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
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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.
ATRAZINE

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DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption-Man
OPPTS Number: 870.7485

OPP Guideline Number: §85-2

DP BARCODE: D232343
P.C. CODE: 08080333

SUBMISSION CODE: S514645
TOX. CHEM. NO.: 63

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

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


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

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 composit ed 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 \(\mu\)Ci/mg (low dose) and 12.8 \(\mu\)Ci/mg (high-dose)
   Lot/Batch: GAN-XXXV-62 (low dose) and GAN-XXXV-63 (high-dose)

   \[
   \begin{array}{c}
   \text{Cl} \\
   \text{CH}_3 \\
   \text{N} \\
   \text{N} \\
   \text{N} \\
   \text{N} \\
   \text{N} \\
   \text{N} \\
   \text{CH}_3 \\
   \end{array}
   \]

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 \([^{14}\text{C}]\)atrazine in blank formulation (AATREX-4L\(^\circledR\)) and diluting with deionized water. Both dose solutions contained \([^{14}\text{C}]\)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 \([^{14}\text{C}]\)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:

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Target Dose (µg/cm²)</th>
<th>Actual Dose (µg/cm²)</th>
<th>Number of volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose</td>
<td>0.2 (8)</td>
<td>0.17 (6.7)</td>
<td>6 (^a)</td>
</tr>
<tr>
<td>High dose</td>
<td>2.0 (80)</td>
<td>1.98 (79)</td>
<td>6</td>
</tr>
</tbody>
</table>

\(a\) 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 to 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 stripplings, 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 [\(^{13}\)C]atrazine at 6.7 or 79 μg/cm\(^2\)/volunteer.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Low dose (6.7 μg/cm(^2)/volunteer)</th>
<th></th>
<th></th>
<th></th>
<th>Total % Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>Skin washes</td>
<td>Tape strips</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.02±2.87</td>
<td>0.57±0.24</td>
<td>95.37±3.83</td>
<td>0.007±0.003</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>Skin washes</td>
<td>Tape strips</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.11±0.92</td>
<td>0.10±0.14</td>
<td>91.02±2.71</td>
<td>0.0011±0.0003</td>
</tr>
</tbody>
</table>

\(^a\) 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 immunoactive 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-¹⁴C]atrazine (98.0-98.4% a.i.) at 6.7 (4 volunteers) or 79 μg/cm² (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [¹⁴C]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.
ATRAZINE

SignOff Date: 1/7/1998
DP Barcode: D240618
HED DOC Number: 012444
Toxicology Branch: TOX2

Dermal Absorption §85-2