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Analytical Performance of Accelerator Mass Spectrometry and Liquid Scintillation Counting for Detection of ^{14}C-Labeled Atrazine Metabolites in Human Urine

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Accelerator mass spectrometry (AMS) has been applied to the detection of ^{14}C-labeled urinary metabolites of the triazine herbicide, atrazine, and the analytical performance of AMS has been directly compared to that of liquid scintillation counting (LSC). Ten human subjects were given a dermal dose of ^{14}C-labeled atrazine over 24 h, and urine from the subjects was collected over a 7-day period. Concentrations of ^{14}C in the samples have been determined by AMS and LSC and range from 1.8 fmol/mL to 4.3 pmol/mL. Data from these two methods have a correlation coefficient of 0.998 for a linear plot of the entire sample set. Accelerator mass spectrometry provides superior concentration (2.2 vs 27 fmol/mL) and mass (5.5 vs 54 000 amol) detection limits relative to those of LSC for these samples. The precision of the data provided by AMS for low-level samples is 1.7%, and the day-to-day reproducibility of the AMS measurements is 3.9%. Factors limiting AMS detection limits for these samples and ways in which these can be improved are examined.

More than 70 million pounds of the triazine herbicide, atrazine (Figure 1), are applied annually in the United States to agricultural lands.[1] Low-level human exposure to atrazine is likely to occur, especially to agricultural workers and to people utilizing watersheds that drain agricultural areas subject to heavy atrazine application.[2−4] Although atrazine exhibits very low acute toxicity[4,5] and has been found to be nonmutagenic in most studies,[4,6,7] there is concern about human exposure to atrazine based on a study that found that high chronic doses of atrazine increased the incidence of mammary tumors in female Sprague−Dawley rats.[4,8] An immunoassay-based method to monitor human exposure to atrazine has been developed and has been shown to be capable of detecting atrazine exposure in pesticide applicators.[9] Our primary interest in atrazine is as an indicator of work practice as related to general agrochemical pesticide exposure. We selected atrazine because of its exceptionally low toxicity to mammals and its widespread use.

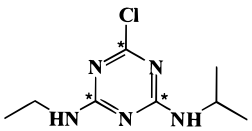

Figure 1. Structure of atrazine, 6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine. The test compound for this study was labeled with ^{14}C at the three ring carbons as indicated by asterisks.

The metabolism and excretion of triazine herbicides have been well studied in test animals[10−16] and, less commonly, in vitro with

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human tissues.[15,17,18] However, minimal published information exists about in vivo human metabolism and excretion of atrazine.[9,14,19−21] Improved methods of monitoring atrazine exposure, such as immunoassay-based methods, are best derived from direct data from human absorption, metabolism, and excretion of atrazine. In vivo toxicological studies of atrazine in test animals such as rats need normalization to subsets of human data in order to gain relevance. Thus, exposure and metabolism studies are needed using human subjects and relevant doses of the test compound. In practice, analytical limitations severely hinder our ability to directly study human pesticide metabolism at low doses.

This study uses samples from an experiment designed to examine human dermal absorption, metabolism, and excretion of atrazine. Low doses of ^{14}C-labeled atrazine (Figure 1) were administered dermally to human subjects, and urine samples were collected over 7 days. Minimization of exposure to the study volunteers was a concern of the study design, and doses were chosen to provide minimal levels of ^{14}C-labeled atrazine metabolites for analysis by liquid scintillation counting (LSC). In vivo studies of dermal absorption of atrazine in rats[12] and in vitro studies in humans[17] guided the selection of doses for this study, but the data substantially overestimated absorbed doses for the human subjects. The ^{14}C levels in the urine samples were near or below detection limits for LSC at what we considered to be practical sample sizes and analysis times (2 mL of urine, 15-min counting time, 3 replicates). The fractionation of individual urine samples by HPLC to identify and determine concentrations of individual metabolites was not performed because LSC was barely able to detect even unfractionated metabolites in large sample volumes.

We have used accelerator mass spectrometry (AMS) to detect ^{14}C-labeled atrazine metabolites in these urine samples. Unlike LSC, which counts decay events of a radioisotope, AMS is a mass spectrometric technique that counts individual ^{14}C atoms.[22] Accelerator mass spectrometry was developed to detect ^{14}C levels orders of magnitude below modern natural abundance with high precision and accuracy for radiocarbon dating, and AMS is more sensitive than LSC for the detection of ^{14}C by factors of 10^5-10^6. Several studies concerning the use of AMS for ^{14}C detection of samples from in vivo studies in test animals[22−28] and humans[29] have been reported.

This paper reports the results of the detection of total ^{14}C-labeled atrazine metabolites in human urine by accelerator mass spectrometry and liquid scintillation counting. The analytical performances of AMS and LSC are directly compared, and the half-lives of atrazine excretion determined by each technique are compared. This study tests the ability of AMS to detect the levels of ^{14}C expected from HPLC fractionation of these urine samples by comparing the determination of total ^{14}C-labeled metabolites for large-volume samples by LSC (2 mL) to the analysis of samples 800 times smaller (2.5 μL) by AMS.

EXPERIMENTAL SECTION

Clinical. All clinical aspects of this work were performed at the University of California, San Francisco, after approval by the UCSF Committee on Human Research and the Radiation Safety Committee. The volunteers were 12 normal, healthy males aged 43−74. An end use formulation of atrazine (AATREX-4L) prepared by Novartis Crop Protection was applied to a 25-cm^2 area of skin on the left ventral forearm of each volunteer for 24 h at low (6 subjects, 0.167 mg, 6.45 μCi) and high (6 subjects, 1.98 mg, 24.7 μCi) doses. At 24 h, a nonocclusive patch to protect the dosed skin site was removed, and the remaining atrazine was washed from the skin area with soap and water and analyzed by LSC. Urine and feces were collected from the subjects during the 7-day study and analyzed by LSC. Total recovery of radioactivity after 7 days was 92 ± 3% for the high-dose subjects and 101 ± 3% for the low-dose subjects.[30] Urine samples were collected over 7 days (prestudy, 0−4, 4−8, 8−12, 12−24, 24−48, 48−72, 72−96, 96−120, 120−144, and 144−168 h). The total urine volume for each sample was determined. Two volunteers from the low-dose group were removed from the study due to improper collection of the urine samples on the first day of study. Aliquots of each sample from the remaining 10 subjects were frozen and stored in a −20 °C freezer. Samples were allowed to thaw to room temperature before analysis and were immediately returned to the −20 °C freezer after use.

Liquid Scintillation Counting. Liquid scintillation counting used a Wallac model 1409 counter (Wallac, Turku, Finland) with ScintiVerse scintillation cocktail (Fisher Scientific, Fairlawn, NJ). For each sample, 1.00 or 2.00 mL of urine was added to 12 mL of scintillation fluid in a 20-mL glass scintillation vial. All samples were prepared in triplicate and counted for 15 min. Known quantities of ^{14}C-labeled atrazine mercapturic acid (provided by Novartis Crop Protection), spanning the range of ^{14}C levels measured in the sample set, were added to at least one study sample from each subject. In each case, the measured count rate

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from the added ^{14}C was equal to the expected count rate within the experimental error.

Accelerator Mass Spectrometry. Measurements of the ^{14}C concentrations by AMS were made at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory (LLNL). The operation of this facility and the details of the AMS instrument at LLNL have been reviewed.[22,31] Accelerator mass spectrometry measures an isotope ratio of ^{14}C to the total carbon in a sample. Samples were subjected to isotope dilution to minimize the effects of the variable carbon content of urine. The ^{14}C in a volume of urine was derived from the measured isotope ratio and the added stable carbon mass. Set volumes of urine were added to carbon carrier (methanol solutions of tributyrin, glyceryl tributyrate; 2.06 mg of tributyrin in 100 μL of methanol), providing 1.23 mg of carbon per sample. Tributyrin was obtained from ICN Pharmaceuticals (Costa Mesa, CA), and analytical grade methanol was obtained from Fisher Scientific. For prestudy samples, 100 μL of urine was added directly to the quartz sample tubes without the addition of carrier solution. For the remainder of the samples, the urine was first diluted 10× in distilled water, and 25 μL of diluted urine was added to the carrier solution. The methanol and water were removed by vacuum centrifugation.[25] The samples were graphitized for AMS analysis as described by Vogel.[32] Samples were first combusted to CO_2 in sealed quartz tubes at 650 °C using a cupric oxide oxidant. The CO_2 was next reduced to graphite using a combination of zinc and hydrogen reduction (TiH_2) over a cobalt catalyst in sealed Pyrex tubes at 500 (3 h) and 550 °C (2 h). Measurement times for AMS analysis were typically 3 min/sample, with a counting precision of at least 1.5% and a standard deviation among at least three measurements of 2−3%. The $^{14}C/^{13}C$ ratios measured experimentally for samples of interest were normalized to measurements of standards of known ^{14}C concentration (Australian National University Sucrose).[33] The sample and control data were presented to the sample submitter for further analysis in a spreadsheet format in units of fraction modern carbon (1 mod = 97.9 amol of ^{14}C/mg of total carbon). Correlations and regressions for the analyzed data were found using Data Desk 6.0 (Data Description, Ithaca, NY).

Carrier Solution Evaporation. Samples for testing evaporation of carrier solutions were prepared in 20-mL glass scintillation vials identical to those used for preparation of AMS samples, and solution volumes were typical of those used during AMS sample preparation. The vials were weighed, the vial caps were removed, and the vials were left on an open benchtop at room temperature (24 °C). Periodically, the vial caps were closed and the vials were reweighed. Samples prepared were methanol (5.39 mL, 4.26 g), tributyrin (0.110 mL, 0.114 g), and methanol + tributyrin (5.50 mL, 4.38 g). Three replicate samples of each type were prepared and analyzed.

Carbon Analysis. Carbon content of urine samples was determined by adding 5.0 μL of urine to approximately 5 mg of a silica support (Chromsorb W/AW 80% 100 mesh; Carlo Erba/Fisons Instruments, Valencia, CA) in a tin sample foil. The samples were then analyzed for carbon content using a Carlo Erba model 1500 NCS analyzer. Each sample was prepared and analyzed in triplicate, and the density of the urine was assumed to be 1.0 g/mL for the calculation of %C in the urine.

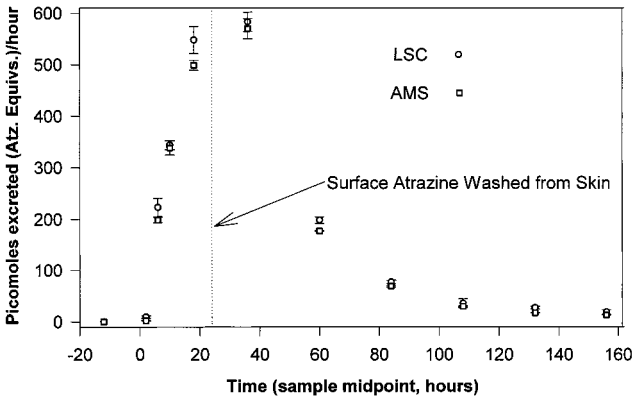

Figure 2. Urinary excretion rates of atrazine metabolites for subject 2 as determined by AMS and LSC. The dose was administered at 0 h, and the dosing patch was removed at 24 h. The data are reported as molar equivalents of atrazine.

RESULTS AND DISCUSSION

All samples (10 prestudy samples, 98 study samples) from the 10 subjects (subjects 1−4, low-dose group; subjects 5−10, high-dose group) were measured in triplicate by liquid scintillation counting and accelerator mass spectrometry. The ^{14}C concentrations for these samples (by AMS) ranged from 1.8 fmol/mL [0.25 disintegrations per minute (DPM)/mL] to 4.3 pmol/mL (602 DPM/mL). The amount of atrazine absorbed dermally by the human subjects during the 24-h dosing period and the total excretion of atrazine metabolites over the 7-day study were substantially lower than estimates used for design of the study based on in vivo data for rats[12] and in vitro data for human skin preparations.[17]

Figure 2 shows the excretion rate of ^{14}C-labled atrazine metabolites over the course of the study for subject 2 (low-dose group) as determined by AMS and LSC. The concentrations of ^{14}C measured by both methods were converted to molar atrazine equivalents, taking into account the volumes of the individual urine samples, and these values were divided by the total time over which the samples were collected. As expected, the atrazine metabolite excretion rate increases during the dosing period (0−24 h) and peaks at a time near the removal of the dermal patch. Plots for the other nine subjects in both dose groups were similar in appearance to that for subject 2.

There is excellent correlation between the AMS and LSC measurements. Table 1 shows the linear correlation coefficients for LSC and AMS measurements. Regression analysis of the data for individual subjects gives slopes ranging from 0.91 to 1.09, with a mean of 0.98. These data indicate that AMS is highly accurate relative to LSC. These correlations are excellent for two measurement techniques that rely on different analytical principles to measure ^{14}C concentrations. Liquid scintillation counting measures β-decay events of individual ^{14}C nuclei by detection of luminescence of reporter molecules in a solution containing the sample of interest. Alternatively, in AMS, ^{14}C and ^{13}C anions from

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Table 1. Linear Correlation between AMS and LSC

low dose		high dose	
subject	correln	subject	correln
1	0.996	5	0.996
2	0.999	6	0.995
3	0.999	7	0.999
4	0.998	8	0.992
		9	0.999
		10	0.998

a Cs sputter source are isolated and individually detected at megaelectronvolt energies, and the ratio of these is used to determine a ^{14}C concentration. Sample volumes differing by almost 3 orders of magnitude have been used for the two techniques (2.0 mL for LSC, 2.5 μL for AMS), and the sample preparation procedures are very different for the two methods. Excellent agreement between AMS and LSC measurements of ^{14}C in archeological samples has been demonstrated previously,[34] but much larger aliquots of chemically well-defined samples were used, and isotope dilution was not used.

Although the correlation between these two methods is outstanding, we attempted to determine why AMS data indicated lower values than LSC measurements, leading to a slope of slightly less than 1 (0.94) for a linear fit of the whole data set. Equation 1 describes the ratio of ^{14}C to total C (R_{sample}) for a sample using isotopic dilution in a carrier solution as was performed for these experiments. The ^{14}C concentration of the labeled atrazine and

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

atrazine metabolites in the urine is represented by $^{14}C_{tracer}/C_{tracer}$. Likewise, ^{14}C concentrations in the urine and carrier tributyrin are $^{14}C_{tissue}/C_{tissue}$ and $^{14}C_{carrier}/C_{carrier}$, respectively. Any sample contamination is represented by $^{14}C_{unknown}/C_{unknown}$. These experiments are designed such that $C_{carrier}$ is much larger than C_{tracer} and C_{tissue} and such that $^{14}C_{tracer}$ is greater than $^{14}C_{tissue}$ and $^{14}C_{carrier}$. One hypothesis to explain the slope of 0.94 is that evaporation of the methanol from the tributyrin carrier solution before and during sample preparation could lead to an underestimation of ^{14}C levels by AMS. If significant amounts of methanol in the carrier solution evaporate during sample preparation before addition of carrier to the sample tubes, the tributyrin concentration will increase and the amount of carrier carbon $C_{carrier}$ added to each sample will increase. This will decrease R_{sample}, and the ^{14}C concentration in the urine samples will be underestimated.

Figure 3 shows the results of an experiment designed to test this hypothesis. Evaporation was monitored by measuring the change in mass of carrier solutions open to the air at room temperature. A solution of methanol and tributyrin identical in composition and volume to that used for sample preparation was used. Pure methanol and pure tributyrin were also examined for comparison. The evaporation of the carrier solution is linear with time over 2 h, and the results for pure methanol and the methanol

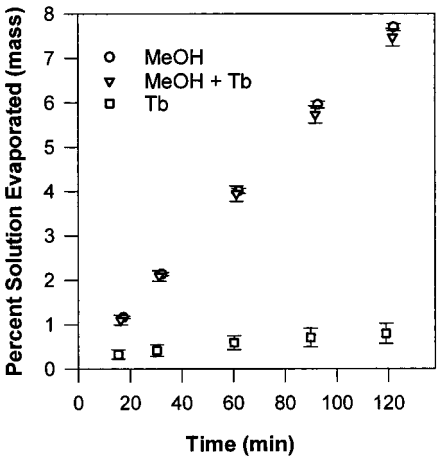

Figure 3. Evaporation over time of the carrier solution used for AMS samples at room temperature. The error bars represent the standard deviation for three replicate samples. MeOH, neat methanol; MeOH + Tb, solution of tributyrin in methanol; Tb, neat tributyrin.

solution containing tributyrin are nearly identical. During AMS sample preparation, the carrier solution was typically transferred to the quartz sample tubes in less than 30 min. Based on the data in Figure 3, evaporation of MeOH from the carrier solution would reduce the calculated ^{14}C concentrations using AMS by 1 or 2% at most under these conditions. Another possible explanation for the slope of less than 1 for the plot of AMS data versus LSC data is chemiluminescence in the LSC samples. Chemiluminescence could increase the apparent concentration of ^{14}C indicated by LSC. However, this is unlikely because the LSC instrument used for this work monitors and corrects for chemiluminescence automatically, and the instrument did not report performance of any such corrections for these samples. No significant, systematic error in LSC or AMS could be ascribed to either of these effects.

Preparation of liquid or carrier diluted samples for AMS requires a vacuum concentration stage. Although tributyrin is chosen as the preferred dilutant because it retains many volatile fractions in solution during this process, a small loss of ^{14}C-labeled volatile metabolites may occur. After elimination of carrier loss and chemiluminescence as causes of this LSC-AMS disparity, such loss of ^{14}C in AMS preparation may be the best explanation.

Precision, Noise Sources, and Detection Limits. Replicate AMS samples ($N \geq 4$) from subject 2 were measured to examine the precision and detection limits for the AMS method used to analyze these samples. The AMS data for the six samples from subject 2 with the lowest ^{14}C concentrations are plotted against the corresponding LSC data in Figure 4. The error bars represent the standard deviation for measurements of quadruplicate samples. Accelerator mass spectrometry gives better precision for low-level samples than LSC, since AMS provides lower detection limits for ^{14}C. The detection limits for AMS and factors contributing to these detection limits will be discussed later in more detail.

The sample for subject 2, day 3 (48−72 h) was prepared and measured on 5 days to examine the day-to-day variability of ^{14}C measurements by AMS. Table 2 summarizes the data for these experiments. The ^{14}C concentrations measured for this sample range from 228.3 ± 0.9 to 252.4 ± 6.1 fmol/mL, with a standard deviation for mean values of all 5 days of 9.2 fmol/mL (3.9%). This

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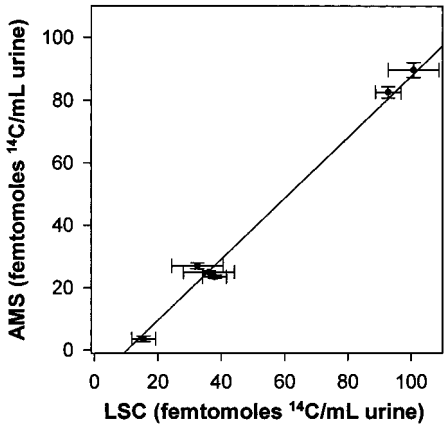

Figure 4. Plot of data acquired by AMS versus data acquired by LSC for subject 2. Only the six samples with the lowest ^{14}C levels are shown. Sample times and corresponding values by LSC (fmol ^{14}C/mL) are as follow: 0−4 h, 15.5; 4−8 h, 100.8; 72−96 h, 92.3; 96−120 h, 32.5; 120−144 h, 37.9; 144−168 h, 36.1. Error bars represent the standard deviation for replicate measurements of each sample ($N \geq 4$). The regression line is for all 10 samples from subject 2.

Table 2. Replicate Measurement of Sample S2, 48−72 h

meas day	concn (fmol of ^{14}C/mL)	SD (fmol of ^{14}C/mL)
1	228.3	0.9
2	236.5	4.1
3	234.3	1.2
4	252.4	6.1
5	243.2	4.3
mean	238.9 ± 9.2 (3.9%)	

represents acceptable stability and reproducibility over this period. The measurement days were spaced over 5 months, and new dilutions (1/10) of the urine samples were prepared and used on different measurement days.

Figure 4 emphasizes that AMS provides more precise measurements relative to LSC for low levels of ^{14}C. To fairly evaluate the analytical advantages offered by AMS relative to LSC for detection of ^{14}C in biomedical research, it is important to examine precision and the lower limits of detection for the two techniques more quantitatively. Equation 1 shows the contributions of different carbon sources to the ratio of ^{14}C to total C for these experiments. To accurately determine $^{14}C_{tracer}$ from this ratio, it is necessary to know the levels of total C and ^{14}C in the urine from all other sources. For the tributyrin used here, $^{14}C_{carrier}/C_{carrier} = R_{carrier}$ is typically 0.10 mod and is measured directly for each experiment. Typically, $^{14}C_{tissue}$ is much less than $^{14}C_{tracer}$, and because the carbon in the urine samples is diluted in tributyrin, C_{tissue} and C_{tracer} are much less than $C_{carrier}$. Care in sample preparation minimizes carbon contamination ($^{14}C_{unknown}/C_{unknown}$). Taking all this into account, eq 1 reduces to eq 2. In practice, eq

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

3 is used to calculate $[^{14}C_{tracer}]$ for the urine samples, where $m_{carrier}$ and m_{urine} are the masses of carbon in the sample from the carrier and urine, and V_{urine} is the volume of urine added to the sample. R_{urine} represents natural ^{14}C in the urine.

$$[^{14}C_{tracer}] = \{[R_{sample}(m_{carrier} + m_{urine}) - R_{carrier}m_{carrier} - R_{urine}m_{urine}] \times 0.0979 \text{ fmol of } ^{14}C/\text{mg of carbon}\}/V_{urine} \quad (3)$$

Natural ^{14}C levels were measured in the prestudy samples from the 10 subjects and ranged from 1.15 ± 0.02 to 1.71 ± 0.04 mod (mean = 1.4 ± 0.2). At the time of the study (late 1995), these values should have been 1.12, based on known natural levels of ^{14}C in the biosphere.[35] It is possible that the elevated background levels for the test subjects are due to prior participation in other medical studies utilizing ^{14}C-labeled compounds. Subjects were tested for such exposure prior to the study. However, LSC was used for this screening, and this technique cannot detect contamination at such low levels. These elevated levels are readily apparent by AMS. Contamination of the urine samples prior to analysis by AMS cannot be ruled out, but again contamination at such low levels is not detectable by LSC and could not have been easily detected or prevented.

Two terms in eq 3, $R_{sample}(m_{carrier} + m_{urine})$ and $-R_{urine}m_{urine}$, depend, in part, on the value of m_{urine}. It is assumed that $m_{carrier}$ is much greater than m_{urine} for these samples, but variability of m_{urine} from urine sample to urine sample potentially could result in significant variability in the determination of $[^{14}C_{tracer}]$. The total carbon content of 20 of the 108 urine samples from this study was measured at 0.66 ± 0.27% carbon (mass/mass), with a range from 0.27 to 1.3%. This means that 16.5 ± 5.8 μg of carbon is added in 2.5 μL of urine. The standard deviation of 5.8 μg represents 0.5% of the carbon carrier mass (1.23 mg). The highest carbon percentage measured for one sample (subject 8, 1.3% C, 65 μg) is only 2.6% of the carbon carrier mass added. The mean carbon content for samples from nine individuals (0.70 ± 0.31%) and 11 samples from subject 2 alone (0.63 ± 0.25%) are not significantly different from the values for all 20 samples. Thus, no significant systematic error is introduced by the assumptions used to reduce eq 1 to eq 3.

The mean precision for the AMS data in Figure 4 and Table 2, as indicated by the relative standard deviation for replicate measurements of individually prepared samples, is 1.7%. As mentioned earlier, typical values for $R_{carrier}$, R_{urine}, $m_{carrier}$, and m_{urine} are 0.10 mod, 1.1 mod, 1.23 mg, and 0.017 mg, respectively. For most of these samples, the magnitude and uncertainty of $R_{sample}(m_{carrier} + m_{urine})$ will be substantially larger than those for $-R_{urine}m_{urine}$ and $-R_{carrier}m_{carrier}$. Therefore, $R_{sample}(m_{carrier} + m_{urine})$ and V_{urine} will be most important for determining the precision of these measurements. Pipetting steps to dilute the urine (m_{urine}) and to add both urine and carrier solution during sample preparation are expected to contribute significantly to the variability of these terms.

The mean relative standard deviation (RSD) for the same low-level samples (subject 2) analyzed in triplicate by LSC is 16%. This is somewhat misleading, however, because these samples are near

(35) White, J. W. C.; Ciais, P.; Figge, R. A.; Kenny, R.; Markgraf, V. *Nature* **1994**, *367*, 153−6.

the detection limit of this method (and could be improved with longer counting times). The mean relative standard deviation for 2-mL samples ranging from 8.4×10^2 (117 DPM/mL) to 4.3×10^3 fmol/mL (602 DPM/mL) in ^{14}C concentration is 1.8%. The mean RSD for replicate measurement of one of the three replicate samples prepared for each sample is 1.0%.

The detection limit for AMS analysis of these urine samples and experimental conditions can be estimated by examining potential sources of variability in the expression used to determine $[^{14}C_{tracer}]$ (eq 3) as it approaches zero. In this limit, R_{sample} becomes $R_{carrier}$. The terms from eq 3 contributing to the background uncertainty are the following: $+R_{carrier}m_{carrier}$, $+R_{carrier}m_{urine}$, $-R_{carrier}m_{carrier}$, and $-R_{urine}m_{urine}$. The RSD of V_{urine} should be on the order of 1−2%, as indicated by pipettor calibrations, and will not be significant at the detection limit. Replicate measurements of only carrier solution give a value of 0.105 ± 0.009 mod. This value includes all background samples for four of the six measurement days included in this paper (28 samples). On two days, the background samples gave distinctively higher and more variable values (0.16 ± 0.03; 0.16 ± 0.03), indicating contamination ($^{14}C_{unknown}/C_{unknown}$) during sample preparation. Measurements of the lowest-level samples on the two days in question were repeated on measurement days where control experiments indicated no significant contamination. The relative standard deviation of the value for the carrier solution is equivalent to the relative uncertainty of $R_{carrier}m_{carrier}$. The magnitude of the uncertainty of this term is 1.2×10^{-2} mod·mg. R_{urine} is, on average, 1.4 mod for these samples, and the average precision for the measurement of R_{urine} in individual samples is 4%. The variability of m_{urine} was discussed earlier. The magnitude of the uncertainty of $+R_{carrier}m_{urine}$ is 0.07×10^{-2} mod·mg. The magnitude of the uncertainty of $-R_{urine}m_{urine}$ is 0.84×10^{-2} mod·mg. The total background uncertainty due to the four terms was calculated, and a definition of the detection limit as 3 times the standard deviation of the background noise was used to estimate concentration and mass detection limits of 2.2 fmol/mL and 5.5 amol, respectively. The lowest ^{14}C concentration measured by AMS for these urine samples is just below this estimated detection limit, at 1.7 fmol/mL (4.3 amol) for two samples from subject 9. All other samples are above the AMS detection limit.

The background noise and detection limit for LSC under these experimental conditions can be evaluated more directly. The mean standard deviation for measurement of 2.00-mL blank urine samples (three replicates) from all 10 subjects was 2.5 DPM (18 fmol of ^{14}C). At 3 times the standard deviation of the background noise, a concentration detection limit of 27 fmol of ^{14}C/mL is calculated, and the corresponding mass detection limit is 54 fmol. Analysis by AMS indicates that 14 of the 98 study samples contain ^{14}C levels below this detection limit. Accelerator mass spectrometry gives a concentration detection limit that is approximately 12 times lower than that for LSC and a mass detection limit that is about 10 000 times lower.

The differences in mass and concentration detection limits are consequences of using different measurement principles. In AMS, individual ^{14}C atoms are detected after isolation by a combination of low-energy and high-energy mass spectrometry. Approximately 1% of the ^{14}C in a graphite sample could be detected in 15 min on the basis of typical detection parameters. Alternatively, LSC detects only ^{14}C atoms that undergo radioactive decay. The 5730-year half-life of ^{14}C means that only 3.4×10^{-7}% of the ^{14}C atoms decay during a 15-min count. Increasing the counting time for LSC will improve detection limits, but counting times would have to approach 80 years to reach the 1% efficiency of AMS.

The concentration detection limits for AMS analysis of these biomedical samples could be improved through reduction of background noise using several strategies, but each improvement would be balanced by increases in analysis time, cost, and complexity. First, more sample could be used for AMS analysis. However, under these experimental conditions, the uncertainty attributable to the background carbon in the urine is approximately equal in magnitude to the uncertainty due to measurement of ^{14}C in the tributyrin carrier, and analysis of larger urine volumes would have a minimal effect on the concentration detection limit. Another approach is to reduce the two major sources of background noise: variability in the carbon content of the urine samples and ^{14}C present in the carrier carbon. The carbon content of each urine sample could be measured to reduce the uncertainty in this value by as much as an order of magnitude. This effort alone would not improve the detection limit significantly because the detection limit would be limited by the tributyrin carrier. Reduction of the carrier ^{14}C could improve concentration detection limits dramatically. If this were achieved, the detection limit would be defined by the error in the measurement of the natural ^{14}C present in the urine and the mass of the urine. Reduction of carrier ^{14}C could be achieved by finding a carrier compound with a lower ^{14}C concentration or a source of tributyrin containing less ^{14}C. A second possibility would be to eliminate the carbon carrier altogether and analyze neat graphitized urine. Sample consumption would increase, and it would be necessary to measure the total carbon content of each sample separately. In this work, 2.5-μL urine samples were analyzed by AMS in 1 mg of carrier carbon, but approximately 150 μL of urine would be required to generate a graphite sample containing 1 mg of total C. For these samples, sufficient volume is available, and the only limitation would be the need to measure total carbon content. In many biomedical applications, analyzing samples without using a carbon carrier is an attractive approach, especially if a rapid or on-line measurement of total carbon can be made.

Excretion Half-Life. Information about excretion kinetics of atrazine metabolites is important for developing sampling strategies for monitoring human exposure. The excretion half-lives determined for each subject by LSC and AMS are summarized in Table 3. For each subject, the elimination half-life was determined from a linear fit of the logarithm of the rate of excretion (picomoles/hour) against the midpoint of the sampling period (for example, 18 h for a 12−24-h sample). Only the five samples for days 3−7 were used for half-life determination. The atrazine dose was applied from 0 to 24 h of the study, the time span over which the first four samples were collected. The plots of the excretion rate vs time reflect this as the rate increases through the first four samples (0−24 h) for each subject (Figure 2). Furthermore, for 3 of the 10 subjects, the highest excretion rate occurs in the day 2 sample, after removal of the dosing patch. This suggests that the renal atrazine concentration may peak due to the applied dose between 24 and 48 h; therefore, the day 2 samples were also excluded from half-life determinations. Subject 10 was eliminated

Table 3. Determination of Excretion Half-Life by AMS and LSC

	half-life (h)		correln coeff	
subject	AMS	LSC	AMS	LSC
Samples Containing Lower Levels of ^{14}C[a]				
1[b]	22.5	27.8	0.92	0.86
2[b]	25.0	28.8	0.96	0.94
6[c]	21.8	26.0	0.99	0.95
7[c]	22.9	29.3	0.99	0.94
9[c]	18.1	28.9	0.99	0.92
Samples Containing Higher Levels of ^{14}C[d]				
3[b]	26.2	24.9	0.96	0.99
4[b]	26.6	29.5	0.98	0.97
5[c]	25.8	29.5	1.00	0.99
8[c]	26.1	27.4	0.99	0.99
10[c,e]	54.4	50.3	0.64	0.59

[a] Mean concentrations ranged from 3.2 to 15.3 DPM/mL. Total range in samples was 0.25−44.3 DPM/mL. [b] Low-dose group. [c] High-dose group. [d] Mean concentrations ranged from 20.2 to 65.3 DPM/mL. Total range in samples was 5.2−218.5 DPM/mL. [e] The data for subject 10 were not included in calculations of half-life.

from the following discussion although the data are included in Table 3. The last sample (day 7) from this subject contained almost twice as much ^{14}C as the previous sample (day 6). None of the 49 other samples from day 3−7 for the 10 subjects had a higher ^{14}C content than the preceding sample.

The data for subjects 1−9 give a mean half-life of 24 ± 3 h by AMS and 28 ± 2 h by LSC. There is no significant difference indicated between the data for the low- and high-dose subjects by either AMS or LSC. If the data are separated into groups with relatively high and low levels of ^{14}C in the samples, as defined in Table 3, differences in the quality of the data from AMS and LSC become apparent. For the data with high levels of ^{14}C, a comparison of the data using a two-tailed paired *t*-test indicates no difference between LSC and AMS ($P = 0.92$). For the samples with low ^{14}C levels and thus poorer signal-to-noise ratios for the data, the same comparison indicates that AMS and LSC give different results ($P = 0.008$). The correlation coefficients for the AMS data are clearly better than those for LSC for the samples low in ^{14}C, but virtually no difference exists for the high-level samples.

CONCLUSION

Accelerator mass spectrometry provides significantly lower *concentration* detection limits (12 times) and dramatically lower *mass* detection limits (10 000 times) compared to LSC for these samples and experimental conditions. The quality of the AMS data in terms of accuracy, precision, and day-to-day reproducibility is equivalent to LSC for reasonable counting times. The quality of the data provided by LSC is significantly worse for low-dose kinetics. The mass detection limit advantage of AMS can be exploited to quantify small fractions of metabolites isolated by separation techniques such as HPLC or TLC to greater than part-per-trillion. The mass detection advantage of AMS is also useful for analysis of more volume-limited samples, such as tissue biopsies or cerebrospinal fluid.

On a broader scale, AMS will impact the usefulness of ^{14}C as a tracer for human biomedical studies in several ways. First, AMS allows the use of lower levels of radioactivity, reducing both health risks to human subjects and radioactive waste. In many cases (e.g., environmental exposure studies), doses can be used that are more relevant to the biological effect being examined. Increased detection sensitivity also allows for the use of less efficiently ^{14}C-labeled test compounds, which can reduce the cost and difficulty of synthesis.

Parallel sample processing for AMS is required to make optimal use of the 300−400 samples/day measurement capacity of the LLNL AMS spectrometer for these types of studies. While sample preparation for AMS is significantly more time-consuming than to LSC, it would take almost 3 days to analyze 400 samples by LSC using a 15-min counting time. Analysis costs are still high on this AMS instrument, so only experiments that must make use of the high sensitivity are feasible at this time. However, spectrometers are now available that are much smaller and less expensive to own and operate for the detection of tritium and radiocarbon. These smaller spectrometers, installed directly in an analytical laboratory, will increase access to AMS and will drive down costs.

For the direct analysis of this particular sample set, nearly equivalent data have been obtained by each method. The goal of the part of the work presented here was to quantitatively compare the analytical performance of AMS and LSC for direct analysis of ^{14}C-labeled metabolites in human biological fluids. We have shown the feasibility of further HPLC analysis of this sample set using AMS for detection at part-per-trillion fractionations of the metabolites into identified species.

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