

Accelerator mass spectrometry in biomedical dosimetry: Relationship between low-level exposure and covalent binding of heterocyclic amine carcinogens to DNA

(¹⁴C detection/2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline)

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Communicated by H. H. Barschall, April 3, 1990 (received for review February 12, 1990)

ABSTRACT Accelerator mass spectrometry (AMS) is used to determine the amount of carcinogen covalently bound to mouse liver DNA (DNA adduct) following very low-level exposure to a ¹⁴C-labeled carcinogen. AMS is a highly sensitive method for counting long-lived but rare cosmogenic isotopes. While AMS is a tool of importance in the earth sciences, it has not been applied in biomedical research. The ability of AMS to assay rare isotope concentrations (¹⁰Be, ¹⁴C, ²⁶Al, ⁴¹Ca, and ¹²⁹I) in microgram amounts suggests that extension to the biomedical sciences is a natural and potentially powerful application of the technology. In this study, the relationship between exposure to low levels of 2-amino-3,8-dimethyl[2-¹⁴C]imidazo[4,5-f]quinoxaline and formation of DNA adducts is examined to establish the dynamic range of the technique and the potential sensitivity for biological measurements, as well as to evaluate the relationship between DNA adducts and low-dose carcinogen exposure. Instrument reproducibility in this study is 2%; sensitivity is 1 adduct per 10¹¹ nucleotides. Formation of adducts is linearly dependent on dose down to an exposure of 500 ng per kg of body weight. With the present measurements, we demonstrate at least 1 order of magnitude improvement over the best adduct detection sensitivity reported to date and 3–5 orders of magnitude improvement over other methods used for adduct measurement. An additional improvement of 2 orders of magnitude in sensitivity is suggested by preliminary experiments to develop bacterial hosts depleted in radiocarbon. Expanded applications involving human subjects, including clinical applications, are now expected because of the great detection sensitivity and small sample size requirements of AMS.

Carcinogens covalently bound to any of the deoxynucleotide bases present in DNA (DNA adducts) have been proclaimed as markers of carcinogen exposure. The relationship between adduct formation and exposure, however, has been primarily established at high carcinogen doses and not at lower, more environmentally relevant, levels because of limitations in assay sensitivity. As a consequence, the significance of using adducts as a measure of carcinogen exposure in the human population is unknown. Currently, the most sensitive technique for adduct detection is the ³²P postlabeling assay. The ³²P postlabeling assay has permitted measurement of 1 adduct in 10¹⁰ nucleotides and has been used to detect carcinogen–DNA binding in occupationally exposed humans and smokers, but accurate quantitative measurement at levels <1 adduct per 10⁷–10⁸ nucleotides is difficult because of variability in adduct recovery (1–3). The ability of accelerator mass spectrometry (AMS) to measure concentrations of rare

isotopes in 20-μg to 1-mg samples suggested to us that its extension to the biomedical sciences was a natural and potentially powerful application of the technology (4). The great enhancement in ¹⁴C detection sensitivity available with AMS offers the distinct advantage of detecting extremely small amounts of covalently bound ¹⁴C-labeled carcinogens to DNA with known stoichiometry over a wide range of carcinogen binding.

AMS was developed as a highly sensitive method for counting long-lived but rare cosmogenic isotopes, typically those having half-lives between 10³ and 2 × 10⁷ years (5). Isotopes with this range of half-lives are too long-lived to detect easily by conventional decay counting techniques but are too short-lived on geological time scales to be present in appreciable concentrations in the biosphere or lithosphere. Assay of these cosmogenic isotopes (¹⁰Be, ¹⁴C, ²⁶Al, ³⁶Cl, ⁴¹Ca, and ¹²⁹I) by AMS has become a fundamental tool in archaeology, oceanography, and the geosciences, but it has not been applied to problems of a biological or clinical nature (6, 7).

Historically, measurement of isotopically tagged materials has been avoided by AMS laboratories due, at least in part, to concerns over facility contamination. Our initial measurements on biological materials have shown that contamination of AMS instrumentation by samples prepared in biomedical laboratories with a history of ¹⁴C usage is indeed a problem (8). In an effort to make this technology available to the biomedical and environmental sciences communities, we devised sample handling protocols to overcome such gross contamination.

In this study, we used these protocols to determine the relationship between carcinogen dose and DNA adduct levels in mice given very low levels of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx; Fig. 1), a carcinogen found in cooked meat (9). This study provides a report of the dynamic range, sensitivity, and general applicability of AMS technology to problems in biomedical and environmental dosimetry, as well as presenting the relationship between DNA adducts and low-dose MeIQx exposure.

MATERIALS AND METHODS

Animals. Male C57BL/6 mice (23–27 g) were obtained from Simonsen Laboratories, Gilroy, CA. Animals were housed individually in disposable polystyrene cages with hardwood bedding on a 12-hr light/12-hr dark cycle. Animals were fasted for 18 hr and then given either [2-¹⁴C]MeIQx (50

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Abbreviations: AMS, accelerator mass spectrometry; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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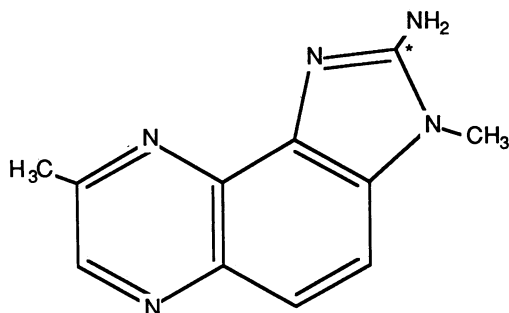


FIG. 1. Structure of MeIQx. The molecule was synthesized with a single ^{14}C atom at the 2-position (*) of the imidazole ring.

mCi/mmol; 1 Ci = 37 GBq), which was synthesized (10) in our laboratory, or 2,3,7,8-tetrachloro[^{14}C]dibenzo-*p*-dioxin (TCDD; 122 mCi/mmol; Cambridge Isotope Laboratories; Woburn, MA). The radiopurity of both compounds was >97% as determined by high-pressure liquid chromatography. Administration of the [^{14}C]MeIQx was in corn oil by stomach intubation (vol, 0.1 ml). [^{14}C]TCDD was given i.p. in *p*-dioxane (10 μl per mouse). Doses of MeIQx were administered from 500 pg to 5 mg per kg of body weight and TCDD was administered from 500 pg to 100 μg per kg of body weight. Animals were sacrificed 24 hr after dosing by CO_2 asphyxiation. Livers were placed on dry ice immediately after removal and stored overnight at -80°C before isolation of the DNA. Three animals were used per dose level and three measurements were made on each hepatic DNA sample.

DNA Isolation. Liver DNA was isolated as described (11). After DNA isolation, the DNA was dissolved in distilled water, extracted 3 times with aqueous 1-butanol (pH 8.0), and dialyzed against sterile distilled water three times to ensure removal of any residual noncovalently bound ^{14}C . Samples were handled with disposable glassware. All equipment was used only once. Gloves were worn throughout the procedures and were changed between samples. The DNA was then diluted from 10:1 to 1000:1 with a 1 mg/ml sodium lauryl sulfate (SDS) solution. SDS was found to be 0.4 Modern (see below for definition of Modern). Approximately 0.5 mg of the DNA/SDS mixture was dried under vacuum in silica tubes and was converted to graphite by using a dedicated system built for handling labeled compounds as described by Vogel *et al.* (12, 13). ^{14}C -free acrylamide and ANU sucrose standards (prepared by the Australian National University) were graphitized along with the samples to monitor for ^{14}C carry-over. Water vapor degassing between preparations helped to remove residual ^{14}C remaining in the instrumentation (8).

Measurement. Measurements are reported in units of Modern or as DNA adducts per 10^{12} nucleotides. Modern is defined as 5.9×10^{10} ^{14}C atoms per g of carbon and is approximately equal to the natural abundance of ^{14}C present in contemporary (1950 A.D.) carbon (14). Determination of adduct levels is based on ^{14}C measurement of the MeIQx DNA adduct. [^{14}C]MeIQx binding was calculated by subtracting the natural radiocarbon content from the measured ^{14}C content of the samples. Adduct frequencies were normalized based on the assumption that 1 μg of DNA = 3240 pmol of nucleotides. The measurements were done by protocols developed for the AMS beamline at the Lawrence Livermore National Laboratory, Multiuser Tandem Laboratory (15, 16). ANU sucrose, with an activity 1.508 times the ^{14}C activity of 1950 carbon, was used as analytical standard.

Growth of Bacteria. *Methylosinus trichosporium* was provided by R. T. Taylor and S. Park (Lawrence Livermore National Laboratory). Briefly, *M. trichosporium* Ob3b was grown in a 5-liter bioreactor with continuous gas flow.

Temperature was maintained at 30°C at pH 6.8–7.2. The methane (petroleum derived) flow rate was kept at 150–500 ml/hr. The flow rate for air containing 10% CO_2 was kept at 450–1500 ml/hr. Approximately 1 mg of bacteria (wet weight) was collected and dried under vacuum. The bacterial sample was converted to elemental graphite and measured as described above.

Statistics. Statistical analysis was done by analysis of variance and regression analysis using Statview II (Abacus Concepts, Berkeley, CA) with a Macintosh computer. Student's *t* tests were used to compare means where appropriate.

RESULTS

Initial measurements of biological samples from ^{14}C tracer studies and of samples prepared in laboratories where ^{14}C is routinely used resulted in instrument contamination (8). As a result, protocols were devised involving careful handling of each sample, use of disposable labware, and isolation of samples from potential sources of gross contamination. In addition, we alternated analytical standards (ANU sugar) with each DNA sample to determine when and if contamination occurred, and water vapor was flushed through the graphitization apparatus between samples to remove any excess ^{14}C left from the previous sample. We also used ^{14}C -free acrylamide samples to test for sample contamination of either the graphitization apparatus or the spectrometer. Actual measurements were made on DNA diluted with ^{14}C -depleted carbon prior to analysis. In one case, an 18,000 Modern sample was measured directly, but no residual ^{14}C from this sample could be detected in any of the subsequent sample preparations and measurements (data not shown). DNA from animals given the 5 mg/kg body weight dose of MeIQx averaged a 46,000-fold enrichment in ^{14}C but actual measurements at this dose were below 53 Modern because of dilution of the DNA with ^{14}C -depleted carbon. In no case did the ^{14}C -enriched samples contaminate either the graphitizer station or the spectrometer, as determined from the lack of a statistical increase in the ^{14}C content of the ANU sugar or acrylamide standards.

Instrument performance was determined by accessing the variation in measurements on replicate treatments and multiple measurements on the same sample. The coefficient of variation in ^{14}C content and number of adducts among animals at each dose level was $\approx 10\%$ by AMS. The average within-sample coefficient of variation in ^{14}C content of these measurements was 2% based on multiple measurements of standard ^{14}C -containing materials, and it was 8% for multiple measurements made on separately prepared aliquots of the same DNA.

The amount of DNA analyzed in these experiments ranged from 1 μg to 1 mg. Adduct levels per 10^{12} nucleotides were found to be dependent ($P < 0.001$) on dose of MeIQx administered (Fig. 2). A linear relationship existed from a dose of 500 ng per kg of body weight to a MeIQx dose of 5 mg per kg of body weight ($P < 0.001$). The ^{14}C content of the DNA of animals at the 5 ng/kg dose is not significantly elevated ($P < 0.10$) over levels detected in unexposed animals.

The measured ^{14}C content of the DNA from control animals, animal food, and other potential sources of ^{14}C used in this study is shown in Fig. 3 in relation to the ^{14}C content of the dosed animals. Carbon from the DNA of animals exposed to 5 ng and 500 pg per kg of body weight varied between 2 and 10 Modern. DNA from animals not given labeled MeIQx ranged between 1.2 and 3 Modern. Animal food was found to be contemporary (1.2 Modern) as was the corn oil (1.15 Modern) used to administer the MeIQx. Samples prepared from ^{14}C -free acrylamide consistently measured 0.01 Modern. Solvents and solutions used in the study were Modern in ^{14}C

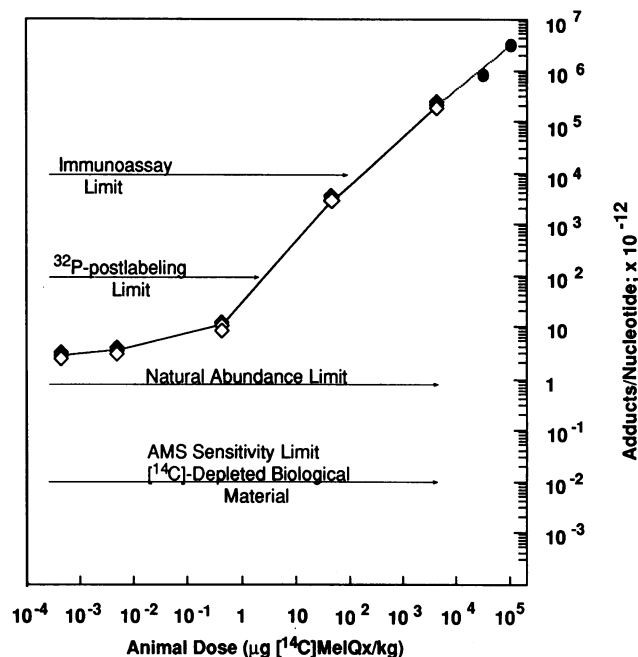


FIG. 2. Effect of MeIQx exposure on DNA adduct levels found in the hepatic DNA of mice as determined by AMS (\diamond). Data (17, 18) from ^{32}P postlabeling of DNA isolated from mice given higher levels of MeIQx (\bullet) demonstrate the linearity of the dose-response above our limit of detection.

content, but radiocarbon levels in surface swipes of work areas showed that some areas were contaminated with ^{14}C from previous tracer experiments. These areas ranged between 18 and 18,000 Modern (data not shown).

The extreme sensitivity of AMS for ^{14}C suggests that very small amounts of noncovalently bound ^{14}C or ^{14}C -bound macromolecular contaminants in the DNA would be detectable and thus could bias the adduct determinations. To evaluate this possibility and to ensure that our procedures adequately purify DNA, we measured DNA adduct formation with TCDD, a carcinogen that has not been found to covalently bind to DNA (19, 20). TCDD absorption is rapid

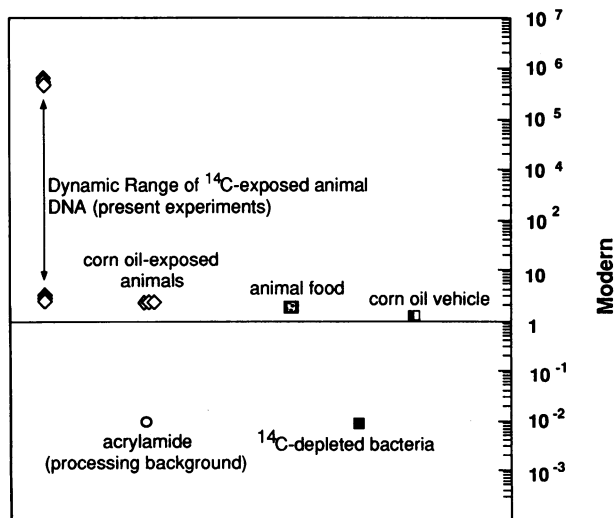


FIG. 3. ^{14}C content of control materials reported as fraction Modern ($\square\square$) in relation to the range in values obtained for the ^{14}C MeIQx-exposed mice (\diamond). CO_2 in present day air is ≈ 1.15 Modern. Measurements of ^{14}C content in methanotrophic bacteria grown on petroleum-derived methane and CO_2 (\blacksquare) show the potential sensitivity obtainable in selected biological systems.

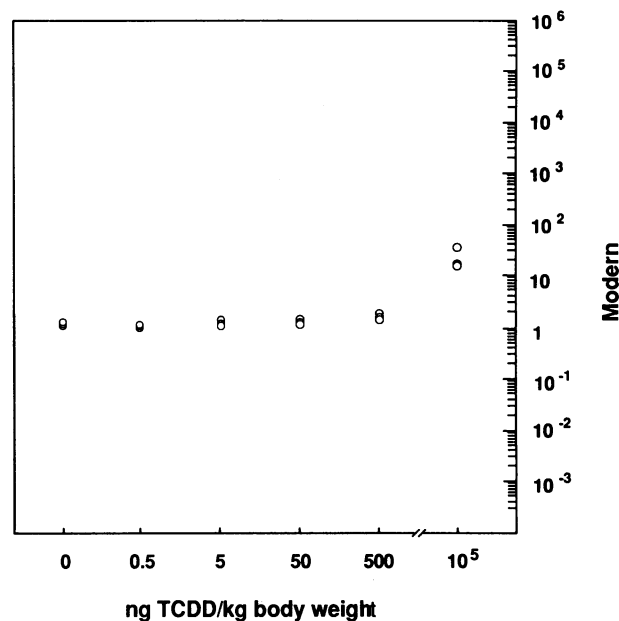


FIG. 4. ^{14}C levels found in the DNA of $[\text{U-}^{14}\text{C}]\text{TCDD}$ -exposed mice. TCDD had 11.7 ^{14}C s per molecule on average as opposed to 1 ^{14}C per molecule for MeIQx. ^{14}C levels were modern at all except the highest TCDD doses, showing that animal handling and DNA extraction procedures were adequate in the removal of ^{14}C noncovalently bound to DNA.

and $\approx 33\%$ of the administered dose should reach the liver tissue within the time frame of this study (21). These distribution kinetics are similar to that expected for MeIQx (22). The TCDD was uniformly labeled with ^{14}C (11.7 ^{14}C s per molecule on average) corresponding to 10 times more ^{14}C at each dose level than the MeIQx-exposed animals. Hepatic DNA samples isolated from the TCDD-exposed mice (Fig. 4) were modern in ^{14}C content (1.06–1.7 Modern) except at the highest TCDD dose level (100 $\mu\text{g}/\text{kg}$; 14 Modern). The highest TCDD dose level is approximately the LD_{50} for TCDD in mice and the significance of the higher ^{14}C DNA values needs to be explored further. These data suggest that no (or little) non-DNA-bound ^{14}C remains after our DNA purifications since all DNA samples, except those from the highest TCDD dose, were modern in ^{14}C content. This suggestion is supported by the fact that ^{14}C levels measured in DNA from the MeIQx-exposed mice ranged from 100- to 4000-fold greater than with the TCDD-exposed mice. Even at the highest TCDD dose, $>99.9\%$ of the ^{14}C distributed to the liver was removed through our DNA purification protocol. In addition, this result shows that animal handling and DNA isolations can be carried out without radiocarbon contamination when appropriate procedures are used.

The limiting factor for biological measurements in the detection of ^{14}C tagged molecules will be the natural abundance of ^{14}C existing in the biosphere. Thus, we felt utilization of ^{14}C -depleted hosts would be valuable for modeling dosimetry. Toward this end, *M. trichosporium* was grown with petroleum-derived methane used as the sole carbon source. Measurement of the ^{14}C content of this organism verified that the ^{14}C content can be easily depleted to an equivalent of 0.01 Modern, which is 200 times less than our lowest mouse sample (Fig. 3).

DISCUSSION

The present detection limit for ^{14}C -labeled DNA adducts by AMS is 1 adduct per 10^{11} nucleotides. This corresponds to a 1 order of magnitude improvement over the very best sensitivity offered to date by the ^{32}P postlabeling assay. AMS

provides a direct measure of the number of adducts present without relying on enzymatic recognition of adducts and/or quantitative extraction recoveries (1, 2, 23). In addition, these measurements are 3–5 orders of magnitude better than other techniques used for quantitative assay of DNA adducts such as ultrasensitive radioimmunoassays (24, 25), surface-enhanced Raman spectroscopy (26), gas chromatography–mass spectrometry (27), laser-induced phosphorescence (28), fluorescence spectrometry (29), fluorescence line narrowing spectrometry (30), and synchronous scanning fluorescence spectrometry (31). Reproducibility of the measurements is very high (within 10%) and is limited by animal to animal variation.

Instrument precision in these measurements is 2% with further improvements likely. Thus, AMS is a uniquely sensitive and reproducible technique for adduct measurement and will easily complement existing methods.

Our results show that the relationship between adduct numbers and MeIQx exposure is linear to a MeIQx dosage of 500 ng/kg based on the treatments used here. The 500 ng/kg dose level, corresponding to 1 adduct per 10^{11} nucleotides, is the lowest dose we can statistically discriminate from the controls ($P < 0.05$). Linear extrapolations of adduct numbers from these exposures to adduct numbers reported previously by our laboratory and others (17, 18) from ^{32}P postlabeling of hepatic DNA from animals given much higher levels of MeIQx (50 and 100 mg per kg of body weight) are similar, further demonstrating a linear dose–response (Fig. 2).

Our inability to measure adducts in animals given 5 ng of MeIQx per kg of body weight and less is seemingly due to contamination of the DNA during isolation and does not represent a biological threshold. It is not the result of our inability to detect modern or lower levels of ^{14}C . Contamination most likely occurred during animal handling and/or DNA isolation. This is evident from comparison of the expected amount of ^{14}C in contemporary materials to the amounts actually found in the control animals (Fig. 3). Measurement of corn oil and animal food corresponded to contemporary carbon (≈ 1.2 Modern) but control animals were 2-fold above contemporary in ^{14}C content. Measurement of acrylamide and the ^{14}C -depleted methanotrophic bacteria demonstrates the sensitivity of the carbon preparation and measurement process and that we can measure up to 100-fold below contemporary levels of ^{14}C . Thus, the relatively high levels of ^{14}C found in animals not given radioisotope are due to sample contamination and to the proximity of the total ^{14}C content of the samples to the ambient ^{14}C content of the DNA itself. Extreme care must be taken to avoid excess contamination above this natural limit. The work station that measured a minimum of 18,000 Modern aptly demonstrates the problems encountered in preparing samples for AMS measurement in laboratories with a history of ^{14}C use. However, the data from the TCDD exposure study show that, with proper handling, contamination problems and nonspecific binding of ^{14}C can be eliminated.

Additional increases of 2- to 10-fold in the sensitivity of DNA adduct detection by AMS will be possible through contamination reduction, but it will certainly be no better than that allowed by the natural abundance of ^{14}C in biological molecules (modern carbon from the biosphere is $1/10^{12}$ ^{14}C). However, enhancement of sensitivity can be gained by using ^{14}C -depleted hosts. Growth of yeast and bacteria on petroleum feedstocks has been reported (32, 33). We have grown ^{14}C -depleted *M. trichosporium* on petroleum-derived methane and verified that the ^{14}C content can be easily depleted to an equivalent of 0.01 Modern, demonstrating a potential 100-fold increase in sensitivity. Such model organisms could be of use in studying the consequences of dose on the metabolism, kinetics, and effects of xenobiotic expo-

sure. Growth of other hosts on petroleum-based foodstuffs should result in similarly low radiocarbon backgrounds.

Clinical applications and research with human subjects can be envisioned with AMS radioisotope tracing. The detection sensitivity and small sample size requirements of AMS make it ideal for measurements of small quantities of easily accessible human cells, in addition to the liver tissue demonstrated here. Therapeutic parameters for individuals could be determined by AMS through administration of small dosages of ^{14}C -labeled pharmaceuticals. Such custom tailoring of effective therapeutic regimens would be particularly valuable for cancer chemotherapy as the extremely small human radiation dose from the drug should not be an issue. The estimated effective [^{14}C]MeIQx radiation dose equivalent in this study, based on a 24-hr biological half-life (22), corresponded to 0.003 milliSieverts at the 500 ng/kg dose level. This exposure is $\approx 0.1\%$ of the total annual adult exposure to ionizing radiation from known natural sources (34). Mutagen exposure in such protocols becomes a more significant issue than the radiation dose incurred.

Use of AMS in the present measurements of low-level DNA adducts provides no structural information on adduct type. Such information is better obtained with the postlabeling assay. However, molecular information on adduct type should be obtainable with AMS when it is used in conjunction with appropriate techniques to purify and separate adducts prior to measuring the ^{14}C ratios. The need for use of radiolabeled compounds with AMS is a limitation, but radiation exposure, because of the extreme sensitivity of the technique, is insignificant, particularly when used in the laboratory and clinical setting where very low levels of isotopically tagged compounds are being measured. AMS also has the advantage of measuring these low ^{14}C levels in small samples. Utilization of rare stable isotopes seems possible as well. Thus, AMS will be useful in any application in which sensitivity of detection is limiting.

The present results with DNA adduct dosimetry demonstrate the utility of AMS for quantitative measurement of low-frequency biomolecular events following exposure to small concentrations of ^{14}C -labeled xenobiotics. The technique will be useful in clinical and laboratory environments where sensitivity of detection is not possible by other assays and in a wide number of applications beyond the adduct detection reported here. The technique has a dynamic range covering many orders of magnitude, is reproducible and sensitive, and ^{14}C contamination is controllable. Furthermore, requirements for ^{14}C enrichment are 5–6 orders of magnitude below traditional decay-counting methods. Other obvious candidate isotopes for low-level biomedical and environmental dosimetry applications are ^3H and ^{41}Ca . These potential applications, coupled with the ^{14}C measurements reported here, show AMS technology to be an important tool for the biomedical and environmental sciences community.

The authors thank D. J. Massoletti and E. E. Stilwell for assistance in sample preparation. This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory (W-7405-ENG-48) and was partially supported by the National Institutes of Environmental Health Sciences (222Y-01-ES-70158), the National Cancer Institute (CA40811), and the Natural Science and Engineering Research Council (Canada).

1. Reddy, M. V. & Randerath, K. (1986) *Carcinogenesis* 7, 1543–1551.
2. Gupta, R. C. (1985) *Cancer Res.* 45, 5656–5662.
3. Gupta, R. C. & Early, K. (1988) *Carcinogenesis* 9, 1687–1693.
4. Elmore, D. (1987) *Biol. Trace Elem. Res.* 12, 231–245.
5. Brown, L. (1984) *Annu. Rev. Earth Planet. Sci.* 12, 39–45.
6. Elmore, D. & Phillips, F. M. (1987) *Science* 236, 543–550.
7. Kielson, J. & Waterhouse, C. (1978) *Radiocarbon Dating*,

- Proceedings 1st International Conference, ed. Grove, H. E. (Nuclear Structure Research Laboratory, University of Rochester, NY), pp. 391–395.
8. Vogel, J. S., Southon, J. R. & Nelson, D. E. *Radiocarbon* **32**, 81–83.
 9. Felton, J. S., Knize, M. G., Shen, N. H., Andresen, B. D., Bjeldanes, L. F. & Hatch, F. T. (1986) *Environ. Health Perspect.* **67**, 17–24.
 10. Grivas, S. (1985) *Acta Chem. Scand. Ser. B.* **39**, 213–217.
 11. Gupta, R.C. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6943–6947.
 12. Vogel, J. S., Nelson, D. E. & Southon, J. R. (1989) *Radiocarbon* **31**, 145–149.
 13. Vogel, J. S., Nelson, D. E. & Southon, J. R. (1987) *Radiocarbon* **29**, 323–333.
 14. Stuiver, M. & Polach, H. A. (1977) *Radiocarbon* **19**, 355–363.
 15. Davis, J. C. (1989) *Nucl. Instrum. Methods* **B40/41**, 705–708.
 16. Proctor, I. D. (1989) *Nucl. Instrum. Methods* **B40/41**, 727–730.
 17. Yamashita, K., Umemoto, A., Grivas, S., Kato, S. & Sugimura, T. (1988) *Mutagenesis* **3**, 515–520.
 18. Turteltaub, K. W., Watkins, B. E., Vanderlaan, M. & Felton, J. S. (1990) *Carcinogenesis* **11**, 43–49.
 19. Randerath, K., Putman, K. L., Randerath, E., Mason, G., Kelley, M. & Safe, S. (1988) *Carcinogenesis* **9**, 2285–2289.
 20. Poland, A. & Glover, D. (1979) *Cancer Res.* **39**, 3341–3344.
 21. Abraham, K., Krowke, R. & Neubert, D. (1988) *Arch. Toxicol.* **62**, 359–368.
 22. Turesky, R. J., Aeschbacher, H. U., Malnoe, A. & Wurzner, H. P. (1988) *Food Chem. Toxicol.* **26**, 105–110.
 23. Dunn, B. P. & San, H. C. (1988) *Carcinogenesis* **9**, 1055–1060.
 24. Hsu, I.-C., Poirier, M. C., Yuspa, S. H., Grunberger, D., Weinstein, I. B., Yolken, R. H. & Harris, C. C. (1981) *Cancer Res.* **41**, 1091–1095.
 25. Harris, C. C., Vahakangas, K., Newman, M. J., Trivers, G. E., Shamsuddin, A., Sinopoli, N., Mann, D. L. & Wright, W. E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6672–6676.
 26. Dinh, T.-V., Uziel, M. & Morrison, A. L. (1987) *Appl. Spectrosc.* **41**, 605–610.
 27. Mohammed, G. B., Nazareth, A., Hayes, M. J., Giese, R. W. & Vouros, P. (1984) *J. Chromatogr.* **314**, 211–217.
 28. Dinh, T.-V. & Uziel, M. (1987) *Anal. Chem.* **59**, 1093–1095.
 29. Rahn, R. O., Change, S. S., Holland, J. M. & Shugart, L. R. (1982) *Biochem. Biophys. Res. Commun.* **109**, 262–268.
 30. Sanders, M. J., Cooper, R. S. & Small, G. J. (1985) *Anal. Chem.* **57**, 1148–1152.
 31. Vahakangas, K., Trivers, G., Rowe, M. & Harris, C. C. (1985) *Environ. Health Perspect.* **62**, 101–104.
 32. Litchfield, J. H. (1977) *Adv. Appl. Microbiol.* **22**, 266–304.
 33. Bassosi, R., Rossi, C., Trizzi, E. & Valensio, G. (1983) *Angew. Chem.* **95**, 55–57.
 34. United Nations Scientific Committee on the Effects of Atomic Radiation Report to the General Assembly (1988) *Sources, Effects and Risks of Ionizing Radiation* (United Nations, New York), Suppl. A/43/45, pp. 49–52.