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

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JAN 7 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Memorandum 12/22/97

Subject:

Atrazine- Reviews of three studies examining: 1. Intravenous metabolism in the monkey; 2. Oral metabolism in the monkey; and 3. Dermal absorption in humans.

DP Barcode: D240618
Case: 838836
Submission: S514636
Chemical: Atrazine
Caswell No.: 063
PC No.: 080803

From: Roger Hawks, Ph.D. Roger Hawks 12/22/97
Toxicology Branch, II
Health Effects Division (7509c)

Thru: Stephen Dapson, Ph.D. Stephen C. Dapson 12/22/97
Branch Senior Scientist
Toxicology Branch, II, HED (7509c)

To: Catherine Eiden
RCAB, Health Effects Division (7509c)
and
Jeff Morris
SRRD (7508w)

Registrant: Novartis Crop Protection
P.O. Box 18300
Greensboro, NC
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Action Requested: Review 3 studies: *1. Disposition of Atrazine in Rhesus Monkey Following Intravenous Administration; 2. Disposition of Atrazine in Rhesus Monkey Following Oral Administration; 3. Dermal Absorption - Man.*

Response: The i.v. administration study in the monkey (MRID 44152112) has been reviewed and found to be acceptable. The oral administration study in monkey (MRID 44152113) has been reviewed and found to be acceptable. The dermal absorption study in man (MRID 44152114) has been reviewed and found to be acceptable. These studies do not satisfy, and were not submitted with the intention of satisfying, guidelines §85-1 or §85-2.

Reviewers:

Dynamac: Primary reviewer for all studies - Mary Menetrez
Secondary reviewer for all studies - Jay Early

EPA: Primary reviewer for all studies - Bod Zendiazin (SAB)
Secondary reviewer for all studies - Roger Hawks (TBII)

Data evaluation records (DERs) are attached and the exeutive summaries are as follows:

Disposition of Atrazine in Rhesus Monkeys Following Intravenous Administration (MRID 44152112)

Executive Summary:

In a metabolism study (MRID 44152112), [triazine ring-U-^{14}C]atrazine (96.5% a.i., 98.1% radiochemical purity) was administered to four female Rhesus monkeys as a single intravenous (i.v.) dose at 0.26 mg/animal (50.8 µCi/mg).

The principal routes of elimination were via the urine and feces. Within 24 hours of dosing, 62.5% of the administered dose was found in the urine. Within 168 hours of dosing, a mean of 98.91% of the administered dose was recovered from the four animals, of which 84.83% was in the urine, 11.73% was in the feces, and 2.35% was in the cage rinses. A 2-hour blood plasma sample was analyzed by HPLC and detected atrazine (9.8% TRR), deethyl atrazine (5.1% TRR), deisopropyl atrazine (10.7% TRR), didealkyl atrazine (12.7%), and an unknown (P-1; 4.3% TRR).

The TLC metabolite profiles for all four animals were similar and no parent atrazine was detected in 0-4 hour whole urine samples. Total chlorotriazines accounted for 25.1-30.9% of the radioactivity in the samples; mercapturic acid conjugates of atrazine and its chlorotriazine metabolites accounted for 7.6-13.04% of the radioactivity.

After isolation and cleanup of the 0-4 urine samples, total atrazine and its chlorotriazine metabolites detected by TLC and GC/MSD accounted for 12.9-33.1% of the TRR and included didealkyl atrazine (3.6-10.6% TRR), deethyl atrazine (7.1-19.9% TRR), and deisopropyl atrazine (0-2.6% TRR). A minor amount of parent atrazine, 0.7% TRR was detected by TLC in one mid-dose sample.

Total mercapturate metabolites detected by HPLC in 0-4 hour urine samples (post-cleanup) accounted for 13.9-19.3% of the TRR and included deethyl atrazine mercapturate (5.2-10.5% TRR), deisopropyl atrazine mercapturate (3.4-5.6% TRR), atrazine mercapturate (3.1-4.4 TRR), and didealkyl atrazine mercapturate (0.3-0.9% TRR).

Enzyme immunoassay techniques, sensitive to atrazine mercapturate, an expected glutathione pathway metabolite, detected the highest levels of the metabolite in the urine within the first 8 hours after dosing and with levels decreasing rapidly thereafter; 1.23-1.72% of the administered dose was detected with one assay. Cross reactivity by other metabolites was observed using the immunoassays.

The data indicate that renal excretion is the primary pathway for the elimination of atrazine from monkeys and that metabolism of [^{14}C]atrazine in the monkey involves dealkylation and glutathione conjugation.

This metabolism study in the monkey is **Acceptable-Non-guideline**. This study was not meant to satisfy guideline §85-1.

Disposition of Atrazine in Rhesus Monkey Following Oral Administration (MRID 44152113)

Executive Summary:

In a metabolism study (MRID 44152113), [triazine ring-U-^{14}C]atrazine (97.5-98.7% a.i., 96.8-97.0% radiochemical purity) was administered to nine female Rhesus monkeys by gavage as a single dose at levels of 1, 10, or 100 mg. Four monkeys were assigned to each dosing group. Three of the low dose monkeys were also dosed at the high-dose level approximately one month later.

The principal route of elimination was via the urine and feces. Within 24 hours, 31.0-46.13% of the administered dose was found in the urine. Within 168 hours of dosing, 91.25-94.73% of the administered dose was recovered from all of the animals, of which 52.95-58.45% was in the urine, 21.40-34.83% was in the feces, and 3.47-13.24 was in the cage rinses.

The metabolic profile in urine was similar between the test groups. Total chlorotriazine residues detected by TLC and GC/MSD in 0-48 hour urine samples accounted for 16.4-34.41% of the TRR in the three dosed groups. The two major chlorotriazine residues identified were didealkyl atrazine (10.7-23.6% TRR) and deethyl atrazine (5.2-10.12% TRR); diisopropyl atrazine (0.39-1.53% TRR) was also detected. The major chlorotriazine metabolites detected in Rhesus monkeys dosed intravenously with atrazine at 0.26

mg/animal were also didealkyl atrazine and deethyl atrazine. Parent atrazine was detected in two urine samples (0.3% TRR in 0-12 hour; 14.2% TRR in 24-36 hour samples), but its presence in urine was attributed to possible fecal contamination.

TLC analyses of 0-24 hour urine samples from all dose groups isolated minor amounts (<2% TRR) of the mercapturic acid conjugates of atrazine and its chlorotriazine metabolites. Enzyme immunoassays sensitive to atrazine mercapturate, an expected glutathione pathway metabolite detected only minor amounts (<1% TRR) of the metabolite. In the study in which monkeys were dosed intravenously with atrazine (MRID 44152112), the four mercapturate metabolites detected in 0-4 hour urine samples accounted for 13.9-19.3% of the TRR.

This metabolism study in the monkey is **Acceptable-Non-guideline**. This study was not meant to satisfy the 85-1 guideline.

Dermal Absorption - Man (MRID 44152114)

EXECUTIVE SUMMARY:

In a dermal absorption study (MRID 44152114), 10 human volunteers were exposed to a single topical dose of [triazine ring-U-^{14}C]atrazine (94.3-96.3% a.i., 98.0-98.4% radiochemical purity) at 6.7 (4 volunteers) or 79 µg/cm^2 (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [^{14}C]atrazine for the low and high doses, respectively.

Overall recoveries of radioactivity from the low- and high-dose groups were 101 and 92%, respectively. The majority (91.1-95.5%) of the dose remained unabsorbed and was detected in skin wash samples taken 24 hours after dosing. After 168 hours, only 5.6% of the dose was absorbed and excreted in the urine and feces of the low-dose group and only 1.2% in the high-dose group. The renal excretion half-life was 19.6-29 hours for the low-dose group and 25.9-31 hours for the high-dose group. In both dose groups, peak urinary elimination occurred at 24-48 hours and peak fecal elimination occurred at 48-72 hours.

Total chlorotriazine residues detected by TLC in a high-dose 0-24 hour composited urine sample accounted for 9.16% of the TRR and included deethyl atrazine (3.88% TRR) and didealkyl atrazine (5.28% TRR). No atrazine was detected. GC/MSD analysis of urine samples also did not detect atrazine or its chlorotriazine metabolites.

Enzyme immunoassays indicated that levels of atrazine mercapturate, an expected glutathione pathway metabolite was near the limit of detection/quantitation for the methods. Mercapturic acid conjugates of atrazine and its chlorotriazine metabolites were not detected by LC/MS/MS in urine samples.

Some similarities in HPLC profiles of urine from i.v. dosed monkeys (MRID 44152112) and dermally treated humans were observed.

This dermal absorption study on humans is **Acceptable-Non-guideline**. This study was not meant to satisfy the 85-2 guideline.

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DATA EVALUATION RECORD

ATRAZINE

Study Type: 85-1; Disposition of Atrazine in Rhesus Monkey Following Intravenous Administration

Work Assignment No. 2-62A (MRID 44152112)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
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Date: 6/27/97

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Program Manager
Mary L. Menetrez, Ph.D.
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Date: 7/9/97

Quality Assurance
Steven Brecher, Ph.D.
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Date: 7/9/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Bob Zendiazin, Ph.D. Stephen C. Dapson for 12/22/97
Science Analysis Branch (7509c)

EPA Secondary Reviewer: Roger Hawks, Ph.D. Roger Hawks 12/12/97
Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Monkey
OPPTS Number: 870.7485 OPP Guideline Number: §85-1

DP BARCODE: D232343 SUBMISSION CODE: S514645
P.C. CODE: 080803 TOX. CHEM. NO.: 63

TEST MATERIAL (PURITY): Atrazine (96.5% a.i.)

SYNONYMS: 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine

CITATION: Hui, X., Wester, R., and Maibach, H.I., (UCSF) Gilman, S.D., Gee, S.J. Hammock, B.D. (UC Davis) Simoneaux, B., Breckenridge, C., Kahrs, R. (Ciba) (1996). Disposition of Atrazine in Rhesus Monkey Following Intravenous Administration. Surge Laboratory, University of California, San Francisco, CA, Department of Entomology, University of California, Davis, CA, and Ciba Geigy Corporation, Greensboro, NC. Laboratory Study Numbers UCSF 95SU04, BDH-081-1, ABR 96066, 96073, September 3, 1996. MRID 44152112. Unpublished.

SPONSOR: Ciba Crop Protection, Ciba-Geigy Corporation, PO Box 18300, Greensboro, NC

EXECUTIVE SUMMARY:

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The TLC metabolite profiles for all four animals were similar and no parent atrazine was detected in 0-4 hour whole urine samples. Total chlorotriazines accounted for 25.1-30.9% of the radioactivity in the samples; mercapturic acid conjugates of atrazine and its chlorotriazine metabolites accounted for 7.6-13.04% of the radioactivity.

After isolation and cleanup of the 0-4 urine samples, total atrazine and its chlorotriazine metabolites detected by TLC and GC/MSD accounted for 12.9-33.1% of the TRR and included didealkyl atrazine (3.6-10.6% TRR), deethyl atrazine (7.1-19.9% TRR), and deisopropyl atrazine (0-2.6% TRR). A minor amount of parent atrazine, 0.7% TRR was detected by TLC in one mid-dose sample.

Total mercapturate metabolites detected by HPLC in 0-4 hour urine samples (post-cleanup) accounted for 13.9-19.3% of the TRR and included deethyl atrazine mercapturate (5.2-10.5% TRR), deisopropyl atrazine mercapturate (3.4-5.6% TRR), atrazine mercapturate (3.1-4.4 TRR), and didealkyl atrazine mercapturate (0.3-0.9% TRR).

Enzyme immunoassay techniques, sensitive to atrazine mercapturate, an expected glutathione pathway metabolite, detected the highest levels of the metabolite in the urine within the first 8 hours after dosing and with levels decreasing rapidly thereafter; 1.23-1.72% of the administered dose was detected with one assay. Cross reactivity by other metabolites was observed using the immunoassays.

The data indicate that renal excretion is the primary pathway for the elimination of atrazine from monkeys and that metabolism of [^{14}C]atrazine in the monkey involves dealkylation and glutathione conjugation.

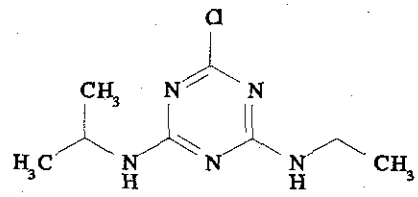
This metabolism study in the monkey is **acceptable**. This study was not meant to satisfy guideline §85-1.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: [Triazine ring-U-^{14}C]Atrazine
   Chemical purity: 96.5% a.i. [determined by GC]
   Radiochemical purity: 98.1% [determined by TLC]
   Specific activity: 50.8 µCi/mg
   Lot/Batch: Not provided
   Structure:



2. Vehicle: 0.9% sodium chloride solution/propylene glycol (2:1) for a final concentration of 0.00385% (w/v)

3. Test animals: Species: Monkey
   Strain: Rhesus
   Age at study initiation: Four females, approximately 20-30 years old.
   Weight at study initiation: Not reported
   Source: U. California San Francisco colony
   Housing: Individually housed in stainless steel cages equipped with containers for collection of urine and feces
   Diet: Purina Monkey Chow (Ralston Purina Company, St. Louis, MO), ad libitum. A fruit supplement was provided daily.
   Water: Tap water, ad libitum
   Environmental conditions:
      Temperature: 66-78 F
      Humidity: 40-60%
      Air Changes: Not reported
      Photoperiod: 12 hours light/12 hours dark
   Acclimation period: Not reported

B. STUDY DESIGN:

The Agency issued a Position Document 1-Initiation of Special Review (PD-1) for three triazine herbicides including atrazine. This study, which was designed to determine [^{14}C]atrazine metabolism and excretion over a 7 day period in monkeys, and the accompanying submissions (MRIDs 44152113 and 44152114) were submitted in partial response to this PD-1. The in-life phase and the measurement of radioactivity were performed by the University of California, San Francisco (UCSF). Additional analyses of urine samples were performed

by the University of California, Davis (UCD), and Ciba-Geigy (CIBA).

1. Experimental Design

Four female Rhesus monkeys were chosen on the basis of good health and body weight.

2. Dosing and sample collection

Radiolabeled atrazine was dissolved in 0.9% sodium chloride solution/propylene glycol (2:1) for a final concentration of 0.00385% (w/v; 1.97 μCi/mL). This stock solution was protected from light and stored at 0-4 C in ambient humidity prior to use on the following day. It was stated that the dose formulation was analyzed for stability by the sponsor, the data however, were not submitted.

Prior to dosing, each animal was sedated with an intramuscular injection of ketamine and xylacine. Catheters were placed in the forearm cephalic vein for i.v. administration and lower saphenous vein for blood sampling. Each monkey received a single i.v. 6.78 mL dose of the dosing solution containing 0.26 mg [^{14}C]atrazine (13.35 μCi). After dosing, the catheter was flushed with saline and then removed.. The delivered dose was quantitated by the weight difference of the dosing syringe before and after dosing.

a. Pharmacokinetics analysis

Blood samples were collected at 0, 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing and separated into plasma and packed blood cells. Urine samples were collected during the 24 hour period immediately prior to dosing and during the following intervals after dosing: 0-4, 4-8, 8-12, 12-24, and at 24 hour intervals during days 2 through 7. Feces samples were collected every 24 hours beginning with the 24 hours prior to dosing. In addition, each cage was washed with water every 24 hours and the cage rinse samples collected for analysis.

Duplicate samples of plasma, urine, and cage rinse were analyzed directly for total [^{14}C] residues using LSC. The packed blood cells and feces samples were each homogenized in distilled water and combusted in duplicate prior to LSC analysis. Confirmatory LSC analyses of all urine samples were performed in triplicate.

b. Metabolite characterization

Frozen blood and urine samples were shipped on dry ice to the University of California, Davis (UCD), and Ciba-Geigy

(CIBA) for further analyses. The blood serum and urine samples arrived at UCD still frozen and were immediately stored at -20 C. The amount of blood serum received by UCD was insufficient for analysis. The report by CIBA did not remark on the integrity of the samples received by them.

Prior to analysis, urine samples were thawed and the pH adjusted to 7.5 using either 1M HCl or 1M NaOH. For metabolite characterization, whole urine samples collected from each of the four monkeys at the 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72 hour intervals were analyzed by 1-D TLC. Radioactive zones were visualized with a radioactivity detector and unlabelled standards were visualized under ultraviolet light (UV). A mixture of unlabelled analytical standards were co-chromatographed with the samples. The standards consisted of atrazine, deethyl atrazine, deisopropyl atrazine, didealkyl atrazine, and their mercapturate conjugates.

Additional metabolite characterizations of the 0-4 hour urine samples were performed using 1-D TLC and reverse phase HPLC after fractionation into neutral and acidic components using anion and cation exchange chromatography. The HPLC eluates were analyzed by radioactive monitoring and UV absorption and radioactivity was determined by LSC.

An enzyme linked immunosorbent assay (ELISA) and an enzyme immunoassay (EIA) for atrazine mercapturate, a glutathione pathway metabolite, were performed on all urine samples.

The 0-24 hour urine samples from each of the animals were composited and fractionated by anion and cation exchange chromatography prior to LC/MS/MS analysis for mercapturate metabolites.

Urine samples with sufficient volume and total radioactive residues were analyzed using GC/MSD in the SIM mode for chlorotriazine metabolites. Prior to GC/MSD analysis, proteins were precipitated from the urine samples with acetonitrile, and the residues extracted from the supernatant by partitioning into ethyl acetate.

Blood plasma samples were assayed by LSC and HPLC after removal of endogenous proteins by dilution with water and centrifugation.

5. Data Analysis

Radioactivity, in terms of concentration (μg equivalents/g), dpm, dpm/g, and the % of administered dose, was reported for individual samples and as the mean (with ± S.D.) of four animals. No statistical comparisons were made.

To determine blood pharmacokinetics parameters, the administered dose was entered as μg equivalents of atrazine to a two compartment pharmacokinetics model.

II. RESULTS

A. Pharmacokinetics analysis

Concentration of radioactivity in plasma and packed blood cell were similar at the various assay intervals and radioactivity in both declined rapidly after dosing (Table 1).

Table 1. Mean concentration (μg atrazine equivalents/g ±SD) of radioactivity in plasma and packed blood cells from 4 monkeys administered one i.v. dose at 0.26 mg/animal. [a]

Time (hours)	Radioactivity (mean μg equivalents/g ±SD)	
	Plasma	Packed Blood Cells
0.5	0.0333 ±0.0045	0.0273 ±0.0028
1	0.0290 ±0.0023	0.0237 ±0.0021
2	0.0231 ±0.0022	0.0229 ±0.0015
4	0.0159 ±0.0014	0.0174 ±0.0008
8	0.0115 ±0.0012	0.0129 ±0.0009
12	0.0088 ±0.0005	0.0123 ±0.0029
24	0.0057 ±0.0016	0.0093 ±0.0018

a Data were obtained from Tables 2b and 4b pages 35 and 41 of the study report.

The time course of [^{14}C]atrazine in the plasma of the animals was adequately described by a two-compartment pharmacokinetics model. The area under the curve (AUC) was calculated to be 0.42 ± 0.03 μg/hr/mL, the plasma clearance (Cl) was 601.08 ± 38.07 mL/hr, the peak plasma concentration (Cmax) was 0.0385 ± 0.0006 μg/mL and the elimination phase half-life ($T_{1/2}ß$) was 17.71 ±2.16 hours.

Within 24 hours of receiving a single i.v. dose of [^{14}C]atrazine at 0.26 mg/animal, 62.52% of the administered

dose was excreted via the urine (Table 2). By the end of the 7-day collection period (168 hours) urinary excretion accounted for 84.83% of the administered dose. Cumulative radioactivity recovered from feces and cage rinses totaled 11.73 and 2.35% of the administered dose, respectively. The overall recovery of radioactivity in monkeys following administration of a single i.v. dose of [^{14}C]atrazine at 0.26 mg/animal was 98.91% by 168 hours.

Table 2. Recovery of radioactivity (mean % dose) from urine, feces and cage washings following administration of a single i.v. dose of [^{14}C]atrazine to 4 monkeys at 0.26 mg/animal.[a]

Period (Hours)	Urine		Feces		Cage Washings	
	Mean % Dose ±SD	Cumulative % Dose	Mean % Dose ±SD	Cumulative % Dose	Mean % Dose ±SD	Cumulative % Dose
0-4	19.95 ±4.62	19.95	-[b]	-	-	-
4-8	13.75 ±4.62	33.69	-	-	-	-
8-12	10.62 ±6.20	44.31	-	-	-	-
12-24	18.21 ±4.59	62.52	-	-	-	-
0-24	-	-	0.62 ±1.14	0.62	1.26 ±0.79	1.26
24-48	9.03 ±0.79	71.54	0.53 ±0.62	1.15	0.64 ±0.28	1.90
48-72	3.94 ±0.74	75.48	7.42 ±1.26	8.57	0.22 ±0.10	2.11
72-96	5.77 ±0.722	81.25	2.22 ±1.04	10.79	0.07 ±0.05	2.19
96-120	2.70 ±0.25	83.95	0.54 ±0.23	11.34	0.05 ±0.02	2.24
120-144	0.47 ±0.09	84.42	0.20 ±0.08	11.54	0.07 ±0.05	2.31
144-168	0.41 ±0.18	84.83	0.20 ±0.06	11.73	0.05 ±0.03	2.35

a Data were obtained from Tables 6C, 8C and 10b; pages 48, 56, and 63, respectively, of the study report.
b - = Not applicable.

Confirmatory LSC data indicated that the excretion rate (nanomoles/hr) for the four animals was highest at 0-4 hours post dosing, the half-life for urine elimination was 20.8 hours, and that by 168 hours, 75.9-90.0% (average of 81%) of the administered dose was excreted in the urine.

B. Metabolite Characterization

The results of TLC analyses of selected whole urine samples are summarized in Table 3. The metabolic profiles for all four animals were similar and no parent atrazine was detected in any of the urine samples. The chlorotriazine metabolites appearing within the first four hours in urine were didealkyl

atrazine (11.3-18.5% of the radioactivity in the samples), deethyl atrazine (9.2-13.0%), didealkyl atrazine mercapturate (4.3-7.3%) and atrazine mercapturate (0-7.17%). The majority of the radioactivity in the urine samples (48.1-59.0%) remained at the origin.

Table 3. TLC metabolite profile in whole urine collected from four monkeys 0-4 hours after a single i.v. dose with [^{14}C]atrazine at 0.26 mg/animal.[a]

Component Common Name (Code)	% Radioactivity in Sample			
	Animal #1	Animal #2	Animal #3	Animal #4
Atrazine (G-30027)	ND [b]	ND	ND	ND
Deethyl atrazine (G-30033)	9.62	11.1	9.2	13.0
Didealkyl atrazine (G-28273)	12.24	11.3	18.5	12.9
Deisopropyl atrazine (G-28279)	6.10	ND	ND	ND
Hydroxyatriazine	ND	2.7	2.9	5.0
Total Chlorotriazines	**27.96**	**25.1**	**30.6**	**30.9**
Deethyl atrazine mercapturate (CGA-246059)	ND	ND	2.7	ND
Didealkyl atrazine mercapturate (CGA-10582)	5.87	5.1	7.3	4.3
Atrazine mercapturate (CGA-359008)	7.17	2.7	ND	3.3
Deisopropyl atrazine mercapturate (CGA-60379)	ND	2.4	ND	ND
Total Mercapturates	**13.04**	**10.2**	**10.0**	**7.6**
Unknown #1 (origin)	59.0	48.1	56.7	49.5
Unknown #2	ND	3.2	2.7	3.6
Unknown #3	ND	ND	ND	3.2
Unknown #4	ND	ND	ND	5.1
Unknown #6	ND	8.6	ND	ND
Unknown #7	ND	4.9	ND	ND
Total Unknowns	**59.0**	**64.8**	**59.4**	**58.2**

a Data extracted from Tables 9 through 12, pages 101-107 of the study report.
b ND = None detected

Prior to additional metabolite characterization, the 0-4 hour urine samples were fractionated into neutral and acidic components by anion exchange chromatography (AEC). TLC analysis of the neutral AEC fractions for chlorotriazines (Table 4) detected deethyl atrazine (7.1-19.9% TRR), didealkyl atrazine (3.6-10.6% TRR), and deisopropyl atrazine (0-2.6% TRR). Parent atrazine was detected in one sample at 0.7% TRR. No residues of atrazine or deisopropyl atrazine were detected in the 0-4 hour urine samples using GC/MSD (Table 4). GC/MSD analyses were comparable to TLC analyses, detecting the two major chlorotriazine metabolites, deethyl atrazine (7.9 and 9.7% TRR) and didealkyl atrazine (4.4 and 5.0% TRR).

HPLC analysis of the AEC acidic fractions of the 0-4 hour urine samples detected four mercapturate metabolites as follows (Table 4): deethyl atrazine mercapturate (5.2-10.5% TRR), deisopropyl atrazine mercapturate (3.4-5.6% TRR), atrazine mercapturate (3.1-4.4 TRR), and didealkyl atrazine mercapturate (0.3-0.9% TRR). Atrazine mercapturate detected using the EIA method accounted for 1.8-3.6 of the TRR. In the 0-4 hour urine samples, total chlorotriazine metabolites accounted for 12.9-33.1% of the TRR and the mercapturate metabolites accounted for 13.9-19.3% of the TRR.

Table 4. Metabolite profiles obtained from urine collected from four monkeys 0-4 hours after a single i.v. dose with [^{14}C]atrazine. Urine samples were cleaned up by ion chromatography prior to analyses.[a]

Compound Fraction (Code)	% TRR [b]			
	Animal #1	Animal #2	Animal #3	Animal #4
Atrazine (G-30027)	0 (0)	0.7	0 (0)	0
Deethyl atrazine (G-30033)	8.6 (9.7)	8.2	7.1 (7.9)	19.9
Deisopropyl atrazine (G-28279)	0.8 (0)	1.5	0 (0)	2.6
Didealkyl atrazine (G-28273)	5.2 (4.4)	3.6	5.9 (5.0)	10.6
Total chlorotriazines	**14.6 (14.1)**	**14.0**	**13.0 (12.9)**	**33.1**
Atrazine mercapturate (CGA-359008)	4.3 (2.1)	4.4 (2.6)	3.1 (1.8)	3.6 (3.6)
Deethyl atrazine mercapturate (CGA-246059)	6.8	5.2	10.5	6.3
Deisopropyl atrazine mercapturate (CGA-60379)	5.6	3.4	5.2	3.7
Didealkyl atrazine mercapturate (CGA-10582)	0.4	0.9	0.5	0.3
Total mercapturates	**17.1**	**13.9**	**19.3**	**13.9**

a Data were extracted from Tables 4 and 9, pages 178 and 183 and Tables 5 through 8, pages 179-182 of the study report.
b For chlorotriazines, data were obtained from TLC and GC/MSD analyses, GS/MSD data listed parenthetically; for mercapturates, data were obtained from HPLC; for atrazine mercapturate, data obtained from HPLC analyses and enzyme immunoassays (EIA), EIA data listed parenthetically.

Immunoassays of urine samples detected the highest levels of atrazine mercapturate within the first 8 hours after dosing and levels decreased rapidly after that; 1.23-1.72% of the administered dose was detected with the ELISA. LC/MS/MS analysis of composited (0-24 hour) urine samples detected the four mercapturic acid conjugates (1.6-3.0% TRR). The amount of atrazine mercapturate determined by EIA in composite 0-24 hour urine samples was greater than that detected by LC/MS/MS indicating cross reactivity by other metabolites using the EIA method. Various techniques were tried to identify the cross reactive metabolites; the efforts were not successful.

HPLC analysis of a 2-hour blood plasma sample detected atrazine (9.8% TRR), deethyl atrazine (5.1% TRR), deisopropyl atrazine (10.7% TRR), didealkyl atrazine (12.7%), and an unknown (P-1; 4.3% TRR).

These data indicate that the metabolic pathway of atrazine involves dealkylation and glutathione conjugation. The sponsor's proposed metabolic pathway for atrazine is presented as an attachment to this DER (study report Figure 39, page 225).

III. DISCUSSION

A. Investigator's Conclusions

Urinary excretion of [^{14}C]atrazine was rapid; 62.5-68.4% of the administered dose was eliminated within the first 24 hours. By the end of the 7-day collection period 81.25-84.83% of the dose was found in urine and 11.7% in feces. The metabolite profile observed in monkeys was comparable to that previously reported for man. The major chlorotriazine residues detected in urine were deethyl atrazine and didealkyl atrazine; four mercapturic acid conjugates of the chlorotriazines were also detected in the urine. The total chlorotriazines accounted for more of the urine radioactive residues than the mercapturates. The majority of the radioactive residue was accounted for by polar and unidentified components. The ELISA technique can be used to measure atrazine mercapturate in urine for at least the first 3 days after exposure. The main biotransformation of [^{14}C]atrazine in the monkey involves dealkylation and glutathione conjugation.

B. Reviewer's Discussion

[^{14}C]Atrazine (96.5% a.i.) was administered to four female Rhesus monkeys as a single i.v. dose at 0.26 mg/animal (50.8 μCi/mg). The principal routes of elimination were via the urine and feces. In urine, within 24 hours of dosing, 62.5-68.4% of the administered dose was found and within 168 hours, 81.25-84.84% was recovered. Cumulative radioactivity recovered from feces (168 hours) was 11.73%. The overall recovery of radioactivity from the four monkeys, including urine, feces, and cage rinses, was 98.92 ± 5.87%. A 2-hour blood plasma sample was analyzed by HPLC and detected atrazine (9.8% TRR), deethyl atrazine (5.1% TRR), deisopropyl atrazine (10.7% TRR), didealkyl atrazine (12.7%), and an unknown (P-1; 4.3% TRR).

The TLC metabolite profiles for all four animals were similar and no parent atrazine was detected in any of the (0-4 hour) whole urine samples. Total chlorotriazines accounted for 25.1-30.9% of the radioactivity in the whole urine (0-4 hour) samples; mercapturate metabolites accounted for 7.6-13.04% of the radioactivity.

After isolation and cleanup of the 0-4 urine samples, chlorotriazine and mercapturate metabolites were analyzed

using TLC, GC/MSD, HPLC, and immunoassay techniques. Total chlorotriazine residues detected by TLC and GC/MSD accounted for 12.9-33.1% of the TRR and included didealkyl atrazine (3.6-10.6% TRR), deethyl atrazine (7.1-19.9% TRR), and deisopropyl atrazine (ND-2.6% TRR). A minor amount of parent atrazine, 0.7% of the total radioactive residue (TRR) was detected by TLC in one mid-dose sample.

Total mercapturate metabolites detected by HPLC in 0-4 hour urine samples accounted for 13.9-19.3% of the TRR and included deethyl atrazine mercapturate (5.2-10.5% TRR), deisopropyl atrazine mercapturate (3.4-5.6% TRR), atrazine mercapturate (3.1-4.4 TRR), and didealkyl atrazine mercapturate (0.3-0.9% TRR).

Immunoassays of urine samples detected the highest levels of atrazine mercapturate within the first 8 hours after dosing and levels decreased rapidly after that; 1.23-1.72% of the administered dose was detected with the ELISA. The amount of atrazine mercapturate determined by EIA in composite 0-24 hour urine samples was greater than that detected by LC/MS/MS indicating cross reactivity by other metabolites with the EIA method.

The data indicate that renal excretion is the primary pathway for the elimination of atrazine from monkeys and that metabolism of [^{14}C]atrazine in the monkey involves dealkylation and glutathione conjugation.

This metabolism study in the monkey is **acceptable**. This study was not meant to satisfy guideline 85-1.

IV. STUDY DEFICIENCIES

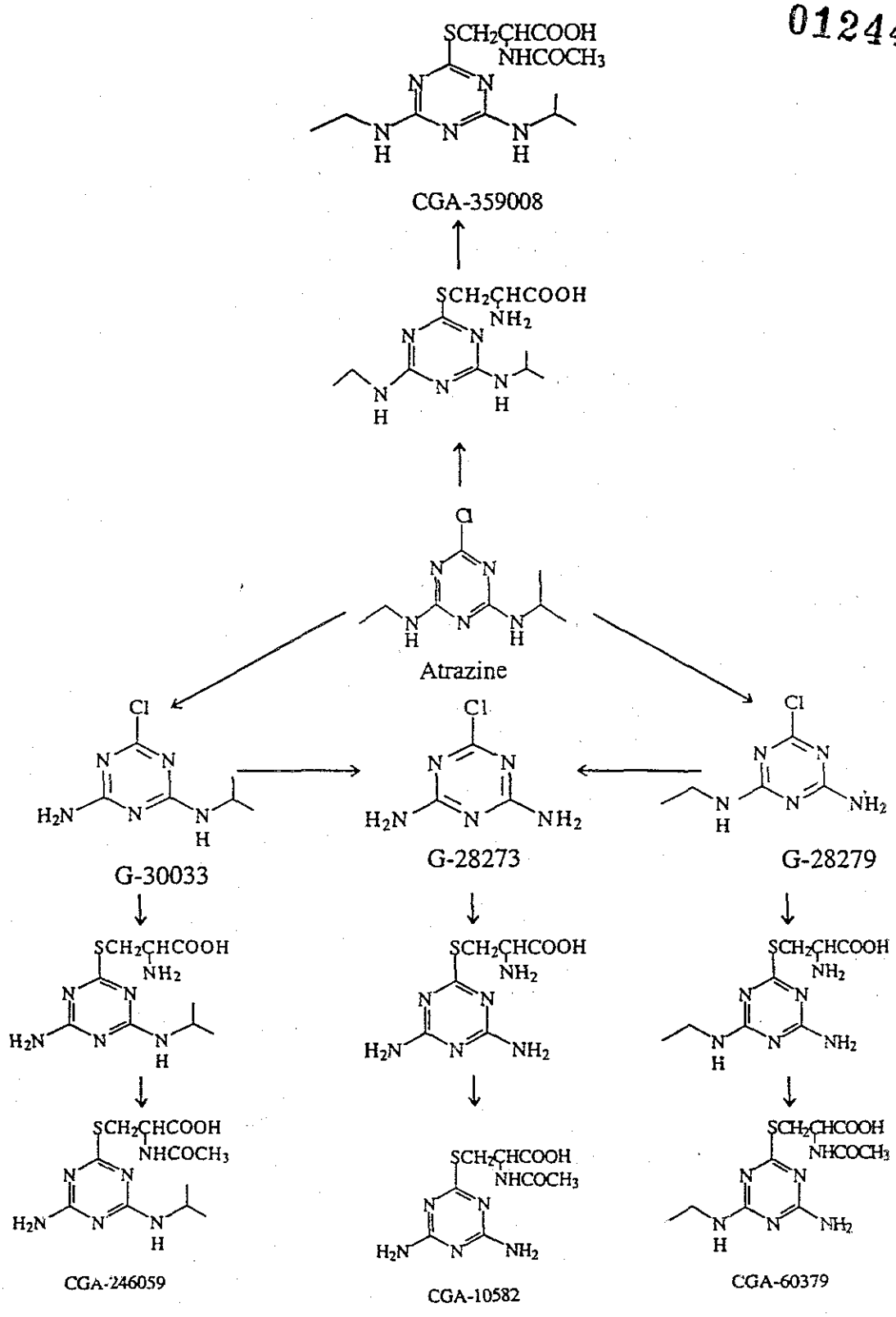
None noted.

ATTACHMENTS

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

FIGURE 39. PROPOSED PATHWAY FOR ATRAZINE METABOLISM IN MONKEYS

012444



DATA EVALUATION RECORD 012444

ATRAZINE

Study Type: 85-1; Disposition of Atrazine in Rhesus Monkey Following Oral Administration

Work Assignment No. 2-62B (MRID 44152113)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Science Analysis Branch (7509c)

EPA Secondary Reviewer: Roger Hawks, Ph.D. Roger Hawks 12/17/97
Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Monkey
OPPTS Number: 870.7485 OPP Guideline Number: §85-1

DP BARCODE: D232343 SUBMISSION CODE: S514645
P.C. CODE: 080803 TOX. CHEM. NO.: 63

TEST MATERIAL (PURITY): Atrazine (97.5-98.7% a.i.)

SYNONYMS: 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine

CITATION: Hui, X., Wester, R., Maibach, H.I. (UCSF) and Simoneaux, B., Breckenridge, C., (Ciba Crop Protection) (1996). Disposition of Atrazine in Rhesus Monkey Following Oral Administration. Surge Laboratory, University of California, San Francisco, CA and Ciba-Geigy Corporation, Greensboro, NC. Laboratory Project Identifications UCSF 96SU01, ABR-96094, Study Number 306-96, October 30, 1996. MRID 44152113. Unpublished.

SPONSOR: Ciba Crop Protection, Ciba-Geigy Corporation, PO Box 18300, Greensboro, NC

EXECUTIVE SUMMARY:

In a metabolism study (MRID 44152113), [triazine ring-U-^{14}C]atrazine (97.5-98.7% a.i., 96.8-97.0% radiochemical purity) was administered to nine female Rhesus monkeys by gavage as a single dose at levels of 1, 10, or 100 mg. Four monkeys were assigned to each dosing group. Three of the low dose monkeys were also dosed at the high-dose level approximately one month later.

The principal route of elimination was via the urine and feces. Within 24 hours, 31.0-46.13% of the administered dose was found in the urine. Within 168 hours of dosing, 91.25-94.73% of the administered dose was recovered from all of the animals, of which 52.95-58.45% was in the urine, 21.40-34.83% was in the feces, and 3.47-13.24 was in the cage rinses.

The metabolic profile in urine was similar between the test groups. Total chlorotriazine residues detected by TLC and GC/MSD in 0-48 hour urine samples accounted for 16.4-34.41% of the TRR in the three dosed groups. The two major chlorotriazine residues

identified were didealkyl atrazine (10.7-23.6% TRR) and deethyl atrazine (5.2-10.12% TRR); diisopropyl atrazine (0.39-1.53% TRR) was also detected. The major chlorotriazine metabolites detected in Rhesus monkeys dosed intravenously with atrazine at 0.26 mg/animal were also didealkyl atrazine and deethyl atrazine. Parent atrazine was detected in two urine samples (0.3% TRR in 0-12 hour; 14.2% TRR in 24-36 hour samples), but its presence in urine was attributed to possible fecal contamination.

TLC analyses of 0-24 hour urine samples from all dose groups isolated minor amounts (<2% TRR) of the mercapturic acid conjugates of atrazine and its chlorotriazine metabolites. Enzyme immunoassays sensitive to atrazine mercapturate, an expected glutathione pathway metabolite detected only minor amounts (<1% TRR) of the metabolite. In the study in which monkeys were dosed intravenously with atrazine (MRID 44152112), the four mercapturate metabolites detected in 0-4 hour urine samples accounted for 13.9-19.3% of the TRR.

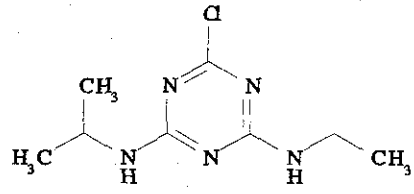
This metabolism study in the monkey is **acceptable**. This study was not meant to satisfy the 85-1 guideline.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: [Triazine ring-U-^{14}C]Atrazine
   Chemical purity: determined by GC; 98.7% a.i. (low and mid dose) and 97.5% a.i. (high dose)
   Radiochemical purity: determined by TLC; 96.8% (low and mid dose) and 97.0% (high dose)
   Specific activity: 28.9 µCi/mg (low and mid dose) and 2.9 µCi/mg (high-dose)
   Lot/Batch: JAK-XIII-23 (low and mid dose) and JAK-XIII-25 (high-dose)
   Structure:



2. Vehicle: Microgranular cellulose

3. Test animals: Species: Monkey
   Strain: Rhesus
   Age at study initiation: Nine females, 6 to 25 years old.
   Weight at study initiation: 4.99-9.53 kg
   Source: U. California San Francisco colony
   Housing: Individually housed in stainless steel cages equipped with containers for collection of urine and feces
   Diet: Purina Monkey Chow (Ralston Purina Company, St. Louis, MO), ad libitum. A fruit supplement was provided daily.
   Water: Tap water, ad libitum
   Environmental conditions:
      Temperature: 66-78 F
      Humidity: 40-60%
      Air Changes: Not reported
      Photoperiod: 12 hours light/12 hours dark
   Acclimation period: Not reported

B. STUDY DESIGN:

The study was designed to determine [^{14}C]atrazine absorption, metabolism, and excretion over a 7 day period in monkeys following a single oral dose at levels of 1, 10, or 100 mg/animal.

1. Group Arrangements

Four animals were assigned to each dosing group. Three of the low-dose monkeys were also dosed at the high-dose level approximately one month later. This was not expected to affect the results obtained in the high-dose group because the low dose received initially was expected to have been eliminated after one month. The authors cited the intravenous dosing study (MRID 44152112), which supports that assumption.

Table 1: Dosing groups for the [^{14}C]atrazine pharmacokinetics study.

Test Group	Dose to Animals (mg/animal)	Number of Animals
Low dose	1	4 [a]
Mid dose	10	4
High dose	100	4

a Three of the low dose monkeys were also dosed at the high-dose level approximately one month after receiving the low dose.

2. Dosing and sample collection

On the day of dosing, each animal was sedated with an intramuscular injection of ketamine, placed in a metabolic chair, and the radioactive doses administered by gavage.

The low and mid treatment groups were dosed with one capsule each containing 1 or 10 mg ±5% of radiolabeled atrazine and microgranular cellulose, respectively. The high-dose treatment group was dosed with two capsules containing 50 mg ±0.5% of [^{14}C]atrazine and 10 mg of microgranular cellulose. The dosing capsules had been placed in glass vials and stored at -15 C until use. Samples of the dosing capsules were shipped to the sponsor for stability testing. The mean concentration of atrazine was 96.9% and the sponsor concluded that the test material was stable.

a. Pharmacokinetics analysis

Blood samples were collected at 0, 2, 4, 8, 24, 48, and 72 hours after dosing and separated into plasma and packed blood cells. Urine and fecal samples were collected during the 24 hour period immediately prior to dosing and at 12 hour intervals after dosing up to 168 hours. In addition, each cage was rinsed with water and

absolute ethanol every 12 hours and the cage rinses were collected for analysis.

Duplicate samples of plasma, urine, and cage rinse were analyzed directly for total [^{14}C] residues using LSC. Packed blood cells and feces samples were each homogenized in distilled water and combusted in duplicate prior to LSC analysis. Additional LSC analyses of urine were performed to calculate percent of total radioactive residue (% TRR) in each urine sample.

b. Metabolite characterization

Frozen urine samples were shipped to the Ciba-Geigy Crop Protection lab for further analyses.

For chlorotriazine metabolite characterization, individual urine samples collected at 12 hour intervals for up to 48 hours were analyzed by 1-D TLC and gas chromatography/mass selective detection (GC/MSD) in the SIM mode. For TLC, isolated metabolites were identified by co-chromatography with parent and seven reference standards. Radioactivity was determined by manual peak area selection or liquid scintillation counting (LSC). Non-radioactive reference standards were visualized under ultraviolet light (UV). Prior to GC/MSD analysis, proteins were precipitated from the urine samples with acetonitrile; the residues were purified using an anion exchange column and then partitioned into ethyl acetate.

To determine the distribution of atrazine, and the chlorotriazine and mercapturic acid metabolites, the 0-12 and 12-24 hour urine samples from one monkey in each dose group were analyzed by 2-D TLC. In addition, an enzyme immunoassay (EIA) for atrazine mercapturate was performed in duplicate on all 0-48 hour urine samples.

5. Data Analysis

Radioactivity, in terms of concentration (μg equivalents/ml), dpm, and the % of administered dose was reported for individual samples and as the mean (with $\pm$ S.D.) of four animals/dose.

Computer software was used to determine blood pharmacokinetics parameters using the mean value of plasma atrazine equivalent levels of each dose group versus time data. The area under the curve (AUC) was determined as the area under the plasma concentration. The oral bioavailability (F) was estimated by the following ratio:

$$F = \frac{oral\ AUC/dose}{I.V.\ AUC/dose}$$

The i.v. AUC was obtained from the study (MRID 44152112) in which each of four monkeys received a single i.v. dose of atrazine at 0.26 mg/animal.

Metabolite data were presented as ppm, percent total radioactive residue (% TRR in a given sample), and % radioactivity of metabolite. Immunoassay results were expressed as atrazine mercapturate equivalents.

II. RESULTS

A. Pharmacokinetics analysis

The levels of radioactivity in plasma after dosing are presented in Table 2. Peak plasma concentrations (Cmax; 0.05, 0.31, and 2.77 µg eq/ml) occurred at 2, 8, and 24 hours (Tmax), in the low, mid, and high-dose groups, respectively. There was a linear correlation (r=1.00; p=0.003) between administered dose and Cmax. The AUC for the low-, mid-, and high-dose groups were calculated at 1.48, 12.16, and 125.8 µg eq/ml/hr; dose proportionality for absorption was linear for AUC (r=1.000; p=0.003). The oral bioavailabilities (F) were calculated to be 92, 75, and 78% of the administered dose for the low- to high-dose levels.

Table 2. Mean concentration (µg atrazine equivalents/ml ±SD) of radioactivity in plasma following a single administration to monkeys via gavage. [a]

Time (hours)	Plasma Concentration (µg atrazine eq/ml)		
	Low dose (1 mg)	Mid Dose (10 mg)	High Dose (100 mg)
2	0.05 ±0.02[b]	0.20 ±0.10	0.36 ±0.27
4	0.04 ±0.01	0.22 ±0.11	1.22 ±0.84
8	0.03 ±0.0	0.31 ±0.03[b]	1.85 ±1.05
24	0.03 ±0.01	0.28 ±0.04	2.77 ±0.75[b]
48	0.01 ±0.00	0.07 ±0.05	1.63 ±0.65
72	0.01 ±0.00	0.06 ±0.03	0.70 ±0.23

a Data were obtained from Table 1 page 27 the study report.
b Indicates time (Tmax) at maximum concentration (Cmax).

Within 24 hours of receiving a single dose of [^{14}C]atrazine at 1, 10, or 100 mg/animal, 31.0-46.13% of the administered dose was excreted via the urine (Table 3). By the end of the 7-day collection period (168 hours) urinary excretion accounted for 52.95-58.45% of the administered dose. Cumulative radioactivity (0-168 hours) from feces totaled 21.40-34.83% of the administered dose; cumulative radioactivity recovered from the cage rinses totaled 3.47-13.24% of the administered dose. The overall recovery of radioactivity in monkeys following administration of a single oral dose of [^{14}C]atrazine at 1, 10, or 100 mg/animal was 91.25-94.73%.

Table 3. Cumulative radioactivity recovered (% dose) in urine, feces, and cage rinses from four monkeys/group following administration of a single dose via gavage of [^{14}C]atrazine at 1, 10, and 100 mg/animal.

Percent Administered Dose				
Low dose (1 mg/animal)				
Interval (hours)	Urine	Feces	Cage Rinse	Total %
0-24 [a]	38.02	10.20	7.60	55.82
0-168 [b]	56.77	21.40	13.24	91.40
Mid dose (10 mg/animal)				
Interval (hours)	Urine	Feces	Cage Rinse	
0-24	46.13	12.65	2.28	61.06
0-168	58.45	24.84	11.45	94.73
High dose (100 mg/animal)				
Interval (hours)	Urine	Feces	Cage Rinse	
0-24	31.0	15.95	1.25	48.20
0-168	52.95	34.83	3.47	91.25

a Data are the mean of four animals at each sampling interval and were calculated by the reviewers from individual animal data obtained from the study report pages 45-83.
b Data extracted from study report Table 2, page 28.

B. Metabolite Characterization

Total chlorotriazine residues determined by TLC and GS/MSD in 0-48 hour urine samples from the three dose groups accounted for 16.4-34.41% of the TRR (Table 4). The major identified chlorotriazine metabolites were didealkyl atrazine, accounting for 10.7-23.60% of the TRR and deethyl atrazine, accounting for 5.2-10.12% of the TRR. Deisopropyl atrazine (0.39-1.53% TRR) was also detected in the 0-48 hour urine samples. Parent atrazine was detected at 0.3 and 14.2% TRR in the 0-12 and 24-36 hour samples, respectively, from one mid-dose monkey. However, the study authors postulated that the appearance of atrazine in these samples was due to fecal contamination.

The mercapturic acid conjugates of atrazine and its chlorotriazine metabolites detected by 2-D TLC accounted for <2% of the TRR in 0-12 and 12-24 hour urine samples. Also detected by 2-D TLC in the high-dose urine samples was an unknown metabolite that accounted for ≤6% of the TRR. Atrazine mercapturate was detected (0.13-0.36% TRR) in 0-48 hour urine samples using the EIA technique.

Table 4. Metabolite profile (mean % TRR) obtained from TLC and GC/MSD analyses of 0-48 hour urine samples collected from monkeys after a single oral dose with [^{14}C]atrazine. [a]

Compound (Code)	Mean Percent TRR					
	Low Dose (1 mg)		Mid Dose (10 mg)		High Dose (100 mg)	
	TLC	GS/MSD	TLC	GS/MSD	TLC	GS/MSD
Atrazine (G-30027)	ND [b]	0.03 [c]	0.9 [d]	0.9 [d]	ND	ND
Deethyl atrazine (G-30033)	5.2	9.96	5.6	9.43	6.9	10.12
Deisopropyl atrazine (G-28279)	0.5	1.53	0.39	1.38	0.9	0.99
Didealkyl atrazine (G-28273)	10.7	13.26	17.7	23.60	13.4	15.74
Total chlorotriazines	**16.4**	**24.75**	**23.7**	**34.41**	**21.2**	**26.85**

a TLC data obtained from Figures 4, 5, and 6 on pages 144-146 from the study report; % TRR for GC/MSD data were calculated by reviewers from data extracted from Tables 4 through 9, pages 135-141 of the study report.
b ND=Not detected.
c Detected in one 24-36 hour sample from one animal.
d Detected at 0.3 and 14.2% TRR in the 0-12 and 24-36 hour samples, respectively; Attributed to fecal contamination.

III. DISCUSSION

A. Investigator's Conclusions

Excretion of [^{14}C]atrazine and its metabolites after oral dosing of monkeys was rapid. By the end of the 7-day collection period, 56.1% of the administered dose was excreted in the urine, 27% in feces, and 9.4% in the cage rinses. EIA analysis detected atrazine mercapturate at <1% of the TRR and the mercapturic acid conjugates of atrazine and its chlorotriazine metabolites detected by 2-D TLC, accounted for <2% of the TRR. The major chlorotriazine residues detected in urine (0-48 hour samples) were deethyl atrazine and didealkyl atrazine. Total chlorotriazines, detected in the 0-24 hour urine samples of the low-, mid-, and high-dose groups, accounted for an average of 11.14% of the administered dose. Total chlorotriazine content in urine is the best biomarker for atrazine exposure.

B. Reviewer's Discussion

[^{14}C]Atrazine (97.5-98.7% a.i.) was administered to female Rhesus monkeys (4 animals/group) by gavage as a single dose at levels of 1, 10, or 100 mg. The principal route of elimination was via the urine and feces. Within 24 hours, 31.0-46.13% of the administered dose was found in the urine and within 168 hours, 52.95-58.45% was recovered from urine. Cumulative radioactivity (168 hours) recovered from feces was 21.40-34.83%. The overall recovery of radioactivity from the three dose groups, including urine, feces, and cage rinses, was 91.25-94.73%.

Total chlorotriazine residues detected by TLC and GC/MSD in 0-48 hour urine samples accounted for 16.4-34.41% of the TRR and included didealkyl atrazine (10.7-23.60% TRR), deethyl atrazine (5.2-10.12% TRR), and diisopropyl atrazine (0.39-1.53% TRR). A minor amount of parent atrazine (0.3 and 14.2% TRR) was detected in two samples (0-12 and 24-36 hour) from one mid-dose monkey, possibly due to fecal contamination of the urine samples.

The mercapturic acid conjugates of atrazine and its chlorotriazine metabolites detected by 2-D TLC in 0-24 hour urine samples accounted for <2% of the TRR. Atrazine mercapturate detected using the EIA technique accounted for only 0.13-0.36% of the TRR in 0-48 hour urine samples from the three treated groups. In the study where monkeys were treated intravenously with [^{14}C]atrazine (MRID 44152112), the four mercapturate metabolites detected in 0-4 hour urine samples accounted for 13.9-19.3% of the TRR.

This non-guideline metabolism study in the monkey is **acceptable**. This study was not meant to satisfy guideline 85-1.

IV. STUDY DEFICIENCIES

None noted.

012444

DATA EVALUATION RECORD

ATRAZINE

Study Type: 85-2; Dermal Absorption - Man

Work Assignment No. 2-62C (MRID 44152114)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by
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Date: 7/9/97

Quality Assurance
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 7/9/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

012444

EPA Reviewer: Bob Zendiazin, Ph.D. Stephen C. Dapson Jr 12/22/97
Science Analysis Branch (7509c)

EPA Secondary Reviewer: Roger Hawks, Ph.D. Roger Hawks 12/2/97
Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption-Man
OPPTS Number: 870.7485 OPP Guideline Number: §85-2

DP BARCODE: D232343 SUBMISSION CODE: S514645
P.C. CODE: 08080333 TOX. CHEM. NO.: 63

TEST MATERIAL (PURITY): Atrazine (94.3-96.3% a.i.)

SYNONYMS: 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine

CITATION: Hui, X., Wester, R., Maibach, H.I., (UCSF), Gilman, S. D., Gee, S. J., Hammock, B.D. (UC Davis), Simoneaux, B., Breckenridge, C., Kahrs, R., (Ciba) (1996). In Vivo Percutaneous Absorption of Atrazine in Man. Surge Laboratory, University of California, San Francisco, CA, Department of Entomology, University of California, Davis, CA and Ciba-Geigy Corporation, Greensboro, NC. Laboratory Study Numbers H832-11835-01, BDH-081-2, ABR 96067, 96073, August 29, 1996. MRID 44152114. Unpublished.

SPONSOR: Ciba Crop Protection, Ciba-Geigy Corporation, PO Box 18300, Greensboro, NC

EXECUTIVE SUMMARY:

In a dermal absorption study (MRID 44152114), 10 human volunteers were exposed to a single topical dose of [triazine ring-U-^{14}C]atrazine (94.3-96.3% a.i., 98.0-98.4% radiochemical purity) at 6.7 (4 volunteers) or 79 µg/cm^2 (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [^{14}C]atrazine for the low and high doses, respectively.

Overall recoveries of radioactivity from the low- and high-dose groups were 101 and 92%, respectively. The majority (91.1-95.5%) of the dose remained unabsorbed and was detected in skin wash samples taken 24 hours after dosing. After 168 hours, only 5.6% of the dose was absorbed and excreted in the urine and feces of the low-dose group and only 1.2% in the high-dose group. The renal excretion half-life was 19.6-29 hours for the low-dose group and 25.9-31 hours for the high-dose group. In both dose groups, peak urinary elimination occurred at 24-48 hours and peak fecal elimination occurred at 48-72 hours.

Total chlorotriazine residues detected by TLC in a high-dose 0-24 hour composited urine sample accounted for 9.16% of the TRR and included deethyl atrazine (3.88% TRR) and didealkyl atrazine (5.28% TRR). No atrazine was detected. GC/MSD analysis of urine samples also did not detect atrazine or its chlorotriazine metabolites.

Enzyme immunoassays indicated that levels of atrazine mercapturate, an expected glutathione pathway metabolite was near the limit of detection/quantitation for the methods. Mercapturic acid conjugates of atrazine and its chlorotriazine metabolites were not detected by LC/MS/MS in urine samples.

Some similarities in HPLC profiles of urine from i.v. dosed monkeys (MRID 44152112) and dermally treated humans were observed.

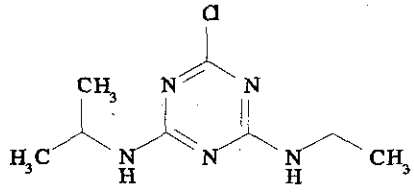
This non-guideline dermal absorption study on humans is **acceptable**. This study was not meant to satisfy the 85-2 guideline.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: [Triazine ring-U-^{14}C]Atrazine
   Chemical purity: determined by GC: 94.3% a.i. (low dose) and 96.3% a.i. (high dose)
   Radiochemical purity: Determined by TLC; 98.4% (low dose) and 98.0% (high dose)
   Specific activity: 38.7 μCi/mg (low dose) and 12.8 μCi/mg (high-dose)
   Lot/Batch: GAN-XXXV-62 (low dose) and GAN-XXXV-63 (high-dose)
   Structure:



2. Vehicle: Blank formulation diluted with deionized water

3. Test animals: Species: Human volunteers
   Age at study initiation: Males, 43 to 74 years old.
   Weight at study initiation: Not reported.

4. Preparation of dosing formulations: The two dose solutions were prepared by mixing the [^{14}C]atrazine in blank formulation (AATREX-4L®) and diluting with deionized water. Both dose solutions contained [^{14}C]atrazine and unlabeled atrazine. The dose formulations were prepared by the sponsor and their stabilities were determined before and after dosing. The formulations were kept at room temperature prior to dosing.

B. STUDY DESIGN:

The study was designed to determine [^{14}C]atrazine percutaneous absorption and metabolism over a 7 day period in humans. The volunteers, chosen from the U. of California, San Francisco and surrounding community, were free from any significant disease as determined by medical history.

1. Group Arrangements

Table 1: Dosing groups:

Test Group	Target Dose mg ($\mu g/cm^2$)	Actual Dose mg ($\mu g/cm^2$)	Number of volunteers
Low dose	0.2 (8)	0.17 (6.7)	6 [a]
High dose	2.0 (80)	1.98 (79)	6

a Two volunteers from the low dose group were dismissed on the second day of the study due to failure to properly collect the first urine sample.

2. Dosing and sample collection

A single topical application of the radioactive dose formulation was delivered to the left ventral forearm of each volunteer using a Teflon coated micro syringe. The delivered dose was quantitated by the weight difference of the dosing syringe before and after dosing. After dosing, the area was allowed to air dry and protected for 24 hours by a non-occlusive plastic cover.

Twenty-four hours after dosing, the plastic covers were removed and the dosed sites and the plastic covers were washed with soap solution (50% v/v) and distilled deionized water using gauze pads. At 168 hours, the dosed sites were tape stripped (10 times) to measure the residual in the stratum corneum. The gauze pads and the cellophane tape strips were subsequently assayed for radioactivity by liquid scintillation counting (LSC).

a. Pharmacokinetics analysis

Urine samples were collected one hour prior to dosing, and at 0-4, 4-8, 8-12, 12-24 hours post dosing and every 24 hours thereafter for up to 168 hours after dosing. Fecal samples were collected one hour prior to dosing and every 24 hours for up to 168 hours after dosing.

Duplicate samples of urine were analyzed directly for total [^{14}C] residues using LSC. The fecal samples were each homogenized and combusted in triplicate prior to LSC analysis.

b. Metabolite characterization

Frozen urine samples were shipped on dry ice tc the Ciba-Geigy Crop Protection lab in NC for further analyses. The samples were received by Ciba still frozen and were immediately stored in a freezer (-20 C). Prior to

analysis, the samples were thawed at room temperature and their pH adjusted to 7.5 by the addition of 3M NaOH. Confirmatory LSC analyses were performed in triplicate. For characterization of atrazine and its chlorotriazine metabolites, a 0-24 hour composited urine sample from a high-dose volunteer was analyzed using 1-D TLC. Prior to TLC analysis, the urine residues were fractionated into neutral components using anion exchange chromatography and partitioned against ethyl acetate; the organic fraction was then analyzed by TLC. Unlabelled analytical standards (atrazine, deethyl atrazine, diisopropyl atrazine, and didealkyl atrazine) were co-chromatographed with the samples and visualized under ultraviolet light (UV). The radioactive zones were visualized with an imaging system and radioactivity was determined by manual peak area selection.

Two enzyme linked immunosorbent assays (ELISA) and an enzyme immunoassay (EIA) for atrazine mercapturate, a glutathione pathway metabolite, were performed on the urine samples.

Residues in the high-dose composited 0-24 hour urine sample were fractionated by ion exchange chromatography prior to LC/MS/MS analysis for the mercapturic acid conjugates of atrazine and its chlorotriazine metabolites.

Urine samples with sufficient volume and total radioactive residues were analyzed using GC/MSD in the SIM mode for atrazine and its chlorotriazine metabolites. Prior to GC/MSD analysis, proteins were precipitated from the urine samples with acetonitrile, and the residues extracted from the supernatant by partitioning into ethyl acetate.

The residues in the 8-12 hour urine sample from a high-dose volunteer were partitioned into acidic ethyl acetate prior to analysis by HPLC.

A 0-4 hour urine from a high-dose human volunteer was compared to a 0-4 hour monkey urine sample after cation exchange chromatography.

5. Data Analysis

Radioactivity, in terms of concentration (µg equivalents/mL), total µg equivalents, µg equivalents/g, dpm, dpm/g, dpm/mL, and the percent of administered dose was reported for individual samples and as the mean (± S.D.).

Computer software was used to perform statistical analyses (t-test) on the data.

II. RESULTS

A. Pharmacokinetics analysis

Table 2 presents the mean percent administered dose recovered from urine, feces, skin site washings and strippings, and plastic cover washing samples from the 4 low-dose and the 6 high-dose volunteers. The total mean percent administered dose recovered from the low- and high-dose groups were 101 and 92%, respectively. The majority (95.5-91.1%) of the dose remained unabsorbed and was detected in the skin wash samples. Throughout the 168 hours, only 5.6% of the dose was absorbed and excreted in the urine and feces of the low-dose group and 1.2% of the dose in the high-dose group. The urine and fecal elimination by the high-dose group (1.2%) was significantly ($p=0.0190$ and 0.0039, respectively) lower than for the low-dose, but the calculated flux between the two groups were not significantly different.

The average renal excretion half-lives were 19.6 hours and 25.9 hours for the low- and high-dose groups, respectively. The confirmatory LSC data indicated renal excretion half-lives of 29 and 31 hours for the low- and high-dose groups, respectively. In both dose groups, peak urinary elimination occurred at 24-48 hours and peak fecal elimination occurred at 48-72 hours.

Table 2. Cumulative radioactivity recovered (mean % dose) from urine, feces, skin washes, tape strips, and plastic covers from human volunteers following administration of a single topical dose of [^{14}C]atrazine at 6.7 or 79 $\mu g/cm^2$/volunteer.[a]

Mean Percent Administered Dose					
Low dose (6.7 $\mu g/cm^2$/volunteer)					
Urine	Feces	Skin washes	Tape strips	Plastic covers	Total % Recovered
5.02±2.87	0.57±0.24	95.37±3.83	0.007±0.003	0.15±0.06	101.12
High dose (79 $\mu g/cm^2$/volunteer)					
Urine	Feces	Skin washes	Tape strips	Plastic covers	Total % Recovered
1.11±0.92	0.10±0.14	91.02±2.71	0.0011±0.0003	0.06±0.06	92.29

a Data extracted from the study report, Tables 2b, 4b, 6b, 12b, 14b, 16b, 8e, 10e, 18e, and 20 e; pages 42, 48, 54, 63, 72, 80, 88, 96, 107, and 118.

B. Metabolite Characterization

After fractionation of [^{14}C]-residues from the high-dose 0-24 hour composited urine sample on an anion exchange column, partitioning against ethyl acetate and analysis by TLC, deethyl atrazine (3.88% TRR) and didealkyl atrazine (5.28% TRR) were detected. Parent atrazine was not detected. Atrazine and its chlorotriazine metabolites were not detected by GC/MSD in selected urine samples.

Enzyme immunoassays of the urine samples indicated that levels of the immunoreactive atrazine metabolites were near the limit of detection/quantitation for the methods. The mercapturic acid conjugates of atrazine and its chlorotriazine metabolites were not detected (<1 ppb) by LC/MS/MS in the 0-24 hour composited urine sample.

HPLC analysis of a 8-12 hour high-dose urine sample detected two unknowns (1b and 2). These two unknowns found in the human urine sample eluted in a similar manner as two unknowns detected in a 24-48 hour composite monkey urine sample (MRID 44152112). These unknowns were proposed to be a labile mixture of modified chlorotriazine metabolites.

The authors stated that similarities were noted when human and monkey urine were compared after cation exchange chromatography; providing further evidence for the use of the monkey as a model for the human.

III. DISCUSSION

A. Investigator's Conclusions

Atrazine may be metabolized in a similar manner in humans and monkeys. The following were similar in both the monkey i.v. study (MRID 44152112) and this human study: (i) all of the radioactivity was eliminated via urinary and fecal excretions after 168 hours; (ii) all of the applied dose was recovered (iii) the principal route of elimination was via the urine; and (iv) the ratio of radioactivity excreted in urine:feces were similar in both species (7:1 and 9:1).

The percutaneous absorption of atrazine in humans was 1.2-5.6% of the applied dose; the absorption by the rat was 24-26% of the dose. Human skin is not considered an important reservoir of atrazine after dermal exposure and the rat is not a good model to predict atrazine absorption by humans.

B. Reviewer's Discussion

Ten human volunteers were exposed dermally to a single topical dose of [triazine ring-U-^{14}C]atrazine (98.0-98.4% a.i.) at 6.7

(4 volunteers) or 79 $\mu g/cm^2$ (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [^{14}C]atrazine for the low and high doses, respectively.

The overall recovery of radioactivity from the low- and high-dose groups was 101 and 92%, respectively. The majority (91.1-95.5%) of the dose remained unabsorbed and was detected in the skin wash samples. After 168 hours, only 5.6% of the dose was absorbed and excreted in the urine and feces of the low-dose group and 1.2% in the high-dose group. The renal excretion half-life was 19.6-29 hours for the low-dose group and 25.9-31 hours for the high-dose group. In both dose groups, peak urinary elimination occurred at 24-48 hours and peak fecal elimination occurred at 48-72 hours.

Total chlorotriazine residues detected by TLC in a high-dose 0-24 hour composited urine sample accounted for 9.16% of the TRR and included deethyl atrazine (3.88% TRR) and didealkyl atrazine (5.28% TRR). No atrazine was detected. GC/MSD analysis of urine samples also did not detect atrazine or its chlorotriazine metabolites.

Enzyme immunoassays of the urine samples indicated that levels of the immunoreactive atrazine metabolites were near the limit of detection/quantitation for the methods. The mercapturic acid conjugates of atrazine and its chlorotriazine metabolites were not detected by LC/MS/MS in urine samples.

HPLC analysis of the 8-12 hour high-dose urine sample detected two unknowns (1b and 2) that eluted in a similar manner as two unknowns detected in a 24-48 hour composite monkey urine sample from a previous study (MRID 44152112).

This non-guideline dermal absorption study in humans is **acceptable**. This study was not meant to satisfy guideline 85-2.

IV. STUDY DEFICIENCIES

None noted.

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Chemical:	**Atrazine (ANSI)**
PC Code:	**080803**
HED File Code	**13000 Tox Reviews**
Memo Date:	**01/07/1998**
File ID:	**TX012444**
Accession Number:	**412-01-0120**

HED Records Reference Center
02/09/2001