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

JUN - 7 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBST

MEMORANDUM

Subject: Further Review of Atrazine Metabolism Reports

TO: Robert Taylor (PM 25)
Registration Division (TS-767)

THRU: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 6/3/88
Section Head, Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769C)

FROM: Sanford W. Bigelow, Ph.D.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

for WHS 6/7/88

Upon further review of the rat metabolism studies (MRID Nos. 404375-01 and 404313-06) submitted by the registrant, the urinary metabolites of atrazine in the rat have been identified as chlorinated compounds, not hydroxylated compounds as stated in the original data evaluation reports (DER). In kind, an addendum has been written to these DERs reflecting these new findings.

PC1/atrazine/atrametl.mem

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Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

6/2/88

ADDENDUM TO THE DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-06

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87115

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism).

AUTHOR: B.J. Miles

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

After further review of MRID No. 404313-06 and the data evaluation report on MRID No. 404313-06, it was found that the major urinary metabolites in the female rat are chlorinated triazines, not hydroxylated triazines as stated obstensibly in MRID No. 404313-06. The registrant states that the hydroxylated metabolites of atrazine are artifacts of the procedure used to isolate the metabolites. Therefore, the major urinary metabolites of atrazine in female rats reported in MRID No. 404313-06 are:

- 2-chloro-4-amino-6-isopropylamino-s-triazine (13),
- 2-chloro-4-ethylamino-6-amino-s-triazine (14), and
- 2-chloro-4,6-diamino-s-triazine (15).

The molecular structures of the above atrazine metabolites are shown in Figure 1 (the numbers in Figure 1 correspond to those associated with the above metabolites). Of the metabolites listed above, 2-chloro-4,6-diamino-s-triazine (15) is reported to be the major urinary metabolite. The identification of the metabolites above indicates that N-dealkylation is the major metabolic pathway for atrazine in female rats.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

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Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-05

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87115

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism).

AUTHOR: B.J. Miles

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this report.
Batch #: Not provided in this report.
Purity: Not provided in this report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 1.0 microCurie/mg. The purity of the radiolabeled test compound was reported to be ≥ 97%.

B. Test Animals:

Species: Rat (female)
Strain: Sprague-Dawley
Age: Not provided in this report.
Weight (mean): about 0.2 kg
Source: Harlan Sprague-Dawley, Indianapolis, IN

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (day)
1 High	100.0	5	1
2 Mid	16.2 - 19.6	8	1

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 24 hours. Animals were individually placed in metabolism cages for the collection of urine.

B. Dose Method: The rats were allowed a 5-day acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 1% methyl carboxymethyl cellulose and Hi-Sil-233 brand of powdered silica used to suspend the atrazine in solution. The rats were allowed free access to animal feed (Purina) and deionized water.

C. Statistics:

No statistical procedures were used in this study.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, this metabolism study was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because:

(1) "A complete set of biological phase SOPs have not been established.

(2) There was no QA inspection of the study because the QAU was not a fully functional unit at the time the study was conducted.

(3) There was no QA audit of the final report ABR-87115."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: This experiment was conducted to identify the atrazine metabolites in two groups of rats.

As shown in Table 1, one group of female rats was given a single dose of atrazine in an effort to produce sufficient levels of urinary metabolites of atrazine for identification. Five adult female Sprague-Dawley rats (about 0.2 kg) were administered 100 mg/kg ^{14}C-atrazine. Samples of urine and feces were obtained at 24, 48, and 72 hours. After taking samples for 72 hours, the rats were sacrificed and 5 ml of blood and the liver were obtained.

In another group of animals, 8 rats were given a single oral exposure of 16.18 - 19.64 mg/kg ^{14}C-atrazine. Urinary metabolites were collected over a 24-hour period following treatment. The metabolites of atrazine were isolated and identified by the following series of analytical chemistry steps:

(1) charcoal cleanup,

(2) C_{18} Bond-Elut separation,

(3) Aminex A-4 cation exchange column chromatography,

(4) Aminex A-25 anion exchange column chromatography or PRP-1 (reverse-phase) HPLC, and finally

(5) confirmation by comparing to the infrared spectra and mass spectra of authentic synthesized standards.

V. RESULTS:

A. The In Vivo Metabolism of Atrazine.

To examine the metabolism of atrazine in rats, 100 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-labeled metabolites were isolated and identified. A recovery of 103.78% of the total radioactivity was achieved. The urinary route accounted for 47.4% of the elimination whereas 49.3% of the ^{14}C-label was eliminated via the fecal route. The tissues contained 5.75% of the ^{14}C-label while the blood contained the remaining 1.4% of the ^{14}C-label. The amount of ^{14}C-label eliminated via exhalation was not reported.

The molecular structures of the urinary metabolites obtained from the first group rats were unattainable, so a second group of 8 rats were given 16.18-19.64 mg/kg ^{14}C-atrazine. The metabolites were collected within the 0 to 24 hour time period after exposure. The urine was freeze dried. Metabolites were then dissolved in a small amount of water that was acidified with HCl to pH 3.0 and separated with an amino acid analyzer (to detect the amino acid residues of glutathione) coupled with a cation exchange column.

A total of 19 radioactive peaks were detected, three of which were identified as metabolites by comparison of the infrared and mass spectra. The identity of two other metabolites was postulated based on additional mass spectral information. The molecular structures of some of the atrazine metabolites are shown in Figure 1 and the numbers in this figure correspond to the metabolites discussed in the text. Only four of these metabolites were identified and were reported, they were:

- o 2-hydroxy-atrazine (7),
- o 2-hydroxy-4-amino-6-isopropylamino-s-triazine (8),
- o 2-hydroxy-4-ethylamino-6-amino-s-triazine (14), and
- o 2-hydroxy-4,6-diamino-s-triazine (3).

The identification of the four metabolites above indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Because several other minor metabolites that possess omega-carboxyl moieties were identified (5, 10, 11, 12),

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oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor and secondary metabolic route.

B. The In Vitro Metabolism of Atrazine.

The author of this study offers the results of a published study on atrazine metabolism performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219) in an effort to account for the covalent binding of atrazine in RBCs.

The method published by Dauterman and Muecke is reported as the following steps. Radiolabeled atrazine was incubated with rat liver microsomes with or without the addition of the metabolic cofactors, glutathione and NADPH. Six metabolites were identified by chromatography against synthetic standards. The results corroborate the findings in the in vivo experiment that N-dealkylation is the major metabolic pathway. Also, the isopropyl moiety is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites previously discussed when cytosolic cell fractions were included in the in vitro reactions.

Covalent binding in RBCs. The author argues that the glutathione-containing metabolites of atrazine may be catalyzed by a "carbon-sulfur lyase," an enzyme that cleaves the glutathione residue and leaves a thiol group on the atrazine metabolite. However, the author has not presented evidence whether lyase is present in red blood cells.

V. DISCUSSION:

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivisior F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

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6/2/88

ADDENDUM TO THE DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404375-01

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87116

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

After further review of MRID No. 404375-01 and the data evaluation report on MRID No. 404375-01, it was found that the major urinary metabolites in the female rat are chlorinated triazines, not hydroxylated triazines as stated superficially in MRID No. 404375-01. The registrant states that the hydroxylated metabolites of atrazine are artifacts of the procedure used to isolate the metabolites. The major urinary metabolite of atrazine in female rats reported in MRID No. 404375-01 is 2-chloro-4,6-diamino-s-triazine (15). The molecular structure of this atrazine metabolite is shown in Figure 1 (the number in Figure 1 correspond to number '15' with the above metabolites). The identification of the metabolites above indicates that N-dealkylation is the major metabolic pathway for atrazine in female rats.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting (1) the identity of urinary metabolites of atrazine in female rats as well as (2) the distribution and excretion of atrazine in male and female rats. However, all of the data requirements for metabolism studies set forth in Subdivision F §85-1 have not been reported, i.e., (a) the urinary and fecal metabolites of atrazine in male rats and (b) the fecal metabolites of atrazine in females must be identified to satisfy completely the §85-1 data reporting requirements for the metabolism of atrazine in the rat.

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