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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

012495

FEB 23 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Memorandum 2/19/98

Subject: Atrazine - Review of metabolism study (MRID 4431454-05) in the rhesus monkey using ^{14}C-atrazine. This study is an amendment to a previous monkey metabolism study - MRID 441521-13.

DP Barcode: D239250
Case: 838836
Submission: S530488
Chemical: Atrazine
Caswell No.: 063
PC No.: 080803
Registrant: Novartis Crop Protection
P.O. Box 18300
Greensboro, NC
27419-8300

From: Roger Hawks, Ph.D. Roger Hawks 2/19/98
Toxicology Branch II
Health Effects Division (7509c)

Thru: Stephen Dapson, Ph.D. Stephen C. Dapson 2/19/98
Branch Senior Scientist
Toxicology Branch II, HED (7509c)

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

Action Requested: Review MRID 443154-05, "Disposition of Atrazine in Rhesus Monkey following Oral Administration -- Amendment 1."

Response: This study has been reviewed and found to be acceptable - non-guideline. This study does not satisfy a guideline requirement and was not submitted with the intention of satisfying a guideline requirement.

Reviewers:
Dynamac: Primary Reviewer - Joan Harlin, M.S.
Secondary Reviewer - Guy Beretich, Ph.D.
EPA: Reviewer - Roger Hawks, Ph.D.
Work Assignment Manager - Sanjivani Diwan, Ph.D.

The Data Evaluation Record is attached and the executive summary follows:

EXECUTIVE SUMMARY:

The disposition of atrazine in monkeys was further investigated in an amended report (MRID 44315405) to a metabolism study (MRID 44152113). In the metabolism study, female Rhesus monkeys received a single oral dose of [triazinyl-U-14C] atrazine (≥96.8% a.i.) by gavage at 1, 10 or 100 mg/animal. For all dose groups at 168 hours post-dose, an average of 56.1% of the radioactivity was excreted in urine, 27.0% was in feces, and 9.4% was in cage washes.

Urine (0-12 hour samples) from the high-dose group contained three chlorotriazine metabolites, 6-chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine (G-30033), 6-chloro-N-ethyl-1,3,5-triazine-2,4-diamine (G-28279), and 6-chloro-1,3,5-triazine-2,4-diamine (G-28273), as confirmed by Aminex A-4 cation exchange profiles. A chlorotriazine doublet (approximately 6% TRR) consisting of 6-chloro-2-ethylamino-1,3,5-triazin-4-yl-alanine and 6-chloro-2-(methylethyl)amino-1,3,5-triazin-4-yl-glycine (75:25 ratio), as confirmed by MS and proton NMR, was also isolated. Five immunologically reactive components in high-dose urine (0-48 hour samples) analyzed by an enzyme immunoassay each had a cross-reactivity of <10% compared to atrazine mercapturate. Plasma from the high-dose group (0-72 hour samples) contained only one chlorotriazine, G-28273, based on HPLC and cation exchange chromatography. The data indicate that in orally-dosed monkeys, atrazine is completely metabolized via oxidation, dealkylation, and conjugation.

This metabolism study in monkeys is classified **acceptable (non-guideline)** as it is not a required guideline study. It is acceptable for the purposes for which it is intended.

DATA EVALUATION RECORD

012495

ATRAZINE

Study Type: (N/A) Disposition of ^{14}C-Atrazine in Rhesus Monkey Following Oral Administration

Work Assignment No. 3-39A (MRID 44315405)

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
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
Joan Harlin, M.S.

Signature: Joan L Harlin
Date: 1/30/98

Secondary Reviewer
Guy Beretich, Ph.D.

Signature: Guy Beretich
Date: 1/28/98

Program Manager
Mary Menetrez, Ph.D.

Signature: Mary L Menetrez
Date:

Quality Assurance
Reto Engler, Ph.D.

Signature:
Date:

Disclaimer

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

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

Roger Hawks 2/19/98

Work Assignment Manager: Sanjivani Diwan, Ph.D.
Toxicology Branch I (7509C)

Stephen C. Dapson Jr
2/19/98

DATA EVALUATION RECORD

012495

STUDY TYPE: Disposition of atrazine in Rhesus monkey following oral administration

OPPTS Number: N/A OPP Guideline Number: N/A

DP BARCODE: D239250 SUBMISSION CODE: S530488
P.C. CODE: 080803 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine (≥96.8% a.i.)

SYNONYMS: Atrazine; G-30027

CITATION: Simoneaux, B.J. (1996) Disposition of atrazine in Rhesus monkey following oral administration. Final Report Amendment 1 Contributing Report to MRID 44152113. Norvartis Crop Protection, Inc., Greensboro, NC. Report No. ABR-96094. Novartis Study Number 306-96. October 30, 1996. MRID 44315405. Unpublished.

SPONSOR: Norvartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419-8300.

EXECUTIVE SUMMARY:

The disposition of atrazine in monkeys was further investigated in an amended report (MRID 44315405) to a metabolism study (MRID 44152113). In the metabolism study, female Rhesus monkeys received a single oral dose of [triazinyl-U-^{14}C]atrazine (≥96.8% a.i.) by gavage at 1, 10 or 100 mg/animal. For all dose groups at 168 hours post-dose, an average of 56.1% of the radioactivity was excreted in urine, 27.0% was in feces, and 9.4% was in cage washes.

Urine (0-12 hour samples) from the high-dose group contained three chlorotriazine metabolites, 6-chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine (G-30033), 6-chloro-N-ethyl-1,3,5-triazine-2,4-diamine (G-28279), and 6-chloro-1,3,5-triazine-2,4-diamine (G-28273), as confirmed by Aminex A-4 cation exchange profiles. A chlorotriazine doublet (approximately 6% TRR) consisting of 6-chloro-2-ethylamino-1,3,5-triazin-4-yl-alanine and 6-chloro-2-(methylethyl)amino-1,3,5-triazin-4-yl-glycine

(75:25 ratio), as confirmed by MS and proton NMR, was also isolated. Five immunologically reactive components in high-dose urine (0-48 hour samples) analyzed by an enzyme immunoassay each had a cross-reactivity of <10% compared to atrazine mercapturate. Plasma from the high-dose group (0-72 hour samples) contained only one chlorotriazine, G-28273, based on HPLC and cation exchange chromatography. The data indicate that in orally-dosed monkeys, atrazine is completely metabolized via oxidation, dealkylation, and conjugation.

This metabolism study in monkeys is classified **acceptable (non-guideline)** as it is not a required guideline study. It is acceptable for the purposes for which it is intended.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

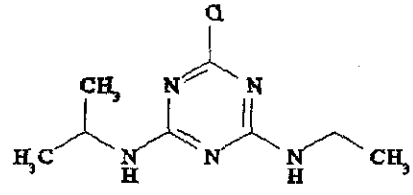
1. Test Material: [Triazinyl-U-^{14}C]atrazine

Table 1. Description of test material.

Dose Group	Radiochemical Purity[a] (%)	Chemical Purity[b] (%)	Specific Activity (μCi/mg)	Lot/Batch Number
Low	96.8	98.7	28.9	JAK-XIII-23
Mid	96.8	98.7	28.9	JAK-XIII-23
High	97.0	97.5	2.9	JAK-XIII-25

[a] Determined by GC analysis.
[b] Determined by TLC analysis.

Structure:



CAS No.: 1912-24-9

2. Vehicle: Microgranular cellulose

3. Test animals: Species: Monkey
   Strain: Rhesus
   Age at study initiation: 6-25 years old (all female)
   Weight at study initiation: 4.99-9.53 kg
   Source: University of California at 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 as an amendment to a metabolism study (MRID 44152113) which investigated the absorption, metabolism, and excretion of [^{14}C]atrazine in monkeys. In that study, female Rhesus monkeys (4/group) received a single dose of [^{14}C]atrazine by gavage at 1, 10 or 100 mg/animal; three of the low-dose monkeys also received a single dose at 100 mg/animal approximately one month later. At 168 hours post-dose, animals from all dose groups rapidly excreted [^{14}C]atrazine and its metabolites; an average of 56.1% of the dose was excreted in urine, 27.0% was in feces, and 9.4% was in the cage washes.

The present study was conducted to identify unknown chlorotriazines in urine, to characterize any unknown immunologically reactive analytes in urine, and to characterize atrazine metabolites in plasma.

Metabolic profiles of urine samples (0-12 hours) from the high-dose animals were obtained using Aminex A-4 cation exchange chromatography. To identify urine metabolites, a combined urine sample (0-48 hours) from one high-dose animal was partitioned sequentially with chloroform, ethyl acetate, and acidic ethyl acetate, and the organosoluble fractions were analyzed by 2-D TLC. [^{14}C]Compounds were identified by co-chromatography with parent and fourteen non-radioactive reference standards that were visualized under UV (254 nm) light (see Attachments 1 and 2).

To characterize unknown immunologically reactive components in urine, a combined urine sample (0-48 hours) from all high-dose animals was analyzed by enzyme immunoassay (EIA) method AG-638 specific for atrazine mercapturate. Following partitioning of a urine subsample with methylene chloride and ethyl acetate, the organic fraction was cleaned up on a diol solid-phase exchange cartridge. The diol load and eluate were each analyzed by Aminex A-4 cation exchange chromatography; the diol load was also analyzed by 2-D TLC. Five major peaks were resolved from the diol eluate and were subsequently analyzed using EIA method AG-638. The EIA diol fractions were compared using 1-D TLC to confirm the presence of the chlorotriazines, G-30033 and G-28273 and an unknown (UD) in the diol load, and a region of polar unknowns in the diol eluate.

To identify unknown chlorotriazine metabolites in urine, a combined urine sample (0-48 hour) from one high-dose animal was sequentially partitioned with chloroform, ethyl acetate, and acidic ethyl acetate. The acidic ethyl acetate fraction was dried with sodium sulfate and hexane, applied to a diol solid-phase extraction cartridge, and processed using EIA method AG-638. Radioactive Band 3 in the load fraction (which contained most of the radioactivity) was consistent with an unknown chlorotriazine doublet. To identify the doublet, Band 3 Zones 1 and 2 were prepped by 1-D TLC, HPLC, and/or AG50W-X8 cleanup prior to MS and proton NMR analyses.

To characterize atrazine metabolites in plasma, samples (0-72 hour) of plasma from the high-dose group were combined, centrifuged, and the final sample was profiled by Aminex A-4 cation exchange chromatography, HPLC, and liquid-liquid partitioning.

b. Data analysis

Statistical analyses were limited to means and standard deviations. Metabolite data were presented as percent total radioactive residue (% TRR) and percent (%) of organic fractions. Immunoassay results were expressed as atrazine mercapturate equivalents.

II. RESULTS

A. Metabolite characterization

1. Identification of chlorotriazines in urine - Urine (0-12 hour samples) from the high dose group contained three chlorotriazine metabolites, G-30033, G-28279, and G-28273, as confirmed by Aminex A-4 cation exchange profiles. An unknown chlorotriazine doublet that was isolated using

cation exchange and 2-D TLC profiles comprised ≤6% of the total [^{14}C]residues. This doublet contained the metabolites, 6-chloro-2-ethylamino-1,3,5-triazin-4-yl-alanine and 6-chloro-2-(methylethyl)amino-1,3,5-triazin-4-yl-glycine (75:25 ratio), as confirmed by MS and proton NMR.

2. Characterization of immunologically reactive analytes in urine - Immunologically reactive components in urine (0-48 hour samples) from the high dose group were detected using an EIA technique following Aminex A-4 chromatography. These multiple components were isolated in the diol column eluate, and had a cross-reactivity of <10% compared to atrazine mercapturate when assayed using an EIA technique. The diol column load contained G-30033, G-28273, and an unknown (UD) as the major components.

3. Characterization of atrazine metabolites in plasma - HPLC and cation exchange chromatograms of plasma (0-72 hour samples) from the high-dose group showed G-28273 as the only major chlorotriazine present. Low-level components resolved by HPLC were not attributed to atrazine or other chlorotriazine metabolites. Quantitative data were not provided.

Atrazine is rapidly and completely metabolized via oxidation, dealkylation, and conjugation. The registrant's proposed metabolic pathway for atrazine is presented as Attachment 3 to this DER.

III. DISCUSSION

A. Investigator's Conclusions

Atrazine and its metabolites are rapidly excreted in monkeys following a single oral dose at 1, 10 or 100 mg/animal. For all dose groups, an average of 56.1% of the radioactivity was in urine, 27.0% was in feces, and 9.4% was in cage washes. Cation exchange and 2-D TLC profiles of whole urine identified the chlorotriazines, G-30033, G-28273, and G-28279. Two chlorotriazines, 6-chloro-2-ethylamino-1,3,5-triazin-4-yl-alanine and 6-chloro-2-(methylethyl)amino-1,3,5-triazin-4-yl-glycine were identified in an unknown doublet (75:25) that comprised approximately 6% of the [^{14}C]residues in whole urine (0-24 hours) from the high-dose animals. In plasma from the high-dose group, the metabolite, G-28273, was the only major chlorotriazine identified. Total chlorotriazine residues are considered the most effective biomarkers for atrazine exposure due to their predictable proportion in urine over a wide range of internal doses. The metabolic pathway for atrazine in monkeys involves oxidation, dealkylation, and conjugation.

B. Reviewer's Discussion

[^{14}C]Atrazine was rapidly excreted in monkeys after they received a single oral dose at 1, 10 or 100 mg/animal by gavage. For all dose groups, the majority of radioactivity was excreted in urine (average 56.1%) followed by feces (27.0%), with lesser amounts in the cage washes (9.4%). In high-dose urine (0-12 hour samples), three chlorotriazine metabolites, G-30033, G-28279, and G-28273, and a chlorotriazine doublet (≤6% of TRR) consisting of 6-chloro-2-ethylamino-1,3,5-triazin-4-yl-alanine and 6-chloro-2-(methylethyl)amino-1,3,5-triazin-4-yl-glycine in an approximate 75:25 ratio, were identified. Five immunologically reactive components isolated in high-dose urine (0-48 hour samples) each had a cross-reactivity of <10% compared to atrazine mercapturate when assayed using an EIA technique, indicating that none were major compounds. In plasma (0-72 hour) from the high-dose group, G-28273 was the only chlorotriazine identified. The data indicate that in orally-dosed monkeys, atrazine is completely metabolized via oxidation, dealkylation, and conjugation.

IV. STUDY DEFICIENCIES

No scientific deficiencies were noted in this study.

ATTACHMENT 1
Not available electronically

TABLE I. REFERENCE SUBSTANCE INFORMATION FOR ATRAZINE AND ITS POSSIBLE METABOLITES

Atrazine: 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
CAS Registry #: 1912-24-9
Code: S86-0894-12
Purity: 98.7%
Source: Novartis Analytical and Product Chemistry Department, Novartis Crop Protection
Storage: Room temperature or refrigerated
Reassay Date: 2/97

G-30033: 6-chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine
CAS Registry #: 6190-65-4
Code: 92-1618 and S85-0710
Purity: 94% and 99%
Source: Novartis Analytical and Product Chemistry Department, Novartis Crop Protection
Storage: Frozen or refrigerated
Reassay Date: 12/97 and 10/96

G-28279: 6-chloro-N-ethyl-1,3,5-triazine-2,4-diamine
CAS Registry #: 1007-28-9
Code: S87-1225-1 and S87-1225-1
Purity: 96% and 98%
Source: Novartis Analytical and Product Chemistry Department, Novartis Crop Protection
Storage: Frozen or refrigerated
Reassay Date: 8/01 and 2/97

G-28273: 6-chloro-1,3,5-triazine-2,4-diamine
CAS Registry #: 3397-62-4
Code: S87-1195
Purity: 97%
Source: Novartis Analytical and Product Chemistry Department, Novartis Crop Protection
Storage: Frozen or refrigerated
Reassay Date: 11/98

CGA-359008: N-acetyl-S-[4-amino-6-(1-methylethyl)amino]-1,3,5-triazin-2-yl, L-cysteine
Lot Number: WFH-I-47
Purity: 94.7%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or frozen
Reassay Date: 12/97

TABLE I. REFERENCE SUBSTANCE INFORMATION FOR ATRAZINE AND ITS POSSIBLE METABOLITES (Continued)

CGA-246059: N-acetyl-S-[4-amino-6-(1-methylethyl)amino]-1,3,5-triazin-2-yl, L-cysteine
Lot Number: TYP-IV-31
Purity: >99.9%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or frozen
Reassay Date: 4/97

CGA-60379: N-acetyl-S-[4-amino-6-ethylamino]-1,3,5-triazin-2-yl, L-cysteine
Lot Number: TYP-IV-33
Purity: 98.5%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or frozen
Reassay Date: 4/97

CGA-10852: N-acetyl-S-(4,6-diamino-1,3,5-triazin-yl), L-cysteine
Lot Number: MCO-XI-81
Purity: >99.9%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or frozen
Reassay Date: 10/97

BPM-XV-19*
Lot Number: BPM-XV-19
Purity: Not Available
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or room temperature
Reassay Date: Not Available

CGA-98571*
Lot Number: BPM-XV-15
Purity: 98.3%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or room temperature
Reassay Date: 10/94

* = Non-GLP reference substances to be used for QUALITATIVE purposes only

TABLE I. REFERENCE SUBSTANCE INFORMATION FOR ATRAZINE AND ITS POSSIBLE METABOLITES (Continued)

CGA-74650*
Lot Number: DAH-XVI-58
Purity: 99.6%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or room temperature
Reassay Date: 10/96

BPM-IV-57*
Lot Number: BPM-IV-57
Purity: 98.8%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or room temperature
Reassay Date: Not Available

GS-12517
Lot Number: BPM-VI-31(P)
Purity: 99.3%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or room temperature
Reassay Date: 8/97

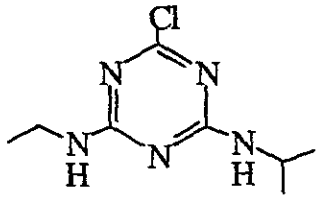
CGA-101248
Lot Number: DAH-XVI-59
Purity: 99.6%
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or room temperature
Reassay Date: 8/97

CAS-X-13*
Lot Number: CAS-X-13
Purity: Unknown
Source: Chemical Synthesis, Novartis Crop Protection
Storage: Refrigerated or room temperature
Reassay Date: Not Available

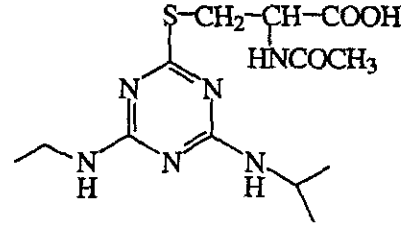
* = Non-GLP reference substances to be used for QUALITATIVE purposes only

ATTACHMENT 2
Not available electronically

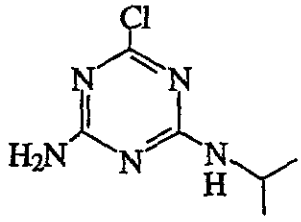
FIGURE 1. CHEMICAL NAMES AND STRUCTURES OF ATRAZINE AND ITS POSSIBLE METABOLITES



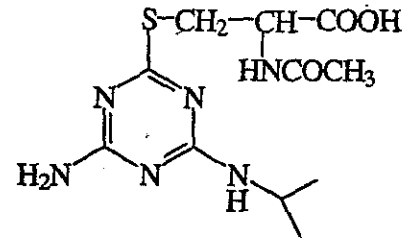
G-30027 (Atrazine)



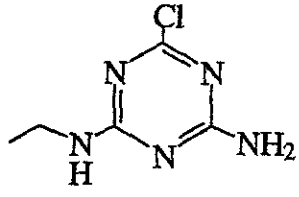
CGA-359008 (Atrazine Mercapturate)



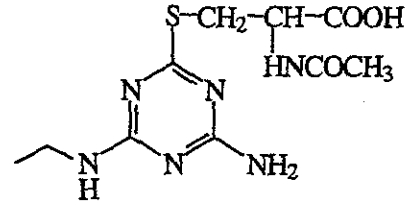
G-30033



CGA-246059 (G-30033 Mercapturate)

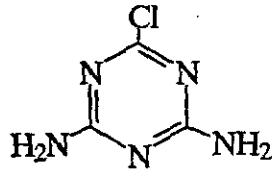


G-28279

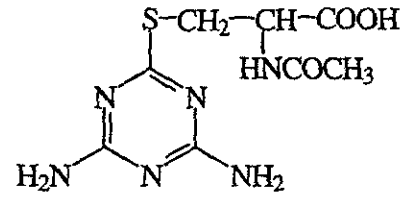


CGA-63079 (G-28279 Mercapturate)

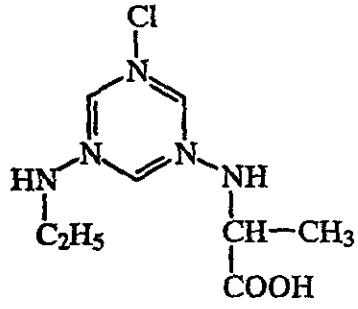
FIGURE 1. CHEMICAL NAMES AND STRUCTURES OF ATRAZINE AND ITS POSSIBLE METABOLITES (Continued)



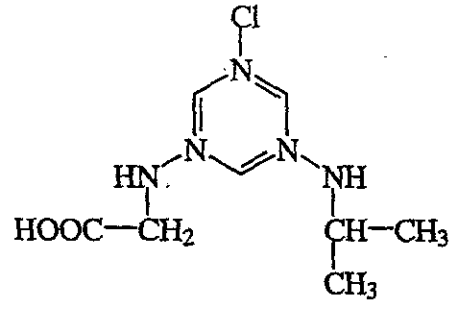
G-28273



CGA-10582 (G-28273 Mercapturate)



Side chain acid*
Major
6-chloro-2-ethylamino-1,3,5-triazin-4-yl-alanine



Side chain acid*
Minor
6-chloro-2-(methylethyl)amino-1,3,5-triazin-4-yl-glycine

* Metabolites formerly known as Unknown Doublet and identified in this report.

ATTACHMENT 3
Not available electronically

FIGURE 21. PROPOSED PATHWAY FOR ATRAZINE METABOLISM IN MONKEYS

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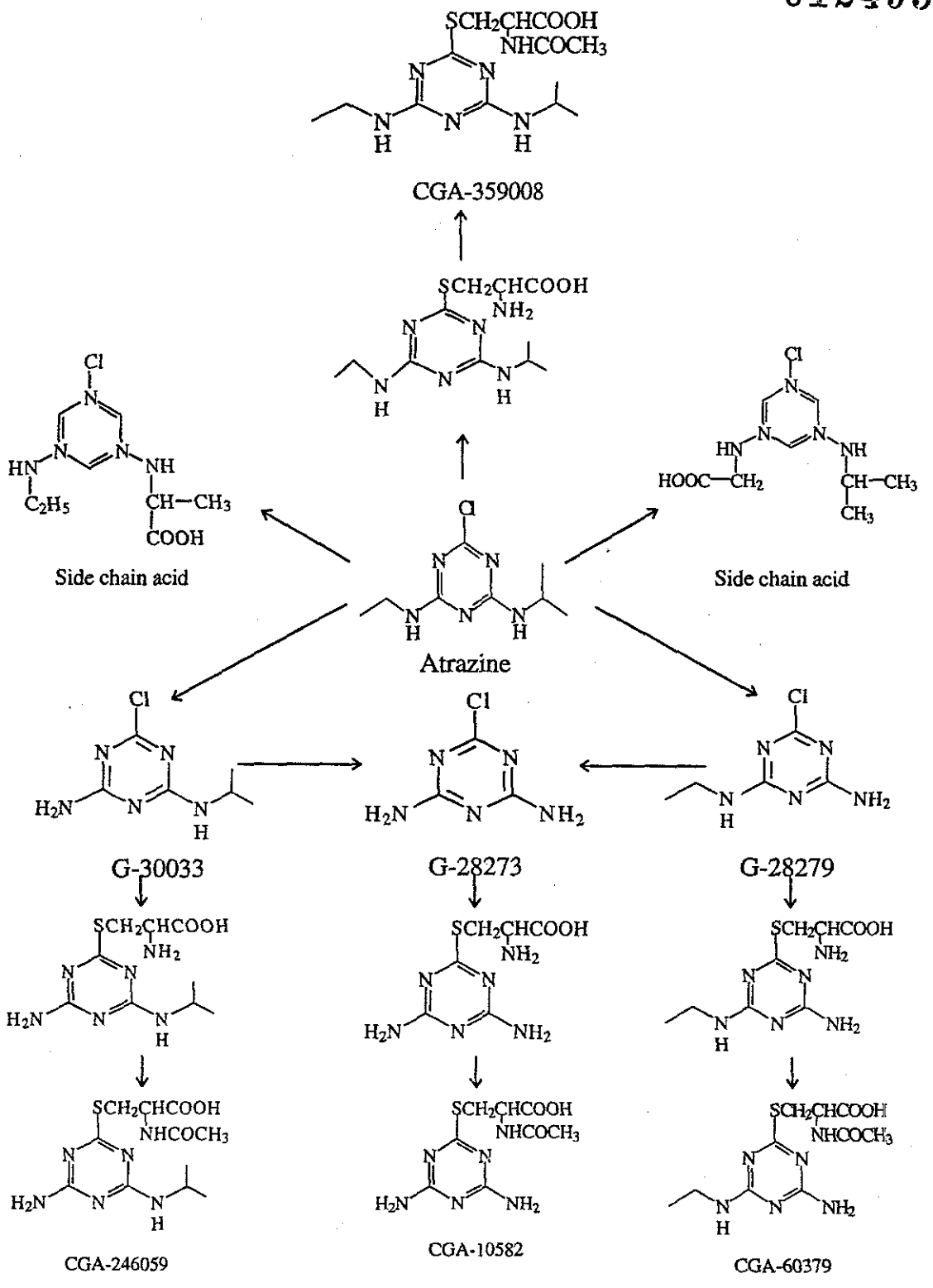

13544

002936

Chemical:	**Atrazine (ANSI)**
PC Code:	**080803**
HED File Code	**13000 Tox Reviews**
Memo Date:	**02/23/1998**
File ID:	**TX012495**
Accession Number:	***412-01-0120***

HED Records Reference Center
02/09/2001