HPLC–Accelerator MS Measurement of Atrazine Metabolites in Human Urine after Dermal Exposure

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Metabolites of atrazine were measured in human urine after dermal exposure using HPLC to separate and identify metabolites and accelerator mass spectrometry (AMS) to quantify them. Ring-labeled [14C]atrazine was applied for 24 h with a dermal patch to human volunteers at low (0.167 mg, 6.45 μCi) and high (1.98 mg, 24.7 μCi) doses. Urine was collected for 7 days. The urine was centrifuged to remove solids, and the supernatant was measured by liquid scintillation counting prior to injection on the HPLC to ensure that <0.17 Bq (4.5 pCi) was injected on the column. A reversed-phase gradient of 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile became less polar with increasing time and separated the parent compound and major atrazine metabolites over 31 min on an octadecylsilane column. Peaks were identified by coelution with known standards. Elution fractions were collected in 1-min increments; half of each fraction was analyzed by AMS to obtain limits of quantitation of 14 amol. Mercapturate metabolites of atrazine and dealkylated atrazine dominated the early metabolic time points, accounting for ~90% of the 14C in the urine. No parent compound was detected. The excreted atrazine metabolites became more polar with increasing time, and an unidentified polar metabolite that was present in all samples became as prevalent as any of the known ring metabolites several days after the dose was delivered.

Knowledge of metabolite dynamics is crucial to developing useful assays for monitoring atrazine exposure in agricultural workers.

Atrazine is among the most prevalently used herbicides worldwide. It is effective at controlling broadleaf grasses and ~70 million pounds is applied annually in the United States.¹ An objective analysis of the net economic benefit of atrazine use is difficult to assess because other herbicides are available, but partial or total bans on its use are projected to cost producers and consumers $224–665 million annually.² The transport of atrazine and its byproducts in soil and in groundwater and surface water has been studied in detail during four decades of commercial use.³,⁴ Often groundwater and surface water moving through agricultural areas where atrazine is used contains atrazine or its byproducts,⁵–⁷ and drinking water standards have been established by state and federal agencies.⁸ Although commonly found, atrazine is generally regarded as effective and safe to the general population when used as directed. As with all pesticides, occupational exposures to agricultural and chemical production workers can be considerably higher than exposures to the general population. A survey of farmers found that pesticide exposures occur commonly by dermal absorption and inhalation but use of personal protective equipment was not routine.⁵ Dermal absorption studies with rats¹⁰ and in vitro¹¹,¹² showed that atrazine is absorbed and metabolized by this exposure route, but little if any parent compound was found in rat urine¹⁰ or in human urine in a limited number of exposure studies.¹³–¹⁶ Development of an assay to assess occupational exposure to atrazine thus requires knowledge

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of the pharmacokinetics and metabolic profile of atrazine in humans.

The ideal exposure assessment method should rely on samples obtained noninvasively (e.g., urine or saliva) and be sensitive to environmental doses. It should identify a specific metabolite or class of metabolites or catabolites most dominant within the time frame of the target exposure and sampling window. An assay that identifies a class of compounds is often more desirable than one that identifies a specific one. Variations in metabolism among individuals is less problematic when an analyst can detect a class of compounds. A wider range of conditions (dose, time after dose) can generally be addressed.

An in vivo human clinical study using 14C-labeled atrazine was designed to determine the metabolites excreted in urine following a dermal exposure to atrazine. Doses were selected based on data from in vitro and rodent dermal absorption experiments.10–12,14

The doses and specific activities were designed for detection by liquid scintillation counting (LSC). The human subjects absorbed the labeled atrazine less than anticipated, so the radioactivity of the collected urine was lower than expected. Although the neat urine could usually be measured by LSC, the activity was insufficient in all but one subject to measure metabolites after HPLC separation.17

We used these archived urine samples in a demonstration of HPLC–AMS: metabolite separation by HPLC followed by 14C quantitation with accelerator mass spectrometry (AMS). AMS can accurately measure <106 atoms (1 amol) of 14C in a sample containing ~1 mg of total carbon and analyze hundreds of samples per day.15,16 Petroleum-derived carbon carrier with a low 14C level can be added to samples with small amounts of carbon17 such as HPLC fractions to bring them to the preferred sample size. Measurements with carrier carbon containing 10% the contemporary 14C level routinely achieve limits of quantitation near 10 amol, sufficient to quantify the 14C content of HPLC eluents. Urine collected at three postexposure time points and a predose control were analyzed from 3 of the 10 subjects who completed the clinical study. The time points were selected to include urine samples whose 14C concentration was >50% of the peak value during application of the dose, the peak value, and <50% of the peak value several days after the removal of the dermal dose. The archived urine samples were selected from subjects receiving low and high doses or those exhibiting different excretion rates.

**EXPERIMENTAL SECTION**

**Chemicals.** All chemicals were checked for 14C content by AMS prior to use to ensure no inadvertently labeled or contaminated solvents were used. The solvents for the HPLC mobile phases were glacial acetic acid purchased from EM Science (Gibbstown, NJ) and acetonitrile purchased from J. T. Baker (Phillipsburg, NJ). A Barnstead low-pressure reverse osmosis system produced 18.2 MΩ deionized water for the aqueous phase. Tributyrin (glycerol tributyrate) purchased from ICN Pharma-

![Figure 1. Ring-labeled atrazine.](image)

centicals, Inc. (Costa Mesa, CA) was dissolved in methanol purchased from Sigma-Aldrich (St. Louis, MO) for use as the carbon carrier for AMS sample combustion and graphitization. The metal powders used in combustion and graphitization were purchased from Alfa Aesar (Ward Hill, MA).

The atrazine was an end use formulation (AATREX-4L) prepared by Novartis Crop Protection (Greensboro, NC). The following HPLC standards were prepared and provided by Novartis: 2-chloro-4-(ethylamino)-6-[(1-methyl-ethyl)amino]-1,3,5-triazine (atrazine, N-acetyl-S-{4-(ethylamino)-6-[(1-methyl-ethyl)-amino]-1,3,5-triazin-2-yl}-L-cysteine (atrazine mercapturate), 2-amino-4-chloro-6-[(1-methyl-ethyl)amino]-1,3,5-triazine (deethylyatrazine), N-acetyl-S-{4-amino-6-[(1-methyl-ethyl)amino]-1,3,5-triazin-2-yl}-L-cysteine (deethylatrazine mercapturate), 2-amino-4-chloro-6-ethylamino]-1,3,5-triazine (deisopropylatrazine), N-acetyl-S-{4-(ethylamino)-6-amino]-1,3,5-triazin-2-yl}-L-cysteine (deisopropylatrazine mercapturate), 2-chloro-4,6-diamino-1,3,5-triazine (2-chloro-4,6-diamoatrazine or didealkylatrazine), N-acetyl-S-{4,6-diamino-1,3,5-triazin-2-yl}-L-cysteine (2-chloro-4,6-diamoatrazine mercapturate or didealkylatrazine mercapturate), 2-(ethylamino)-4-hydroxy-6-[(1-methyl-ethyl)amino]-1,3,5-triazine (hydroxyatrazine), 2,4,6-triamino-1,3,5-triazine (melamine), 2,4-diamino-6-hydroxy-1,3,5-triazine (ammelide), and 2-amino-4,6-dihydroxy-1,3,5-triazine (ammelide).

The atrazine tracer was uniformly labeled with 14C on the ring structure to retain the tag during metabolism (Figure 1). The labeled atrazine was mixed with the end use formulation to make dosing solutions with specific activities of 38.7 and 12.8 µCi mg−1.

**Clinical Procedure.** All clinical work was performed at the University of California, San Francisco after approval by the UCSF Committee on Human Research. Twelve normal, healthy males ages 43–74 served as volunteers with 10 completing the trial. The atrazine was applied for 24 h to a 25 cm2 area of skin on the left ventral forearm of each volunteer at low (subjects 1–4, 0.167 mg, 6.45 µCi) and high (subjects 5–10, 1.98 mg, 24.7 µCi) doses. The protective nonocclusive cover was removed at 24 h, and excess atrazine was washed from the skin with soap and water. A series of 10 cellophane tape stripplings was conducted on the skin at the site of application on day 7 following the dosing to remove any residual atrazine remaining in the epidermis. These tapes and the nonocclusive cover were also measured for 14C by LSC. Urine samples were collected 1 day before the dose and 7 days following it (24 h predose, 0–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 h). Total urine volume for each sample was measured before separating into aliquots and storing at ~20 °C. Samples were thawed at room temperature before analysis and immediately returned to ~20 °C after use. Fecal samples were collected throughout the trial, homogenized, and measured for 14C by LSC.

**HPLC Sample Preparation.** Predose and three postdose samples from three subjects (2, 5, and 6) were selected for
complete HPLC separation and AMS quantitation. For each time point, ~3 mL of urine was centrifuged at 10000g to remove solids and a 500-μL aliquot of the supernatant was measured with a Wallac 1410 liquid scintillation counter (Wallac Oy, Finland) to determine the specific activity. Supernatant was stored in new 1.5-mL Eppendorf tubes at −35 °C and thawed as needed.

We reserve an HPLC system solely for separations prior to AMS quantitation. The HPLC system includes Rainin HPLX pumps, a 250 × 4.6 mm YMC 303 S-5 120A ODS-AQ chromatography column and guard column (YMC, Inc., Wilmington, NC), and a Hewlett-Packard Series 1050 UV detector with a dedicated PC for spectra collection and analysis. The mobile phase used a reversed-phase gradient of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in acetonitrile (solvent B) with a 1 mL/min flow rate. The mobile phase varied as follows: 0 min, 100%A; 0–10 min, 0–5%B; 10–15 min, 5–15%B; 15–30 min, 15–20%B; 30–40 min, 20–100%B; 40–45 min, 100%B, 45–55 min, 0–100%A. The amount of 14C was limited to <0.17 Bq (4.5 pCi) for each injection, restricting injected volumes of supernatant to 25–100 μL. Fractions were collected each minute from 4 to 42 min and were labeled with the end of the collection period (fraction 8 contained eluent from 7 to 8 min). Peaks were tentatively identified based on cochromatography. In this case, radioactive urine samples were mixed with authentic standards and the UV profile of HPLC elution was compared with the histogram of 14C measured by AMS.

**AMS Sample Preparation and Measurement.** Half of each fraction collected was analyzed for 14C content using AMS. Each sample was placed in a quartz tube (6 × 30 mm, 4 mm i.d.) nested inside two borosilicate glass culture tubes (10 × 75 mm in 12 × 100 mm). The insides of the borosilicate tubes were never touched by the researcher. The eluents were dried in two steps. Initially, 300 μL was placed in the small inner quartz vial and the liquid was removed in a vacuum centrifuge. A small glass fiber filter (e.g., Whatman GF/A 21 mm) was stuffed into the top of each set of nested tubes during centrifugation to minimize intersample contamination by aerosols. After 2.5 h, the remaining 200 μL of sample and 50 μL of carrier solution (40.0 mg mL⁻¹ tributyrin in methanol to yield 1.19 mg of carrier C) was added. The remaining water and the added methanol were then removed by further evaporation in the centrifuge, leaving the eluted metabolite dispersed in tributyrin. Tributyrin is used for the carbon carrier because it has a relatively low 14C content, high carbon content (60% by mass), low nitrogen content, low vapor pressure, and low solubility in alcohol and is nonhazardous. Total drying time was ~5 h. Three tributyrin carrier blanks were prepared with each set of samples. AMS measures an isotope ratio, and this addition of a carrier amounts to an isotope dilution. The precision of the 14C determination thus depends on the precision of the added carbon mass.

The inner quartz vials were transferred to quartz combustion tubes which were evacuated and sealed. The samples were combusted to CO₂ and reduced to carbon using Vogel’s method. Graphite samples were packed into aluminum sample holders, and carbon isotope ratios were measured on the LLNL spectrometer. Typical AMS measurement times were 3 min/sample, with a counting precision of 1.4–2.0% and a standard deviation among

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**Table 1. Fate of Dose in Test Subjects**

<table>
<thead>
<tr>
<th>Test subject</th>
<th>dose unabsorbed, recovered after 24 h (%)</th>
<th>dose excreted in urine (%)</th>
<th>dose excreted in feces (%)</th>
<th>dose unaccounted after 7 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₂</td>
<td>93.9</td>
<td>4.0</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>S₅</td>
<td>90.4</td>
<td>2.9</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>S₆</td>
<td>91.5</td>
<td>0.3</td>
<td>0.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Excreted doses are sums of the 7-day trial. Uncertainties depended on counting statistics and were insignificant compared to precision reported. Low dose, 0.167 mg, 6.45 μCi. High dose, 1.98 mg, 24.7 μCi. Less than 0.05%*

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**RESULTS AND DISCUSSION**

All 10 subjects absorbed atrazine similarly, on the basis of the 14C recovered from the skin and nonocclusive cover after 24 h, but the excretion varied considerably. Table 1 lists the fractions of the dose not absorbed and recovered after 24 h, excreted over the 7-day trial, and unaccounted after the 7-day trial for the three subjects selected. The unaccounted dose after the trial may have been retained for more than the 7-day trial, may have been excreted in an insoluble form that could not be measured by LSC, or may have adhered to container surfaces. Urine was the dominant excretion route for dermally absorbed atrazine. Subject 6 excreted ~1/10 the atrazine of subject 5 despite absorbing a similar dose. No blood samples were drawn so it is unclear if the excess [14C]atrazine was residing in the lipids below the skin, circulating in the blood, or deposited in another tissue after absorption in the blood.

The HPLC standards eluted from the column between 3.8 and 45 min (Table 2 and Figure 2). The peak heights of the standards, obtained in different injections, are not scaled in Figure 2. The absorbance at 254 nm and solubility of the standards varied greatly, but elution time could easily be determined. The dealkylated atrazine metabolites and their mercapturates eluted between 17 and 29 min. Although hydroxyatrazine also elutes in this interval, it is produced by hydrolysis with mineral acids and is not expected in urine after dermal exposure. The most highly substituted compounds, melamine, ammeline, and ammelide, were poorly soluble in aqueous and alcohol solutions and unlikely to appear in urine at high concentrations. Metabolites were tentatively identified on the basis of cochromatography of the radioactive fraction from urine with an authentic synthetic standard. Cochromatography involved co-injection of the synthetic standard and urine with monitoring by both AMS and UV absorbance. The best separated standard peaks of likely major metabolites were deisopropylatrazine mercapturate (23.5 min) and atrazine mercapturate (39.2 min). The congestion of metabolite peaks in the central range and the coarse fraction sampling for AMS present difficulties in deconvolution of individual compounds.

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The predose samples of each of the three subjects were analyzed by AMS for 14C content neat and after separation by HPLC. Each urine supernatant contained slightly higher 14C concentrations (118 ± 5, 124 ± 6, and 117 ± 2 amol of 14C/g of C) than the contemporary level at the time of the clinical exposure (112 amol of 14C/g of C). This elevation may be caused by the subjects’ diets, the rate of carbon turnover in their bodies, or slight contamination at the clinic, but it did not affect the HPLC traces. A 14C-labeled polar molecule eluted at 4–5 min and produced a small peak in all HPLC tracings, including blank injections. The primary atrazine metabolites elute later and were not affected.

Tributyrin carrier provides the majority of the carbon contained in each HPLC–AMS sample (1.19 mg of C with 11.7 amol of 14C). The 14C background, and hence the mean detection limit for the fractions, depended on the mass consistency and the reproducible measurement of the tributyrin carrier. The contribution of carrier 14C was subtracted from predose samples and three subjects are pooled to produce a probability plot of undosed urine fractions (Figure 3). Individual samples are more likely to be contaminated by elevated 14C rather than by 14C-depleted carbon, skewing the distribution. The average predose fraction contained 4.2 ± 5.0 amol of 14C above the known carrier. A reliable limit of quantitation 2 standard deviations above the blank is 14 amol of 14C per fraction above carrier.

The measured ratio of 14C to total C for each sample (Rsample) is described in eq 1. The concentration of 14C labeled atrazine,

\[
R_{\text{sample}} = \frac{\text{Amol of } ^{14}\text{C}}{\text{Amol of total C}}
\]

Table 2. HPLC Elution Times for Atrazine and Metabolites

<table>
<thead>
<tr>
<th>elution time (min)</th>
<th>compound (CAS registry number)</th>
<th>R2</th>
<th>R4</th>
<th>R6</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8</td>
<td>melamine (108-78-1)</td>
<td>NH2</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>17.4</td>
<td>deethylatrazine (6190-65-4)</td>
<td>Cl</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>18.7</td>
<td>diethylatrazine (3397-62-4)</td>
<td>Cl</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>19.3</td>
<td>diethylatrazine mercapturate</td>
<td>SCH2CH(NHAc)COOH</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>22.6</td>
<td>hydroxy atrazine (2163-68-0)</td>
<td>OH</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>23.5</td>
<td>desisopropylatrazine mercapturate</td>
<td>SCH2CH(NHAc)COOH</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>27.3</td>
<td>deethylatrazine mercapturate</td>
<td>SCH2CH(NHAc)COOH</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>28.8</td>
<td>desisopropylatrazine (1007-28-9)</td>
<td>Cl</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>39.2</td>
<td>atrazine mercapturate (138722-96-0)</td>
<td>SCH2CH(NHAc)COOH</td>
<td>NHCH2CH3</td>
<td>NH2</td>
</tr>
<tr>
<td>42.1</td>
<td>atrazine (1912-24-9)</td>
<td>OH</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>45.0</td>
<td>ammelide (645-93-2)</td>
<td>OH</td>
<td>NH2</td>
<td>NH2</td>
</tr>
<tr>
<td>not soluble</td>
<td>ammeline (645-92-1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The UV absorbance peaks of the standards were narrow (see Figure 2) and stable. Elutions times were within 0.1–0.2 min and the entire spectrum shifted.

Figure 2. Elution times of HPLC standards detected with UV absorbance (254 nm). Peak heights are not normalized. The UV absorbance spectra were used to identify peaks but not quantify levels. In order of retention time shown on peaks, the standards were melamine (3.8), deethylatrazine (17.4), 2-chloro-4,6-diaminoatrazine (18.7), 4,6-diaminoatrazine mercapturate (19.3), hydroxyatrazine (22.6), desisopropylatrazine mercapturate (23.5), deethylatrazine mercapturate (27.3), desisopropylatrazine mercapturate (28.8), atrazine mercapturate (39.1), atrazine (42.1), and ammelide (45.0).

Figure 3. Predose HPLC fractions of three subjects pooled (n = 111) contained on average 4.2 ± 5.0 amol of 14C, with a median of 2.5 amol of 14C, excluding the 4–5-min peak. A Gaussian distribution would yield a straight line with the slope corresponding to the standard deviation. This distribution is slightly skewed to higher 14C concentrations.
tissue and greater than \( ^{14}C \) carrier for samples with a signal. If \( ^{14}C \) carrier/\( ^{14}C \) carrier, respectively. The possibility of contamination is represented by \( ^{14}C \) tracer/\( ^{14}C \) tracer. The contributions from the urine metabolites, and atrazine catabolites in the urine is indicated as \( ^{14}C \) unknown/\( ^{14}C \) unknown.

Some components of eq 1 can be minimized by experimental design. In the case of HPLC eluent samples, \( C_{\text{carrier}} \) is much larger than \( C_{\text{tissue}} \) and \( C_{\text{tissue}} \). Furthermore, \( ^{14}C_{\text{tracer}} \) is much greater than \( ^{14}C_{\text{tissue}} \) and greater than \( ^{14}C_{\text{carrier}} \) for samples with a signal. If contamination is assumed negligible, eq 1 then reduces to eq 2.

\[
R_{\text{sample}} = \frac{^{14}C_{\text{tracer}} + ^{14}C_{\text{tissue}} + ^{14}C_{\text{carrier}} + ^{14}C_{\text{unknown}}}{C_{\text{tracer}} + C_{\text{tissue}} + C_{\text{carrier}} + C_{\text{unknown}}} \quad (1)
\]

\[
R_{\text{sample}} = \frac{^{14}C_{\text{tracer}} + ^{14}C_{\text{carrier}}}{C_{\text{carrier}}} \quad (2)
\]

Carbon analysis of individual urine samples was not necessary since most of the carbon in the sample was from the tributyrin carrier. Urine typically contains 1% carbon by mass and has a density near 1 g mL\(^{-1}\). The 25, 50, or 100 \( \mu \)L of urine injected on the HPLC column contained 0.25–1.0 mg of carbon spread over 40–50 fractions. The distribution of carbon among the fractions is not known, but urea or inorganic carbon should reside as polar salts and pass through the column quickly. The carbon contributed by the sample \( C_{\text{tissue}} \) in any fraction is estimated to be less than 0.05 mg. This corresponds to only 4% of the total carbon after the addition of 1.19 mg of carrier.

The HPLC–AMS histograms of \( ^{14}C \) content vs time (Figure 4) show the shift in atrazine metabolites from nonpolar to polar over the 7-day sampling period. Metabolite shifts were observed in all subjects as the alkyl groups were cleaved to produce amine groups. The histogram peaks are much wider than the corresponding UV absorbance peaks. Coarse fraction sampling widens peaks that fall on boundaries between fractions. Every \( ^{14}C \) peak was wider than our 1-min collection resolution. AMS has linear response over 4 orders of magnitude and measures the real width of the \( ^{14}C \) peaks, giving a true picture of the elution wave front moving through the column. Conventional UV absorbance is less sensitive than AMS, burying tails of the elution peaks in the background and making the peaks appear thinner. Peak broadening destroys resolution in the 20–30-min elution range in our case, but it does not necessarily cause problems with less complicated mixtures of compounds. In addition, the \( ^{14}C \) elution spectrum could contain metabolites for which we did not have HPLC standards.

Table 3 reports the quantity of atrazine equivalents per milliliter of urine in identifiable peaks. Uncertainties are expressed as the square root of the sum of the squares of uncertainties of individual fractions. Atrazine metabolites and catabolites are quantified in molar atrazine equivalents (AtrEq) in Table 3 because changes in side chains have no effect on the ring-sited \( ^{14}C \) label. The two best separated metabolite peaks were an unknown at 10–13 min and atrazine mercapturate at 39–41 min. Deethylatrazine (17–18 min) can be identified on most plots, and a likely mixture of deisopropylatrazine and didealkylatrazine mercapturate at 19–21 min also appears to be usable as markers of exposure. Other peaks seen in some histogram spectra were 23–24 (deisopropylatrazine mercapturate), 26–28 (deethylatrazine mercapturate and possibly some deisopropylatrazine), and 30–31 min (possibly deisopropylatrazine or an unknown). Parent compound was not observed in any sample, as expected.\(^{14}\)

The AtrEq/ (mL of urine) listed in Table 3 were calculated with eq 3, where the isotope ratios \( R_{\text{sample}}, R_{\text{carrier}}, \) and \( R_{\text{urine}} \) were

\[ \frac{\text{AtrEq}}{\text{mL of urine}} = \frac{R_{\text{sample}} (m_{\text{carrier}} + m_{\text{urine}}) - R_{\text{carrier}} m_{\text{carrier}}}{R_{\text{urine}} m_{\text{urine}} / V_{\text{urine}} F_{\text{label}}} \quad (3) \]

expressed in mol of \( ^{14}C \) / mg of C, the masses \( m_{\text{carrier}} \) and \( m_{\text{urine}} \) were expressed in mg of C, \( V_{\text{urine}} \) is the volume of urine injected on the HPLC column (in mL), and \( F_{\text{label}} \) is the fraction of atrazine labeled in the dosing solution. The mass of urine was assumed...
to be distributed evenly over the 40 HPLC fractions. Although not strictly correct, this assumption has little effect due to m_{carrier} overwhelming the carbon inventory.

The first (10–13 min) and last (39–41 min) peaks are sufficiently well separated that the tails in the AMS spectra could be included in the area under the curve. All other peaks overlapped, so the magnitudes of the peaks between 17 and 31 min are less certain. The small peaks are most vulnerable to tails of neighboring peaks. In all cases, the peaks were well above the background. The 14 amol per fraction limit of quantitation may not be included in the area under the curve. All other peaks are quantified with AMS.

### Table 3. Quantity of Atrazine Equivalents in Separate Peaks Expressed in fmol of AtrEq/(mL of urine)

<table>
<thead>
<tr>
<th>sample</th>
<th>elution timea (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>subject 2</td>
<td>10–13b</td>
</tr>
<tr>
<td>4–8 h</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>2 day</td>
<td>500 ± 4</td>
</tr>
<tr>
<td>7 day</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>subject 6</td>
<td>0–4 h</td>
</tr>
<tr>
<td>4–8 h</td>
<td>175 ± 3</td>
</tr>
<tr>
<td>5 day</td>
<td>65 ± 1</td>
</tr>
<tr>
<td>subject 5</td>
<td>0–4 h</td>
</tr>
<tr>
<td>4–8 h</td>
<td>5110 ± 30</td>
</tr>
<tr>
<td>5 day</td>
<td>318 ± 3</td>
</tr>
</tbody>
</table>

a Elution peaks were tentatively identified by coelution with standards as unknown, bdeethylatrazine, cidealkylatrazine and edidealkylatrazine mercapturate, ddeisopropylatrazine mercapturate, e deethylatrazine mercapturate and deisopropylatrazine, fdeisopropylatrazine and an unknown, and gatrazine mercapturate.

and the large peak including diaalkylatrazine and its mercapturate suggests that mercapturate metabolites of atrazine are suitable biomarkers for dermal exposure. The unknown metabolite that eluted at 10–13 min is well separated and easy to quantify, but it is only a major component several days after exposure when the concentrations of all metabolites are relatively low, possibly too low to be detected with routine assays (e.g., ELISA). Since this peak grows in proportion to the other peaks for the later sampling time points and occurs at the polar end of the elution gradient, it is probably a catabolite.

### CONCLUSIONS

The sensitivity of AMS salvaged the original aim of the clinical experiment: to determine the major metabolites of atrazine in human urine after a dermal exposure. The low levels of 14C in the urine precluded the use of a flow-through radiation detector in conjunction with an HPLC. Fraction collection with liquid scintillation counting was also not possible since the volume of urine injected on the HPLC column is limited to a few hundred microliters in practice. Traditional UV absorbance as a monitoring system for HPLC was useful for determining the retention times of standards, but was not sufficiently sensitive or selective to detect femtomole quantities of atrazine metabolites in fractions of crude urine. It did not detect all the 14C peaks and identified numerous peaks that did not contain any 14C tracer. AMS is the only detection method capable of quantifying the tracer atrazine and its byproducts.

Atrazine mercapturate was the only metabolite that could be identified with certainty. Its dominance at the earliest time points...
ACKNOWLEDGMENT

We thank Stewart P. H. T. Freeman for assistance in running AMS samples and Novartis Crop Protection for providing standards and suggesting HPLC conditions. This work was supported in part by the National Institute of Environmental Health Sciences Superfund Basic Research Program (P42 ES04699), NIEHS RO1 ES02710, NIEHS RO1 ES09690-01, and funds from the University of California and the Department of Energy, LLNL (UC Presidents Campus Laboratory Collaboration 95-103 and Intra-University Agreement B29 1419, LLNL-UCD). S.D.G. was supported by an NIEHS postdoctoral fellowship (ES05697). B.D.H. was a Masters' and NSF Fellow during this period. UC Davis is an NIEHS Center for Environmental Health Sciences (P30 ES05707). Work completed at LLNL was supported under DOE Contract W-7405-ENG-48.

Received for review February 10, 1999. Accepted May 21, 1999.

AC990152G