated, purified and identified by solid phase extraction, high performance liquid chromatography (HPLC), thin-layer chromatography(TLC), gas chromatography/mass spectrometry (GC/MS), fast atom bombardment/mass spectrometry (FAB/MS), thermospray/liquid chromatography/mass spectrometry (TSP/LC/MS), and desorption chemical ionization/mass spectrometry (DCI/MS).

RESULTS AND DISCUSSION

As seen in Table 1, 91 - 96% of the administered radioactivity was recovered from dosed animals. Most was found in the urine and feces, excreted within the first 36 to 48 hours post dosing (Tables 2,and 3). Although there were no statistical differences between results in males and females, the total radioactivity recovered in males was consistently higher than females (Table 1). While both sexes excreted more radioactivity in the urine than feces, males consistently excreted more in the feces than females, and females consistently excreted more in the urine than males (Table 1), suggesting that absorption through the gut is higher in the female than the male. Again, these differences are not statistically significant.

Single Oral High dose group showed higher activity in the urine, for a longer period of time than the other dose groups (Table 2), a pattern not seen in the feces (Table 3).

Radioactivity in carcass and tissue was low (Tables 1, 6 and 7). No significant observations were made in this area.

Thirty-six metabolites, including polar nonconjugated metabolites and conjugated metabolites, were isolated (Table 4, Figure 1). Of these, 17 were identified (Fig. 1). Tables 4 and 5 show the quantities of the 13 major metabolites identified in the urine and feces. Trace levels of nonpolar metabolites, including the parent compound, were detected in feces.

N-Dealkylation of the molecule and glutathione conjugation, which led to mercapturic acid analogs, were the major routes of biotransformation in the rats. Oxidation of the n-isopropyl side chain to n-isopropionate and sulfate conjugation were also observed.

The following conclusions can be drawn from these data:

1. Over 50% of the orally administered ametryn is absorbed through the gut, as evidence by the recovered radioactivity in urine, blood and tissues.

2. Ametryn was distributed into all tissues assayed, including brain, although all tissue residues were generally low.

3. Ametryn is extensively metabolized to hydrophilic derivatives, which can explain why tissue residues were low.

4. Ametryn is excreted through the urine and feces, generally within the first 48 hours after administration.

TABLE 1. FATE OF DOSED RADIOACTIVITY

Distribution	Single Oral Low Dose		Single Oral High Dose		Multiple Oral Low Dose		Single Intravenous Dose	
	Male	Female	Male	Female	Male	Female	Male	Female
Mean Body Wt.(± S.D.)	161.8 5.8	166.2 1.6	159.4 2.2	169.4 4.6	241.8 27.4	198.0 12.5	173.4 13.1	165.8 5.6
% in Urine (± S.D.)	56.87 4.86	50.33 2.89	54.[illegible] 5.[illegible]	50.36 6.08	55.16 5.90	50.27 3.93	60.63 4.49	57.66 4.19
% in Feces (± S.D.)	37.04 2.88	39.29 2.31	39.20 4.50	42.06 5.62	36.36 5.46	40.18 3.90	29.99 4.32	31.43 4.32
% in Carcass & Tissues (± S.D.)	1.38 0.23	1.51 0.23	2.08 0.33	2.10 0.06	1.43 0.29	1.61 0.24	1.32 0.18	1.78 0.20
Total % Recovered (± S.D.)	95.30 6.34	91.10 3.74	96.00 3.58	94.53 1.06	92.95 2.39	92.10 2.28	91.95 5.03	90.37 4.83

TABLE 2. PERCENT OF RADIOACTIVITY EXCRETED IN RAT URINE

Time Post Dosing (Hours)	Single Oral Low Dose		Single Oral High Dose		Multiple Oral Low Dose		Single Intravenous Dose	
	Female	Male	Female	Male	Female	Male	Female	Male
0 - 4	9.73	1.08	2.96	1.57	15.57	12.89	33.21	25.16
4 - 8	18.79	32.06	5.79	9.37	15.34	23.82	8.98	19.28
8 - 12	7.81	13.06	6.96	9.91	6.96	7.56	6.85	7.82
12 - 24	8.56	6.68	17.57	20.23	6.15	5.87	4.11	4.02
24 - 36	2.32	1.36	11.34	8.90	1.94	1.94	1.38	1.66
36 - 48	0.91	0.81	2.86	2.09	1.04	1.04	0.93	0.85
48 - 72	1.01	0.65	1.20	1.02	1.18	0.81	0.69	0.64
72 - 96	0.32	0.37	0.74	0.69	0.96	0.55	0.72	0.50
96 -120	0.37	0.38	0.40	0.41	0.47	0.27	0.34	0.35
120-144	0.21	0.24	0.31	0.27	0.38	0.18	0.19	0.20
144-168	0.23	0.16	0.22	0.23	0.32	0.16	0.25	0.16
FINAL RINSE	0.07	0.02	0.01	0.02	0.05	0.06	0.02	0.02
TOTALS	50.33	56.87	50.36	54.72	50.27	55.16	57.66	60.63

TABLE 3. PERCENT OF RADIOACTIVITY EXCRETED IN RAT FECES

Time Post Dosing (Hours)	Single Oral Low Dose		Single Oral High Dose		Multiple Oral Low Dose		Single Intravenous Dose	
	Female	Male	Female	Male	Female	Male	Female	Male
0 - 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4 - 8	0.51	0.00	0.00	0.00	0.00	0.00	0.05	0.00
8 - 12	0.63	1.43	0.10	0.00	0.01	0.00	1.95	5.88
12 - 24	18.32	24.39	17.26	20.67	29.23	27.78	22.23	17.80
24 - 36	12.28	7.52	14.68	9.90	6.77	4.21	4.04	3.53
36 - 48	5.05	2.04	7.19	5.01	2.41	2.96	1.74	1.86
48 - 72	1.11	0.82	1.99	1.88	0.75	0.65	0.63	0.33
72 - 96	0.74	0.34	0.37	0.74	0.38	0.40	0.38	0.21
96 -120	0.27	0.27	0.18	0.31	0.18	0.11	.021	0.22
120-144	0.17	0.14	0.12	0.54	0.13	0.11	0.10	0.10
144-168	0.23	0.08	0.14	0.15	0.32	0.13	0.11	0.06
TOTALS	39.29	37.04	42.06	39.20	40.18	36.36	31.43	29.99

TABLE 4. MAJOR METABOLITES IDENTIFIED IN RAT URINE (AS % OF DOSE) FOLLOWING AMETRYN ADMINISTRATION

Name	Code	SOLD Day 1		MOLD Day 1		I.V. Day 1		SOLD Day 1,2	
		♂	♀	♂	♀	♂	♀	♂	♀
Atrazine Disulfide	CG13	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.19
6-Methoxy atrazine	M1	0.00	0.00	0.00	0.93	0.00	0.00	0.00	0.00
Mono-de-ethyl atrazine disulfide	M2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N-Acetylcysteine atrazine	CG11	1.34	2.30	2.75	3.08	4.14	5.12	4.04	4.75
Diamino ametryn	CG2	2.75	2.38	2.85	2.50	2.86	2.37	3.75	4.09
N-De-ethyl-N-acetylcysteine atrazine	M3	0.00	1.63	1.98	2.87	1.16	4.73	2.21	2.41
N-De-isopropyl-N-acetylcysteine atrazine	M10	5.20	6.81	5.97	5.13	6.08	7.22	9.62	10.46
N-Isopropanol-N-acetylcysteine atrazine	M4	1.61	1.86	2.26	1.49	1.23	2.20	2.47	2.01
2,4-Dihydroxy-N-acetylcysteine atrazine	M5	2.46	1.37	2.50	0.83	2.92	1.65	1.51	1.27
N-Isopropionate ametryn	M6	1.54	0.86	0.90	1.93	0.81	1.91	1.44	1.35
N-De-ethyl-[illegible]propionate ametryn	M7	9.53	4.08	7.63	3.20	10.11	6.00	5.77	3.92
2-O-Sulfonate ametryn	M8	12.42	6.05	8.19	4.58	10.54	4.91	5.12	3.17
6-Thioglucuronate atrazine	M9	1.20	0.81	1.10	1.33	1.61	1.79	2.56	2.00

SOLD = Single Oral Low Dose
MOLD = Multiple Oral Low Dose
SOHD = Single Oral High Dose
SID = Single Intravenous Dose

TABLE 5. MAJOR METABOLITES IDENTIFIED IN RAT FECES (AS % OF DOSE) FOLLOWING AMETRYN ADMINISTRATION

Name	Code	SOLD Day 1,2		MOLD Day 1,2		I.V. Day 1,2		SOLD Day 1,2	
		♂	♀	♂	♀	♂	♀	♂	♀
Atrazine Disulfide	CG13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6-Methoxy atrazine	M1	1.45	1.61	1.05	1.58	0.89	0.78	0.99	0.88
Mono-de-ethyl atrazine disulfide	M2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N-Acetylcysteine atrazine	CG11	1.32	1.65	1.28	1.33	0.26	0.00	0.99	4.97
Diamino ametryn	CG2	1.90	1.48	1.69	1.55	0.87	1.96	1.29	1.72
N-De-ethyl-N-acetylcysteine atrazine	M3	0.49	1.60	0.35	0.99	0.84	0.00	4.51	0.35
N-De-isopropyl-N-acetylcysteine atrazine	M10	4.25	4.81	5.58	5.89	3.41	4.60	5.19	4.09
N-Isopropanol-N-acetylcysteine atrazine	M4	0.02	0.00	0.00	0.00	0.00	1.18	0.00	5.12
2,4-Dihydroxy-N-acetylcysteine atrazine	M5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.47
N-Isopropionate ametryn	M6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N-De-ethyl-N-isopropionate ametryn	M7	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.79
2-O-Sulfonate ametryn	M8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6-Thioglucuronate atrazine	M9	1.69	3.03	1.28	5.37	0.98	3.89	1.05	2.31

SOLD = Single Oral Low Dose
SOHD = Single Oral High Dose
MOLD = Multiple Oral Low Dose
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