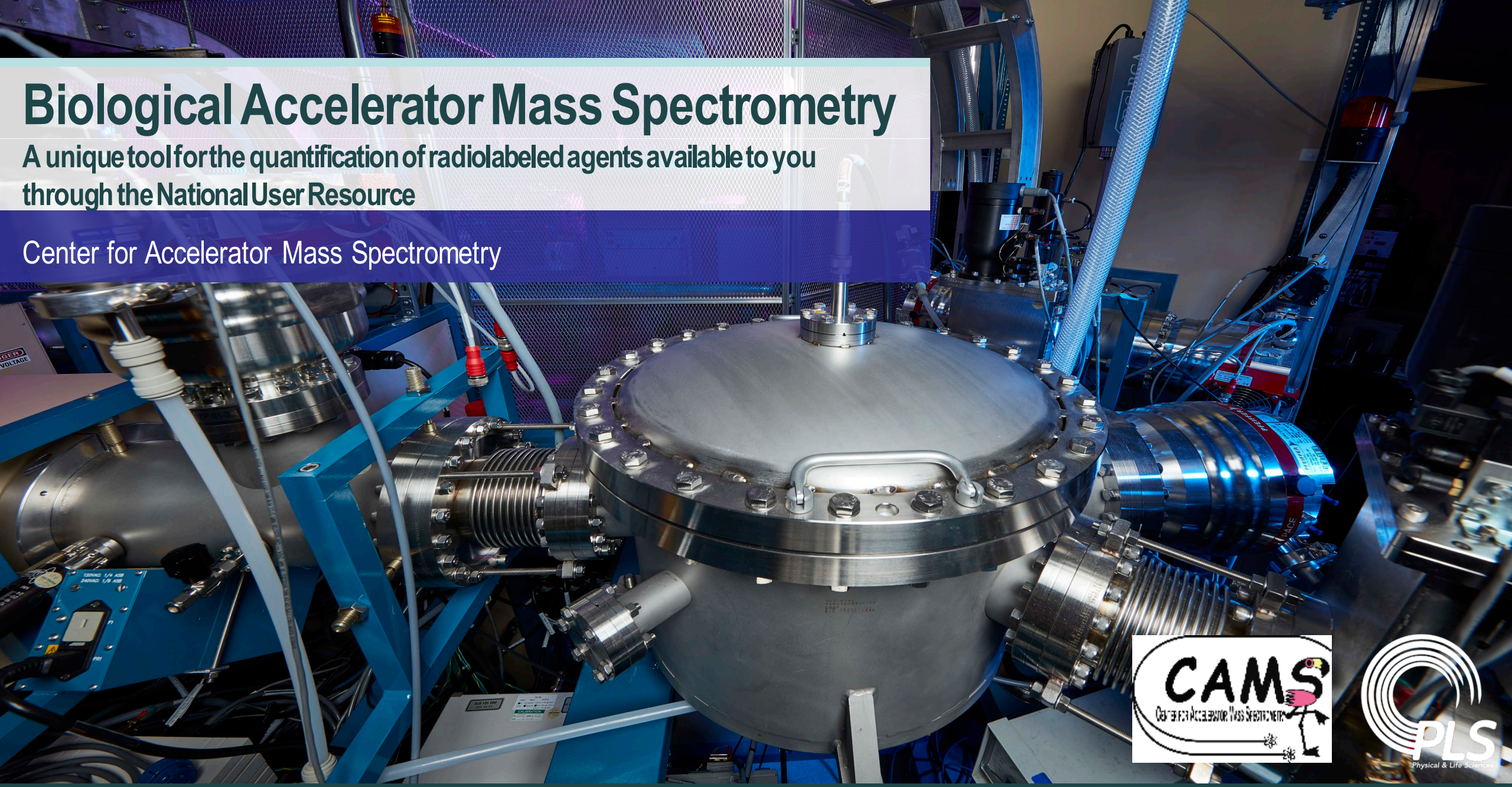
Biological Accelerator Mass Spectrometry

A unique tool for the quantification of radiolabeled agents available to you through the National User Resource

Center for Accelerator Mass Spectrometry



LLNL-PRES-823493

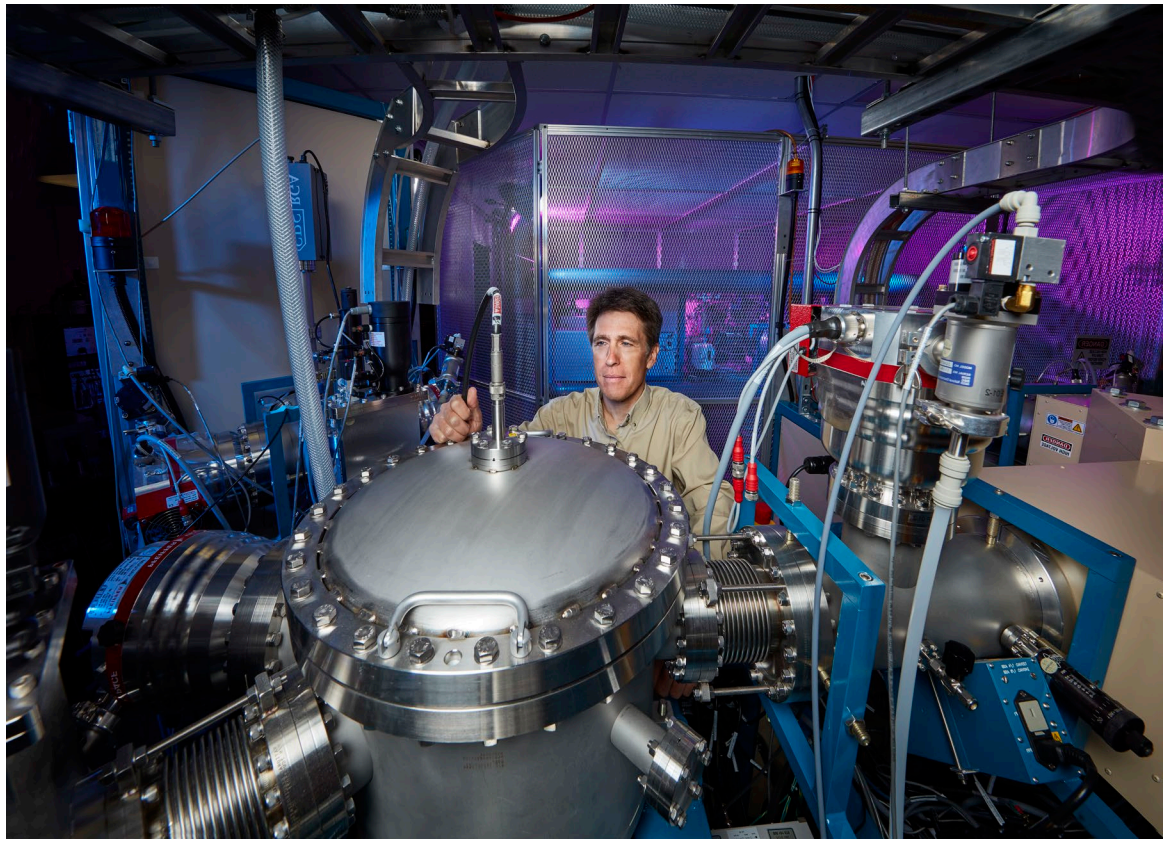
This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344. Lawrence Livermore National Security, LLC

Lawrence Livermore National Laboratory

Accelerator Mass Spectrometry (AMS) measures isotope ratios with high selectivity, sensitivity, and precision

Quantitative and independent of physiochemical properties, this mass spectrometric method:

- Uses a linear acceleration stage to analyze ions at extraordinarily high energies
- Counts atoms of long-lived radioisotopes—carbon-14, or ^{14}C, is most common—to measure isotope ratios
- Enables analysis of attomole quantities of agent, metabolite, or adducts in milligram or microgram-sized samples with 2%–5% precision



AMS differs from conventional mass spectrometry (MS), with unique benefits and challenges

AMS

- Measures radioisotope tracers
- Extreme sensitivity
- Measures radioisotope-labeled chemicals; provides signature for analytes
- Solid or liquid measurement
- Quantitative technique
- Detects isotopes; provides no structural information
- Succeeds at tracer experiments with unrivaled specificity and sensitivity
- Analytes containing tracers are detected even if unknown

MS

- Measures multiple analytes
- High sensitivity
- Measures unlabeled or stable isotope-labeled samples
- Solid, liquid, or gas measurement
- Quantitative or qualitative technique
- Provides structural information; fragment analysis is possible
- Isotope dilution and natural abundance limit tracer experiments
- Unknown analyte tracing is difficult because prior structural knowledge is needed

AMS excels in research that requires radiolabeled agents

The technology is ideal for:

- Studies that require applied doses to remain low
  - Dynamic range: 0.6-600 fCi/mg carbon
  - Limit of Detection: 30 aCi/mg carbon; 3 attomoles agent per sample at 5% precision
- Studies of agents that can't be produced or used in high specific activity or where samples are very small

AMS has a wide range of applications, including:

- Dose-response; mass balance; fate and distribution; and pharmacokinetic and metabolic pathway analysis in experimental cell/animal models and human subjects
- Measurement of absolute bioavailability, pharmacokinetics, protein/nucleic acid binding, and metabolic parameters
- Cell turnover in humans

In the last 20 years, AMS has evolved as an innovative biomedical tool

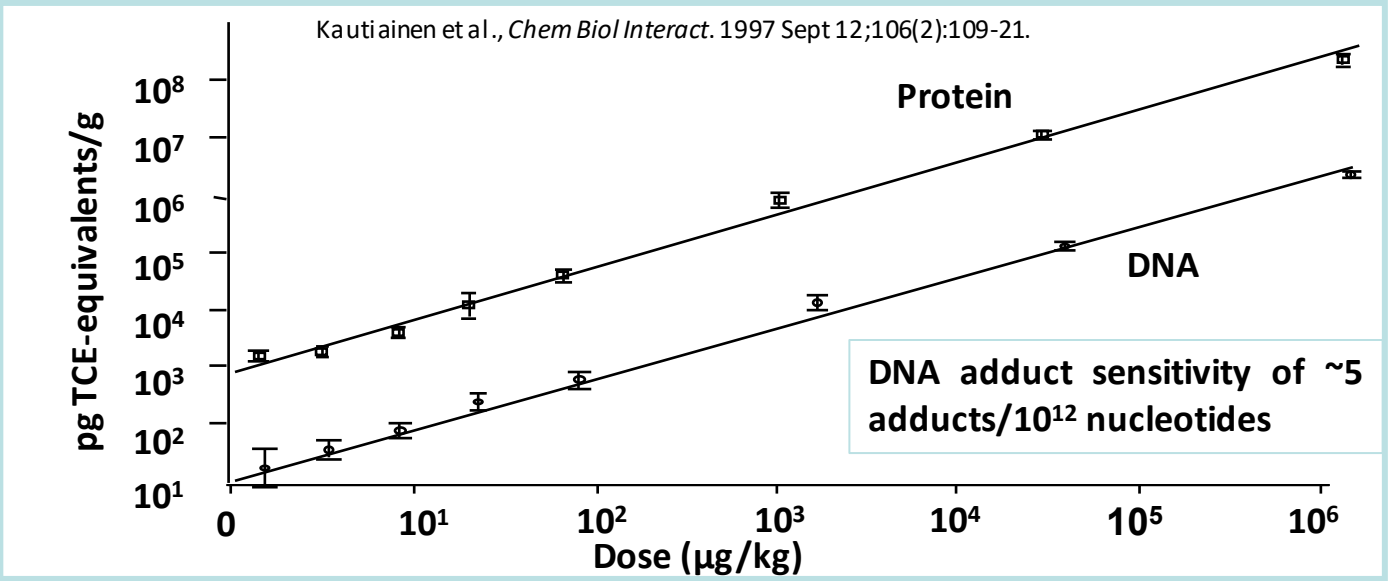
Data developed with AMS technology has helped:

- Improve risk assessment of toxicants
- Address safety and efficacy considerations for therapeutic entities
- Deepen understanding of xenobiotic and intermediary metabolism
- Illuminate the interactions between critical molecular pathways
- Strengthen efforts to model and predict various metabolic and biological states
- Examine the turnover of long-lived cells
- Provide quantitative comparisons of metabolic/dosimetric parameters between species

AMS's sensitivity and dynamic range improves risk assessment of low-dose chemical exposures

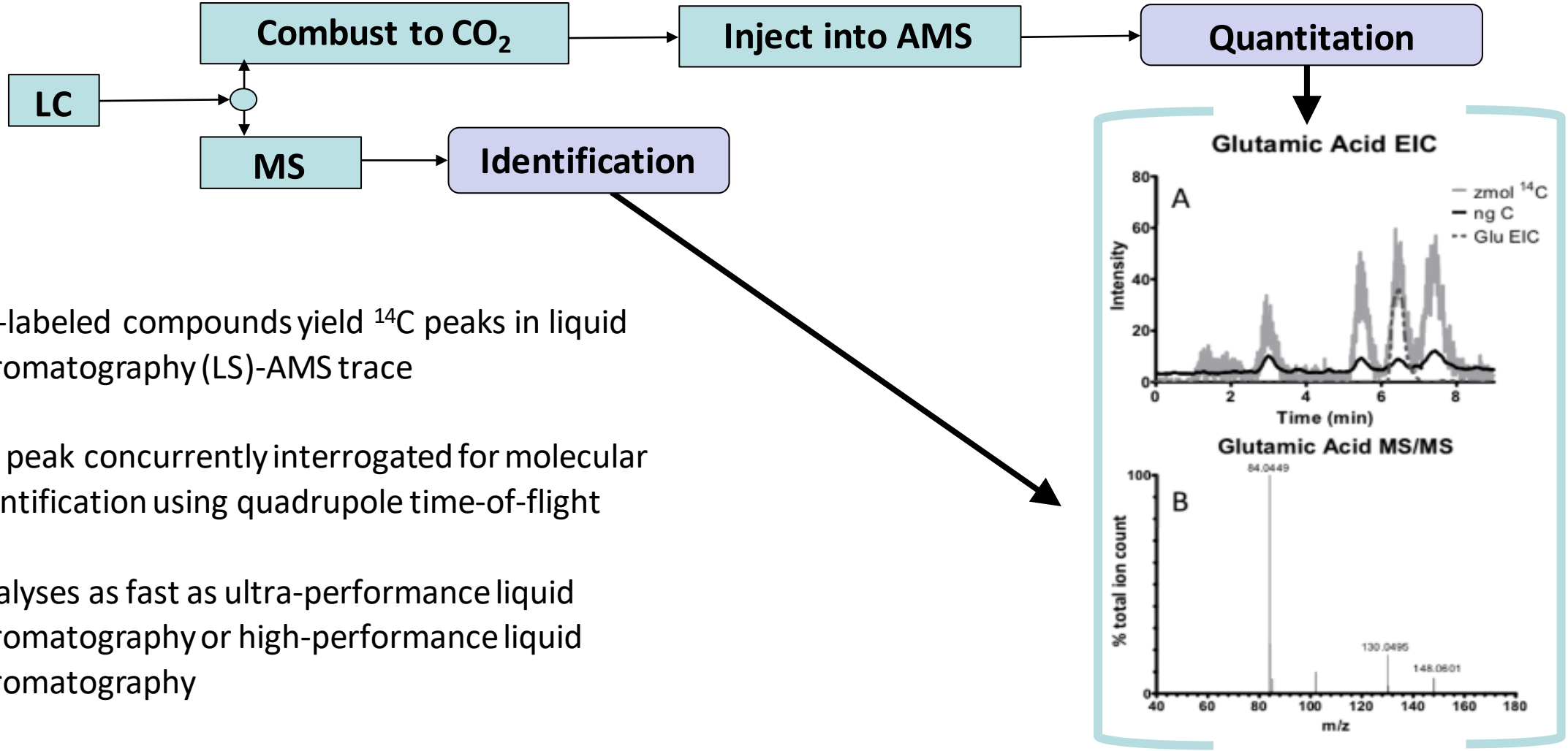
Example: Trichloroethylene (TCE) macromolecular binding to elucidate the mechanism of cancer formation

- TCE is a widely-used industrial chemical and low-level contaminant of surface and ground water
- It is weakly mutagenic in several test systems and carcinogenic in rodents
- The mechanism for cancer production is unknown



AMS studies revealed that protein and DNA adduct formation may play a role in the formation of certain tumors.

Parallel accelerator and molecular mass spectrometry analysis enables analyte identification and quantification



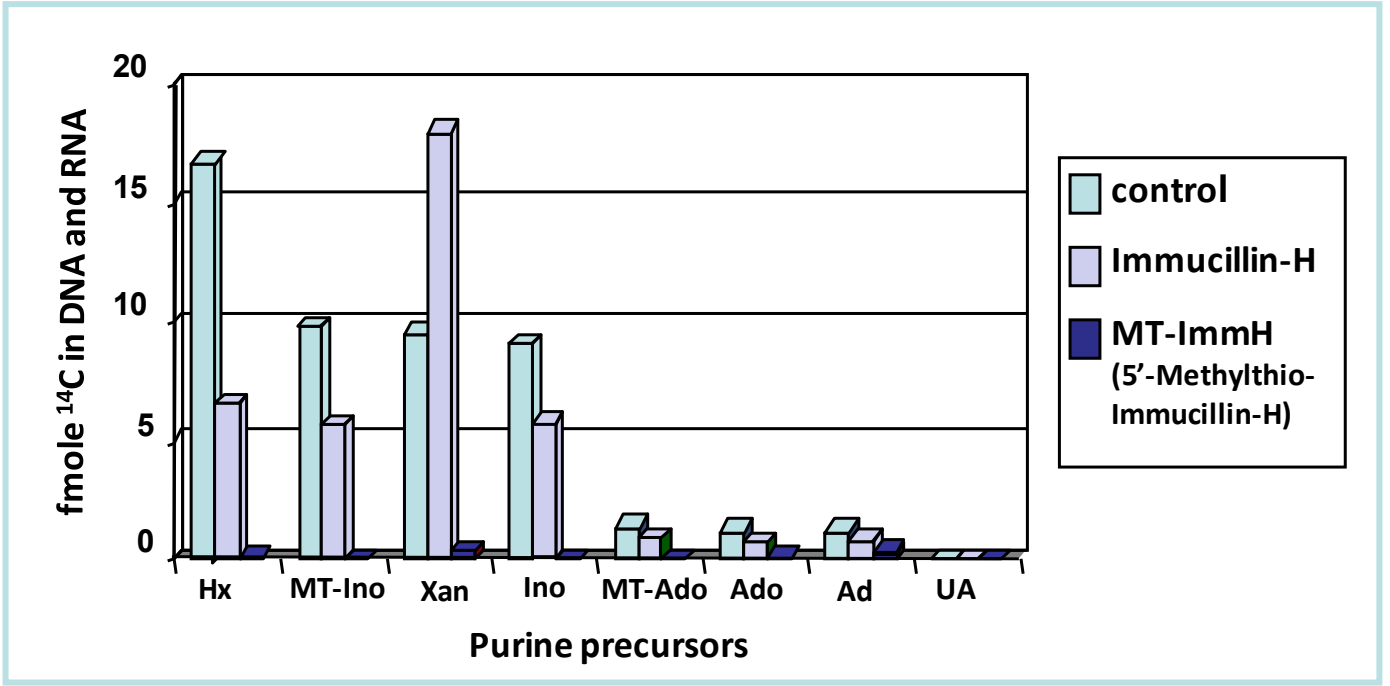
- ^{14}C-labeled compounds yield ^{14}C peaks in liquid chromatography (LS)-AMS trace
- ^{14}C peak concurrently interrogated for molecular identification using quadrupole time-of-flight
- Analyses as fast as ultra-performance liquid chromatography or high-performance liquid chromatography

Malfatti et al., *Toxics*. 2019;7(2):27.
Ognibene et al., *Nucl Instr. & Meth. B.* 2015;361:173-77.

AMS can exploit a labeled precursor to define and quantify metabolic pathways

Example: Quantifying DNA/RNA synthesis in malaria to develop anti-malarials

- *P. falciparum* requires purine, an organic compound, for DNA and RNA synthesis. But the parasite lacks *de-novo* purine synthesis, so purines must be salvaged from the host.
- Researchers wondered: Would immucillins—compounds that inhibit purine-salvaging enzymes—block purine recycling in malaria?
- To find out, they assessed DNA and RNA production from recycled purines by adding ^{14}C-labeled purine precursors to malaria containing blood cultures.



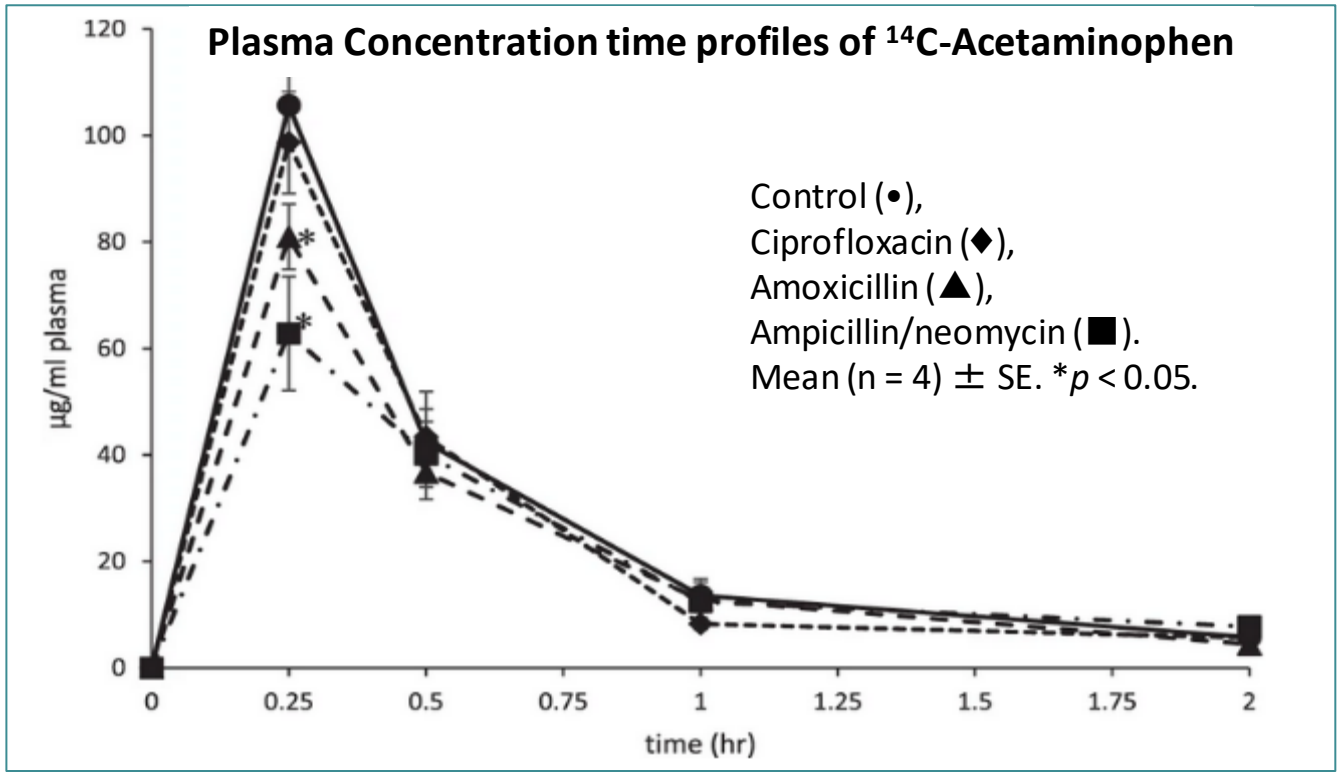
Ting et al., *Journal of Biological Chemistry*. 2005;280:9547-9554

Researchers discovered that immucillins block purine recycling in malaria, kill malaria in culture, and may find application as an anti-malarial.

The sensitivity of AMS enables the precise study of biological interactions

Example: Manipulating the gut microbiome alters acetaminophen biodisposition in mice

- In this experiment, mice received 10-day oral exposure to antibiotics before a single, oral dose (100 mg/kg) of ^{14}C-acetaminophen
- Analysis of gut microbe composition revealed changes in microbe content were associated with changes in acetaminophen absorption and distribution
- Urinary metabolite profiles showed decreases in acetaminophen-sulfate metabolite levels

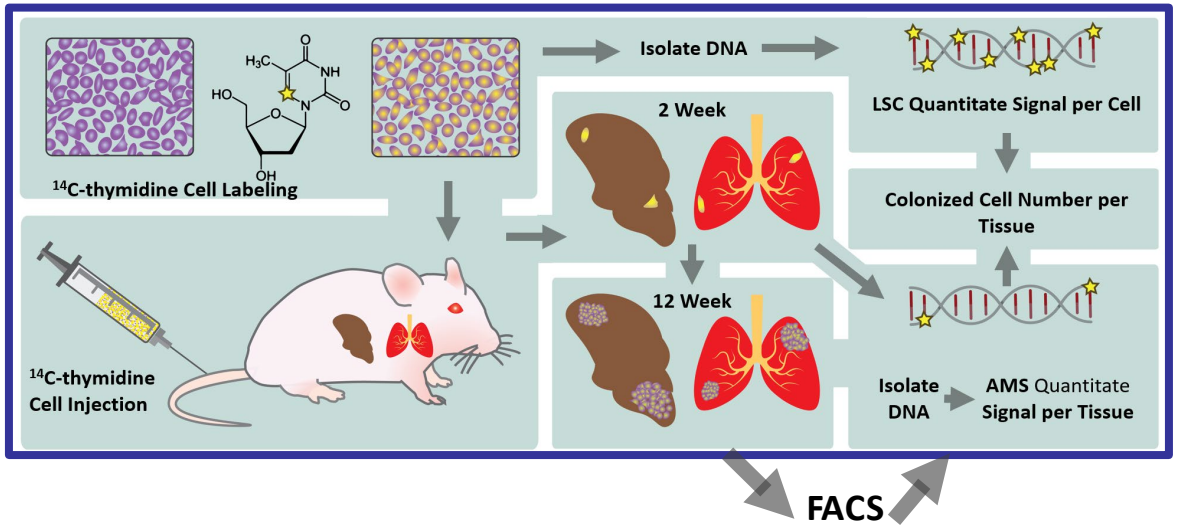


Malfatti et al., *Scientific Reports*. 2020;10:4571.

Exposure to amoxicillin or ampicillin/neomycin can alter the biodisposition of acetaminophen. These alterations could be due to changes in gut microbiome composition.

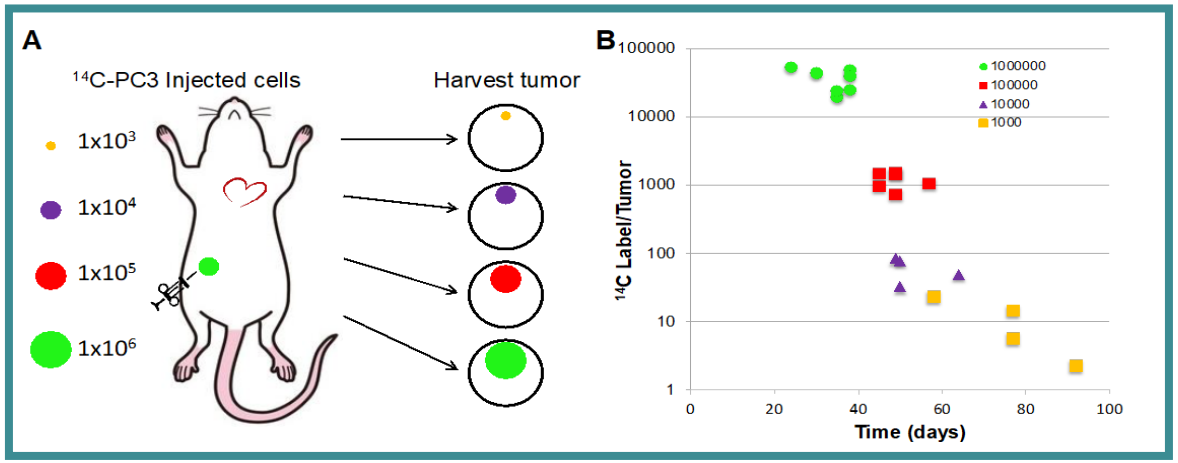
AMS can be used to track highly ^{14}C-labeled cells in animal models

Example: Using ^{14}C-labeled cancer cells to assess their potential to spread



In this study, scientists cultured cells with ^{14}C-thymidine to achieve single-cell resolution

- Single-cell resolution:
  - ✓ Detects tumors derived from a few metastatic cells
  - ✓ Determines whether cancer cells are dormant in tissues



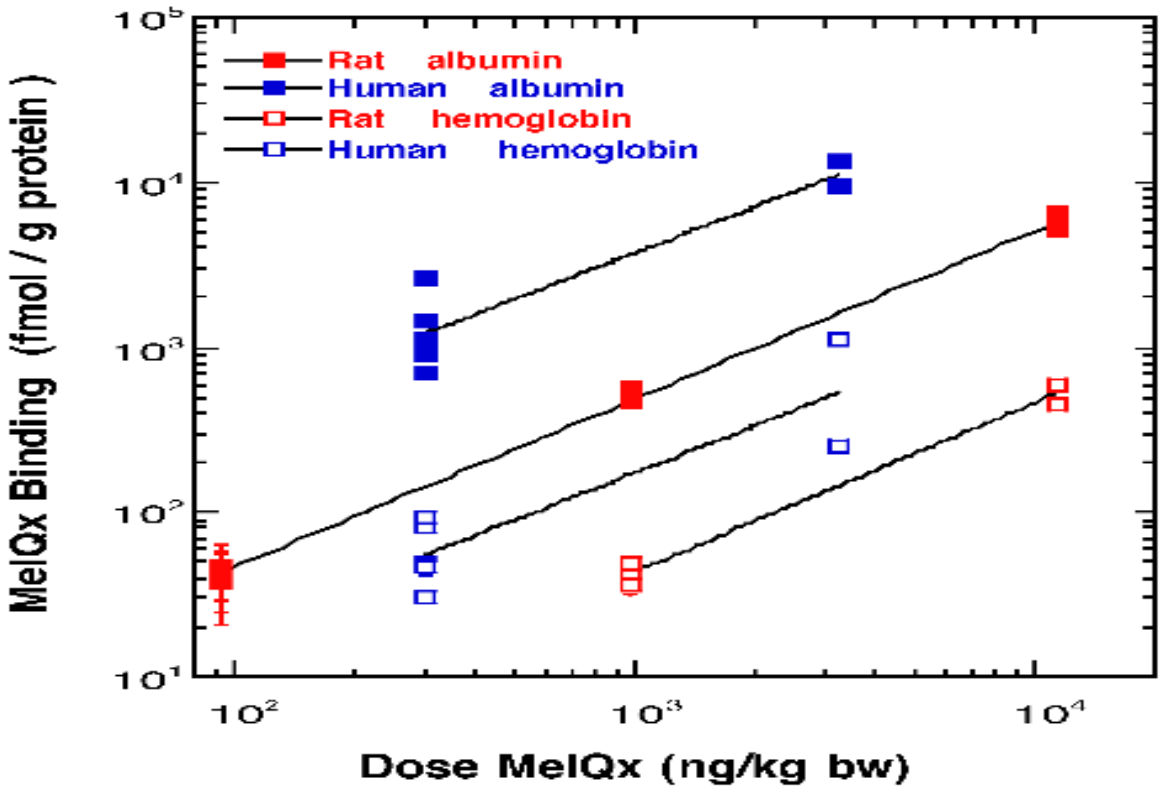
In trace probe metastasis with highly-labeled prostate cancer cells, the scientists:

- Injected different numbers of cells into mice and harvested the eventual tumors
- Calculated the number of cells that led to the tumors

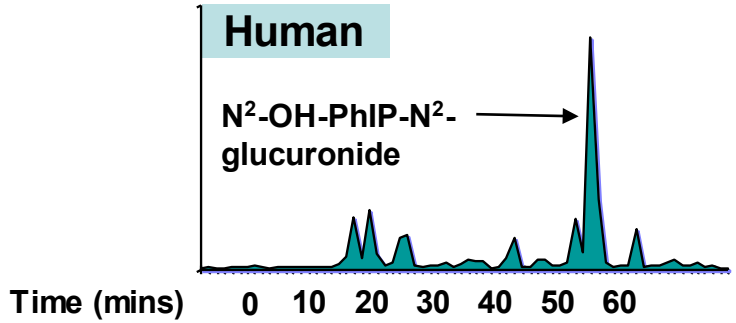
Hum et al., *Scientific Reports*. 2018;8:15013.

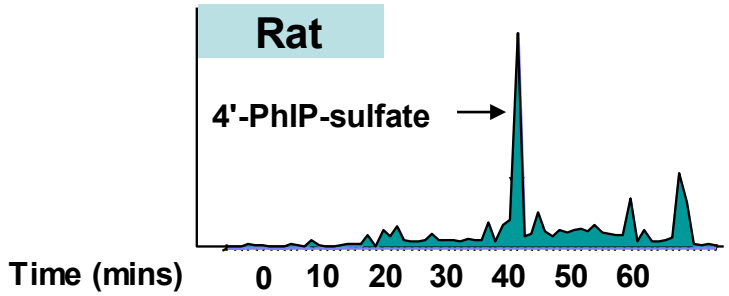
AMS facilitates human–animal comparisons

Example: Dosing human subjects and rodents with the food mutagen MeIQx



Dingley et al., *Cancer Epidemiol. & Biomarkers*. 1999;8:507.



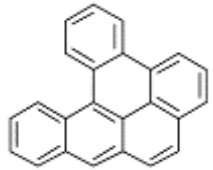


Humans produce more bioactive metabolites than rodents.

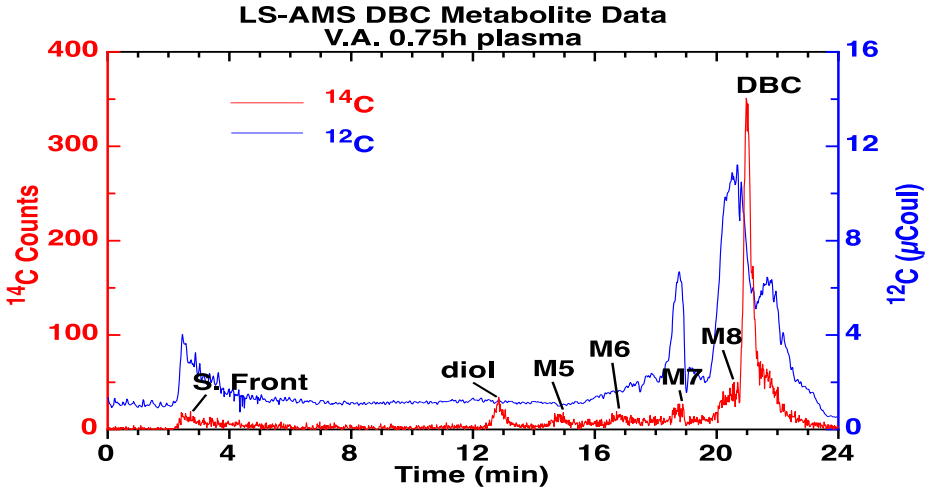
Researchers found that the amount of MeIQx bound to circulating proteins was lower and more reproducible in rats than in humans.

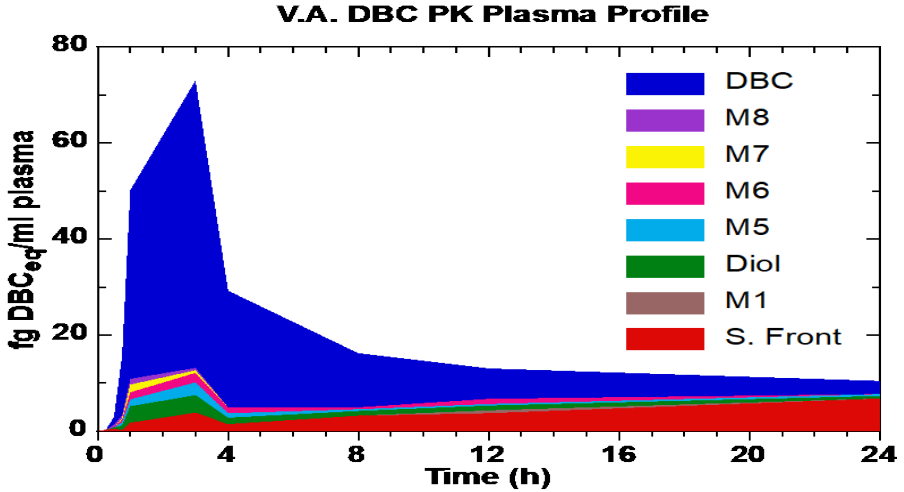
AMS can measure environmentally-relevant doses of carcinogens with *de minimis* health risk to human volunteers

Example: Polycyclic aromatic hydrocarbons (PAHs)



- PAHs, such as dibenzo chrysene (DBC), are ubiquitous environmental pollutants
- DBC is an International Agency for Research on Cancer (IARC) class-2A carcinogen
- In this study, human volunteers were dosed orally with ^{14}C-DBC (29 ng, or roughly 28% of the average daily intake)
- Plasma and urine metabolites were quantified with unprecedented sensitivity





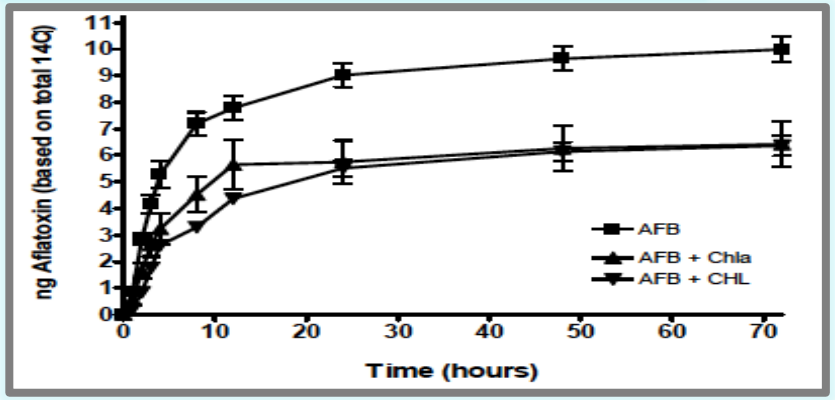
E.P. Madeen et al., *Chem. Res. Tox.*, 2016;29:1641

This was the first controlled study of the *in vivo* human metabolism of an IARC class-2A probable human carcinogen.

AMS has sufficient sensitivity to study, directly in humans, how diet affects the bioavailability of toxins

Example: A study showed that chlorophyll (Chla) and chlorophyllin (CHL) co-consumption limit the bioavailability of ingested aflatoxin (AFB1) in humans

- AFB1 is a potent, naturally-occurring carcinogenic mycotoxin associated with mold foods, including corn, cottonseed, peanuts, and milk
- Chla and CHL reduce AFB1 bioavailability in animal models
- In this experiment, four volunteers ingested a 5 nCi dose of ^{14}C-AFB1, a fraction of that found in a peanut butter sandwich
- Researchers used AMS to measure the concentration of ^{14}C in blood and urine
- Subsequently, the volunteers were given 150 mg of Chla or CHL simultaneously with the same dose of ^{14}C- AFB1, and the measurement protocol was repeated
- Chla and CHL treatment each significantly impeded AFB1 absorption

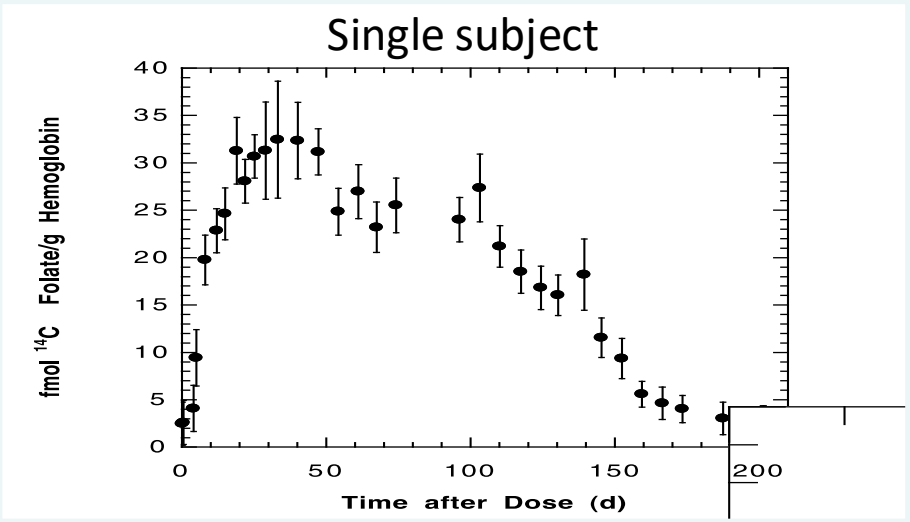


Jubert et al., *Cancer Prevention Research*. 2009;2(12):1015-1022.

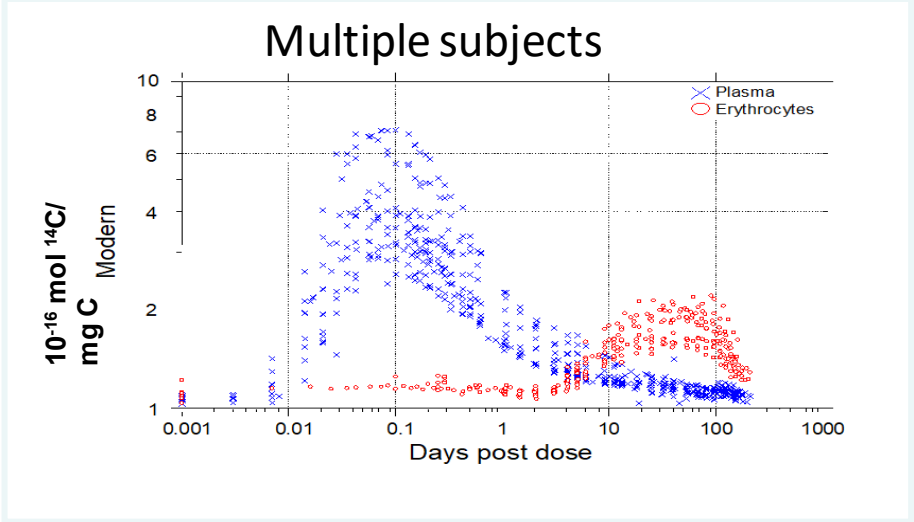
Long-term studies of naturally-occurring compounds in humans is a central advantage of AMS

Example: Dynamics and kinetics of folic acid metabolism

Folate is required for health, growth, and development but its metabolism is poorly understood.



Folate metabolism model constructed from data



Dose of ^{14}C-folic acid (35 mg, 1/12 RDA) ingested and followed for 200 days

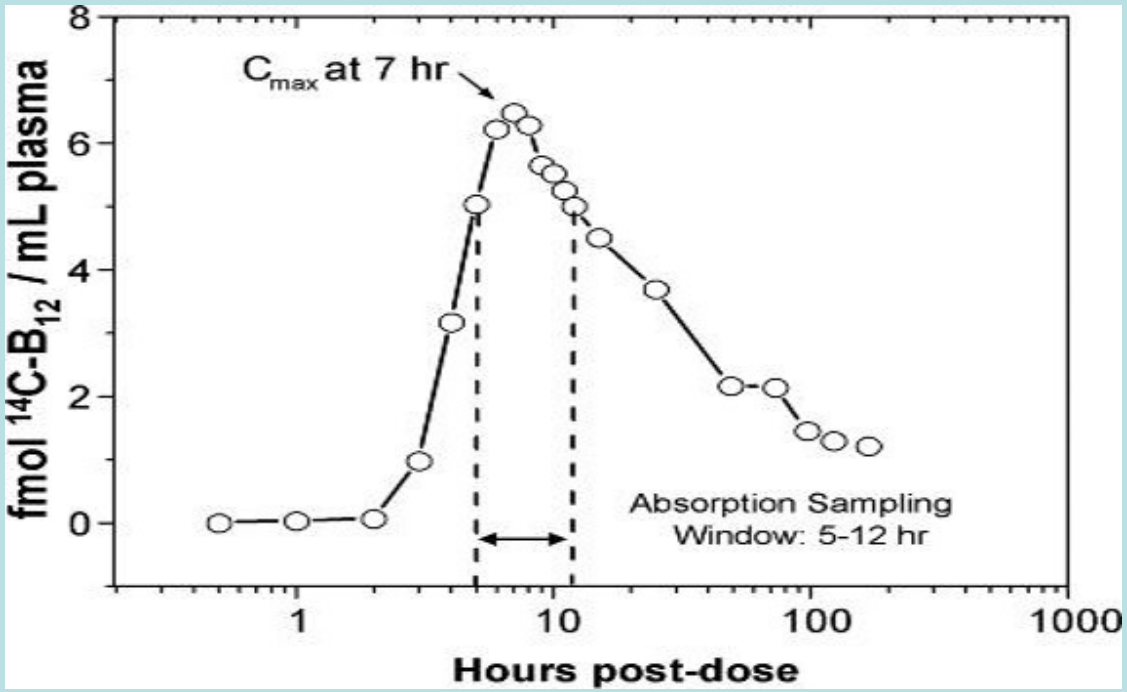
Lin et al., *American Journal of Clinical Nutrition*. 2004;8:680-691.

Folic acid metabolism revealed red blood cell count lifetime and a possible link between hepatitis and birth defects. People with liver disease may need much more folate in their diets.

High sensitivity makes AMS a powerful tool for the diagnosis of nutritional and dietary deficiencies

Example: Vitamin B_{12} deficiency

- Vitamin B_{12} deficiency due to malabsorption, pernicious anemia, and neurological dysfunction afflicts approximately one million Americans over the age of 65
- The current assay (Schilling test) is semiquantitative and has methodological and practical problems
- A microdose of ^{14}C-labeled B_{12} enables a simple test requiring one drop of blood for B_{12} absorption

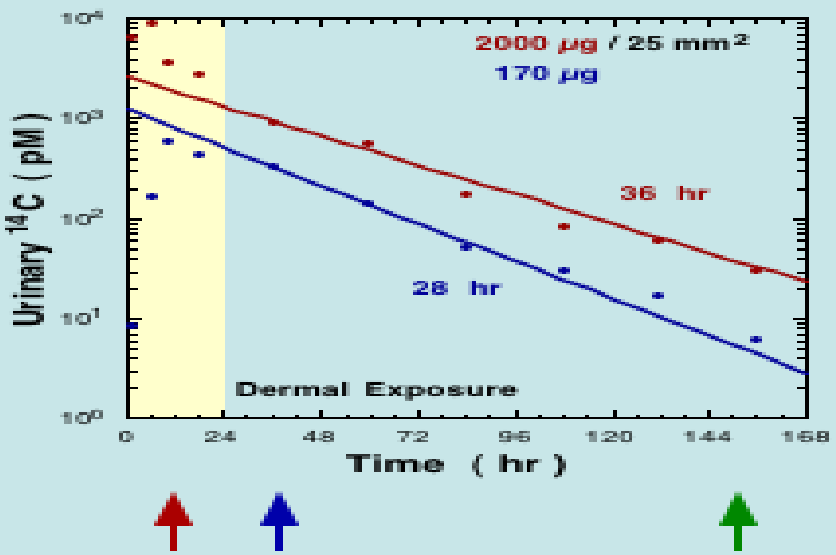


Carkeet et al., *Proc. Natl. Acad. Sci. USA. 2006;*103:5694-5699

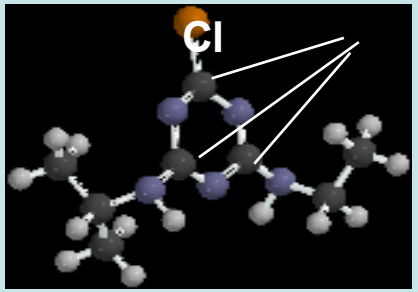
The combined use of ^{14}C-labeled B_{12} and AMS detection yields an improved method for studying the underlying causes of B_{12}-uptake disorders.

AMS is a powerfully sensitive tool for biomarker identification

Example: Determining the biomarkers of dermal exposure to atrazine—a widely-used herbicide—in urine



HPLC-AMS at three time points



^{14}C Atoms

In this study, researchers:

- Applied ^{14}C-atrazine to skin for 24 hours (0.167 mg, 6.45 mCi)
- Collected urine pre-dose and post-dose for seven days
- Separated metabolites by high-performance liquid chromatography (HPLC) to determine distribution
- Quantified by AMS

Gilman et al., *Anal. Chem.* 1998; 70(16):3463-3469.

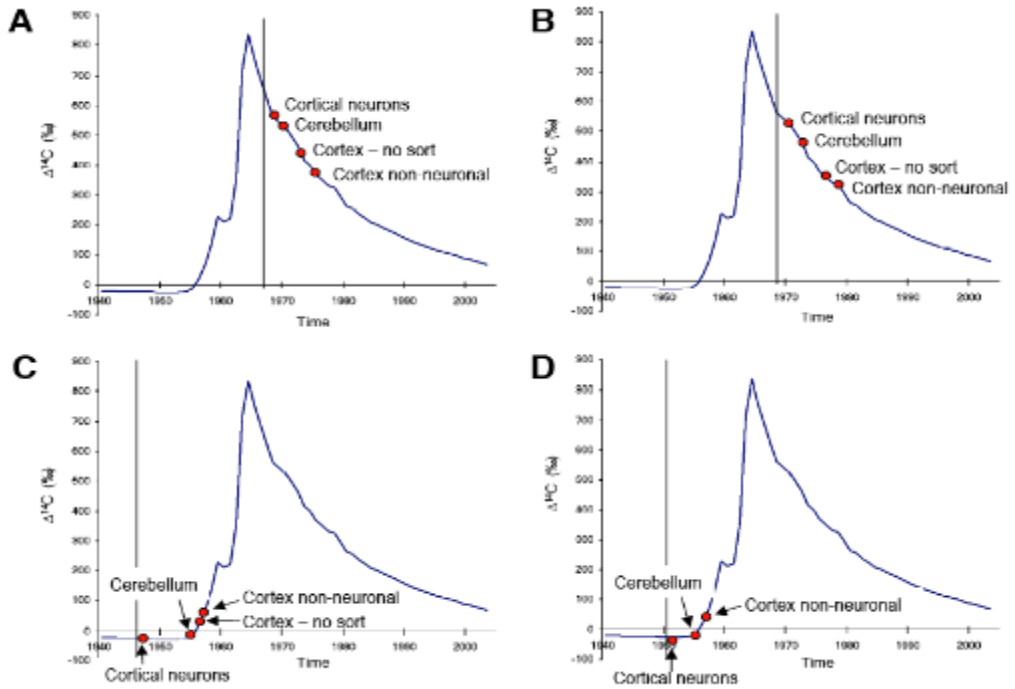
When they compared the analytical performance of AMS and liquid scintillation counting, researchers found that AMS provided superior concentration and mass detection limits for their samples.

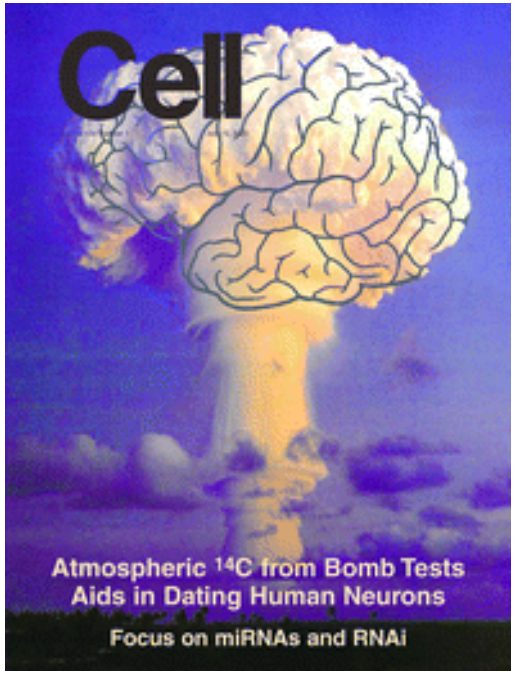
AMS can be used to exploit the bomb pulse of ^{14}C to examine the turnover of long-lived cells

Example: Dating and determining human neuron DNA

Above-ground nuclear weapons testing produced a pulse of atmospheric ^{14}C that labeled all living things

- The level of ^{14}C in genomic DNA is a timestamp of synthesis and can be used to measure cell population age
- Fluorescence-activated cell sorting can be used to sort for specific cell types
- Models of cell turnover can be constructed from cell population ages





Spalding et al., *Cell*. 2005;122:133-143

In this study to determine the age of cortical cells in the human brain, scientists found that cortical neurons are as old as the individual and neurogenesis does not take place in the cerebellum.

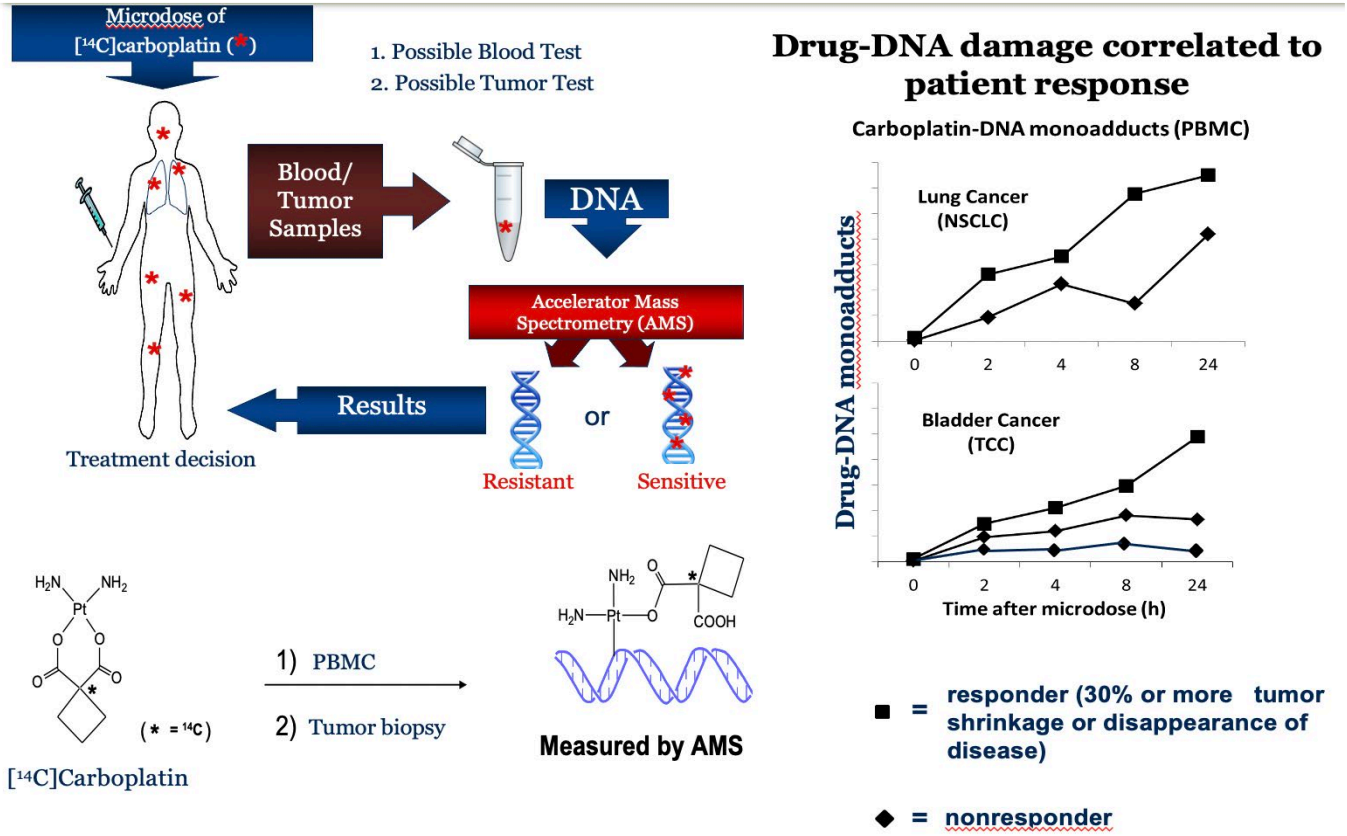
The National User Resource for Biological AMS has sparked a growing interest in using the technology for pharmaceutical development through microdosing

- Microdosing delivers sub-pharmacological levels to healthy volunteers for ADME studies
- A microdose is 1/100 the level calculated to yield a pharmacological effect, or 100 micrograms, whichever is lower
- The dosage yields a cellular response but not a whole-body effect
- AMS has ideal sensitivity for the measurement of ^{14}C in early human studies
  - Microtracer studies
  - Lightly-labeled doses for metabolism studies (MIST)
- Using low microdoses of drug candidates lowers the risk of adverse effects in humans
- The technique is particularly valuable in cases where human *in vivo* performance is difficult to predict from *in vitro* data or animal studies
- Regulatory agencies (FDA and EMEA) have accepted the microdosing approach
  - Reduced bulk drug requirement
  - Reduced formulation efforts
  - Abbreviated safety package
  - Decreased time and cost to FIH studies
  - Aid in the selection of best drug candidates

AMS has the potential to predict chemotherapeutic outcome for individualized therapy

Example: Platinum (Pt)-based chemotherapy

- Pt-based chemotherapy is used to treat various cancers
- Some patients are more resistant to the effects of Pt-based drugs than others
- DNA damage is a critical step in cancer cell response to Pt-based chemotherapy
- Low levels of Pt-induced DNA damage maybe predictive of chemotherapy resistance
- AMS can detect DNA damage and repair using ^{14}C-carboplatin



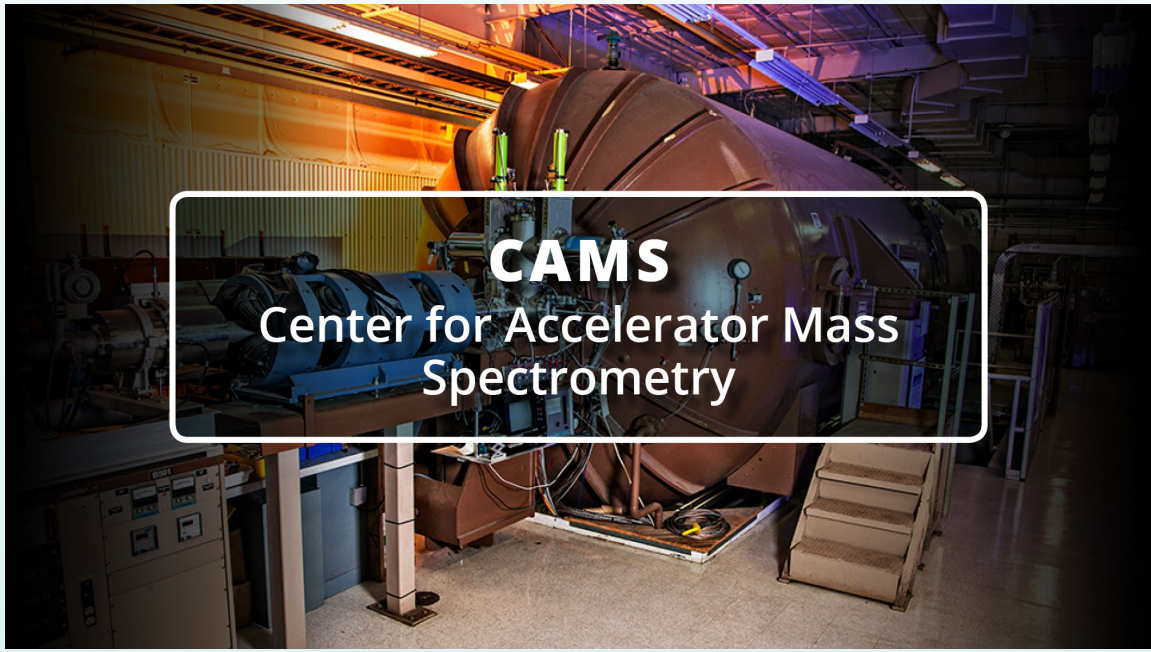
Zimmerman, et al. *Mol Cancer Ther.* **2017;**16(2):3875-3876. Wang, et **al.,** *Int J Cancer.* 2017;141:604-613

Low levels of DNA damage induced by carboplatin microdoses in peripheral blood mononuclear cells predict the levels of damage induced by therapeutic carboplatin.

CAMS combines state-of-the-art tools and expertise to address scientific challenges important to LLNL, external communities, and the nation

Globally recognized, CAMS is at the forefront of AMS technology with competencies in many fields

- Biomedical studies
- Forensic studies
- Terrestrial carbon cycle
- Atmospheric chemistry
- Earth system processes
- Climate change/paleoclimate and geochronology
- Environmental radiochemistry
- Radiation damage/materials modification
- Nuclear science/nuclear chemistry



CAMS is home to the National User Resource for Biological Accelerator Mass Spectrometry (BioAMS)

This NIH National Center (NIH grant R24GM137748) is the only user resource of its kind in the United States.

- With over thirty years of expertise in the development and application of AMS for biomedical sciences, the user resource continues to strengthen AMS analysis
- Our dedicated staff make leading AMS technology available to biomedical researchers
- Access is free for nearly all investigators
- For more information, contact:

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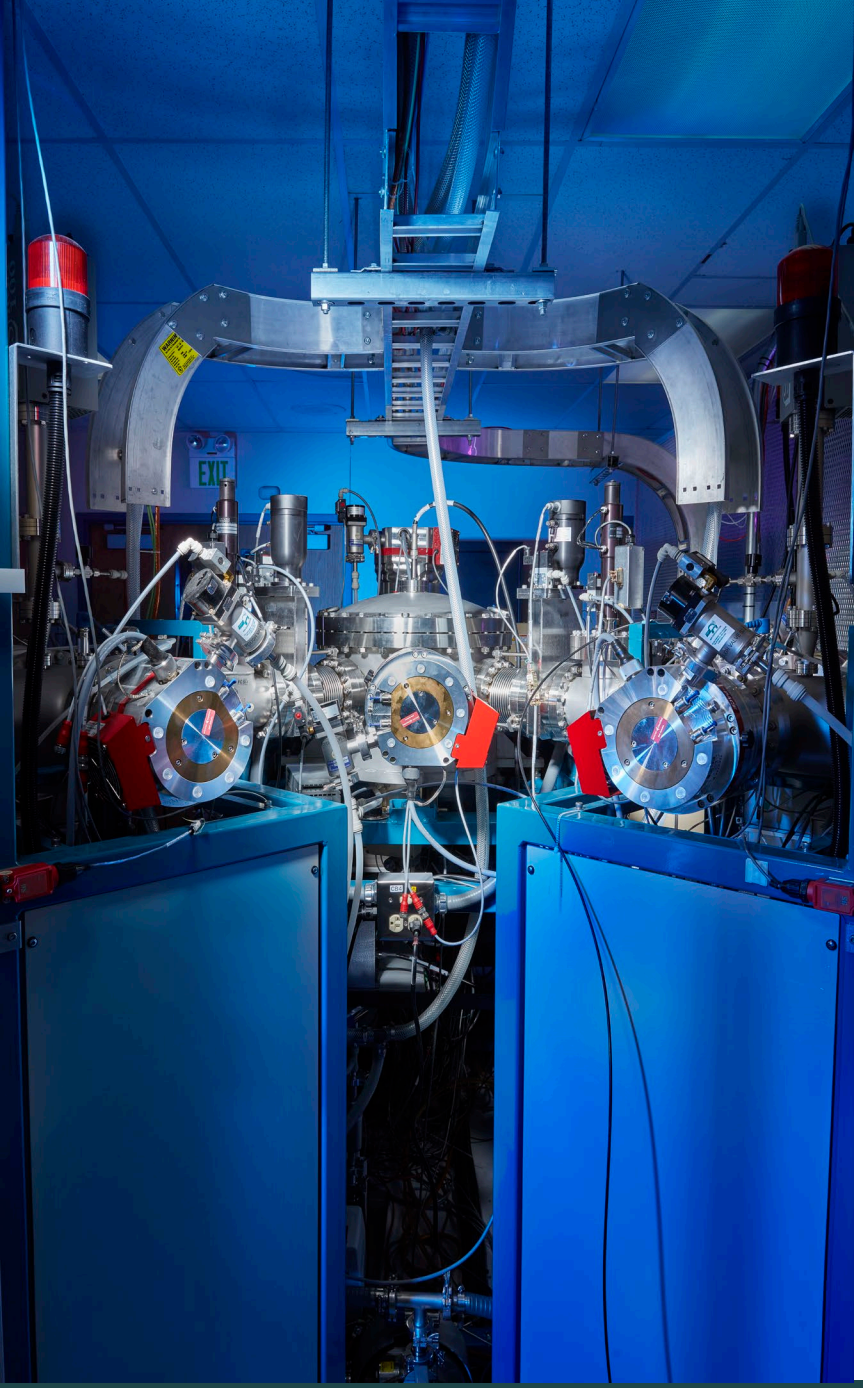

In summary, Accelerator Mass Spectrometry quantifies extremely low concentrations of radioisotope-labeled chemicals

- AMS's niche is research that requires radiolabeled agents
  - Situations where applied doses need to remain low
  - Studies of agents that can't be produced or used in high-specific activity or where samples are very small

- AMS enables a deeper understanding of the origins of human health concerns by:
  - Quantifying pharmacokinetics and other molecular endpoints directly in humans
  - Offering the ability to conduct quantitative studies using biologics such as proteins or lipids
  - Facilitating more relevant studies of metabolic pathways in health and human disease with lower, more biologically-relevant concentrations of metabolic substrates in cells and intact organisms