Metabolite Quantitation and Biomarker Development Using Accelerator Mass Spectrometry

Bruce Buchholz
Center for Accelerator Mass Spectrometry
Lawrence Livermore National Laboratory

May 22, 2017

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-PRES-730881.

Outline

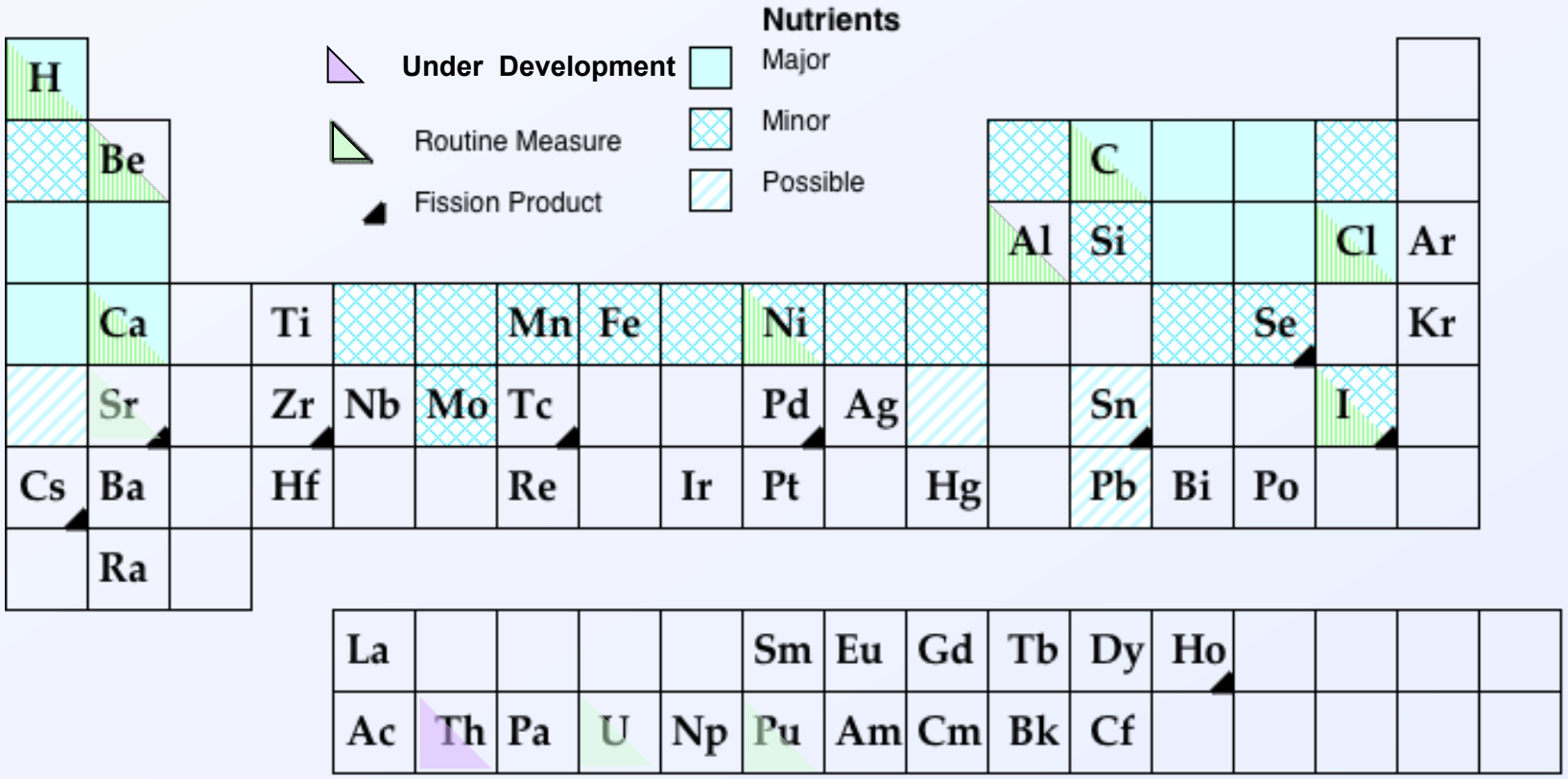
- What is accelerator mass spectrometry (AMS)?
- What can AMS do and how can I use it?
- Applications of ^{14}C-AMS in biological tracing
  - Food carcinogens and toxins
  - Pesticide metabolism and biomarker development
  - PAH ingestion
- Future trends

The National Center for Biomedical AMS was established in 1999

http://bioams.llnl.gov/

- Funded by the National Institutes of Health (NIGMS)
- Based on PI/ Collaborator/User model
- Technology advances
- Scientific collaborations
- Service
- UC Davis SRP
- Oregon State SRP

AMS Counts Atoms of Long-Lived Radioisotopes (10-10^7 y)

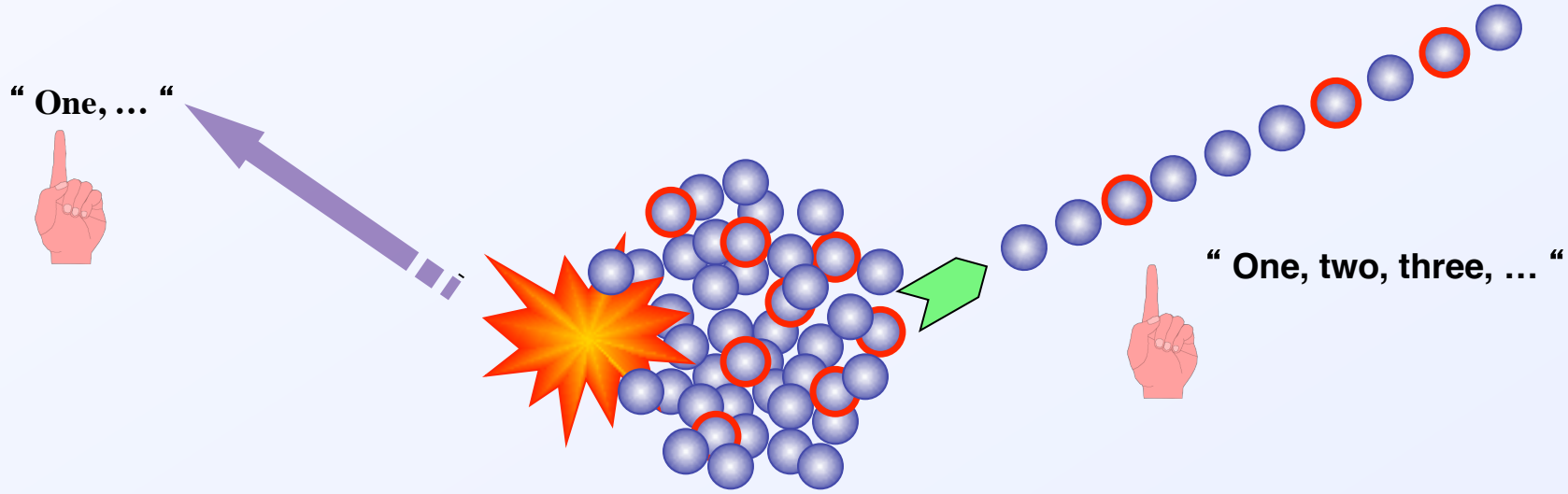


Carbon-14 or ^{14}C is the most commonly used isotope in AMS

Counting radioactivity is inefficient: mass spectrometry counts ≥1% of isotopes present

Decay Counting

AMS



$dN / dt = - N / \tau$

$dt = dN / N * \tau$

Counting 0.1% of ^{14}C in a sample

$dt = 0.1\% * \tau = 0.1\% * 8330\ y = \underline{8.3\ y}$

Count atoms, not decays

10,000 ^{14}C can be counted in <30 sec for contemporary sample

Sensitivity is attomole

*Figure courtesy of John Vogel

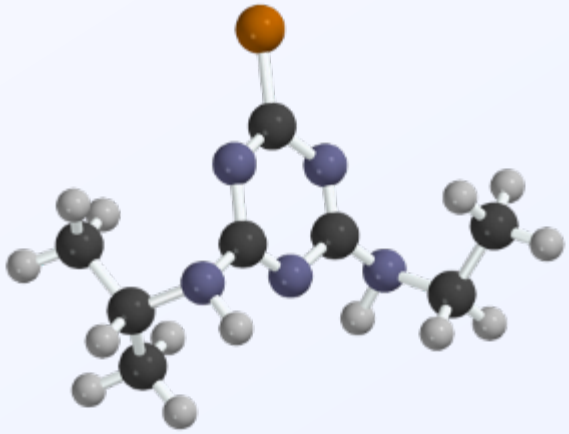
Use isotope label to follow molecules through metabolic processes

- Metabolism alters molecules
- Need intrinsic isotope label to preserve chemical behavior and trace molecule
- All isotopes of carbon follow the same chemistry
  - ^{12}C = 98.9%
  - ^{13}C = 1.1%
  - $^{14}C = 1.2 \times 10^{-12}$
- Exploit the rarity of ^{14}C to trace carbon atoms

Treat long-lived radioisotope like a stable isotope

Why use ^{14}C instead of stable ^{13}C?

Consider tracing atrazine



- Formula weight = 216
- 8 carbon atoms, 3 carbon atoms in stable triazine ring positions
- Obtain labeled atrazine
  - ^{13}C - atrazine with all ring carbon atoms ^{13}C
  - ^{14}C - atrazine with 1/10 molecules containing 1 ^{14}C (specific activity = 6.2 Ci/mol)

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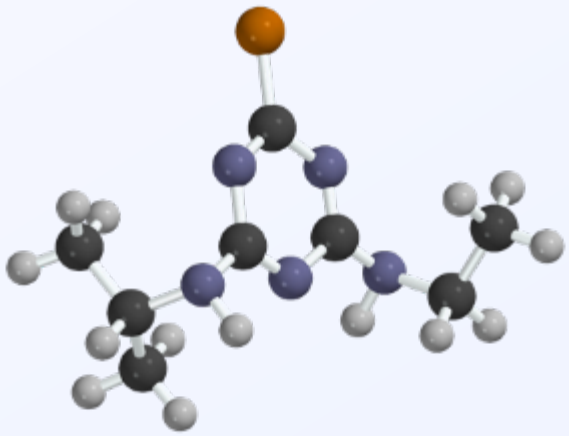

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Quantify metabolite levels in urine (~1 ppm)

[^{13}C] increases by 0.03 %

[^{14}C] increases by 10^5

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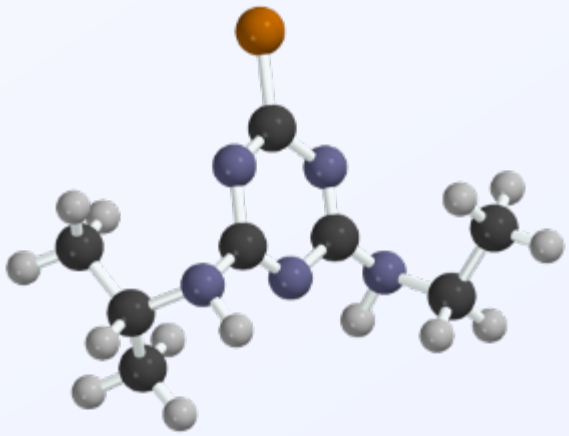

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Low Natural Concentration of ^{14}C Yields Large Signal-to-Noise

Can easily measure ppb to pptr concentrations of tracer compounds

Small, well-defined samples are traditionally converted to graphite for analysis

Tissue needed for AMS analysis (1 mg Carbon)

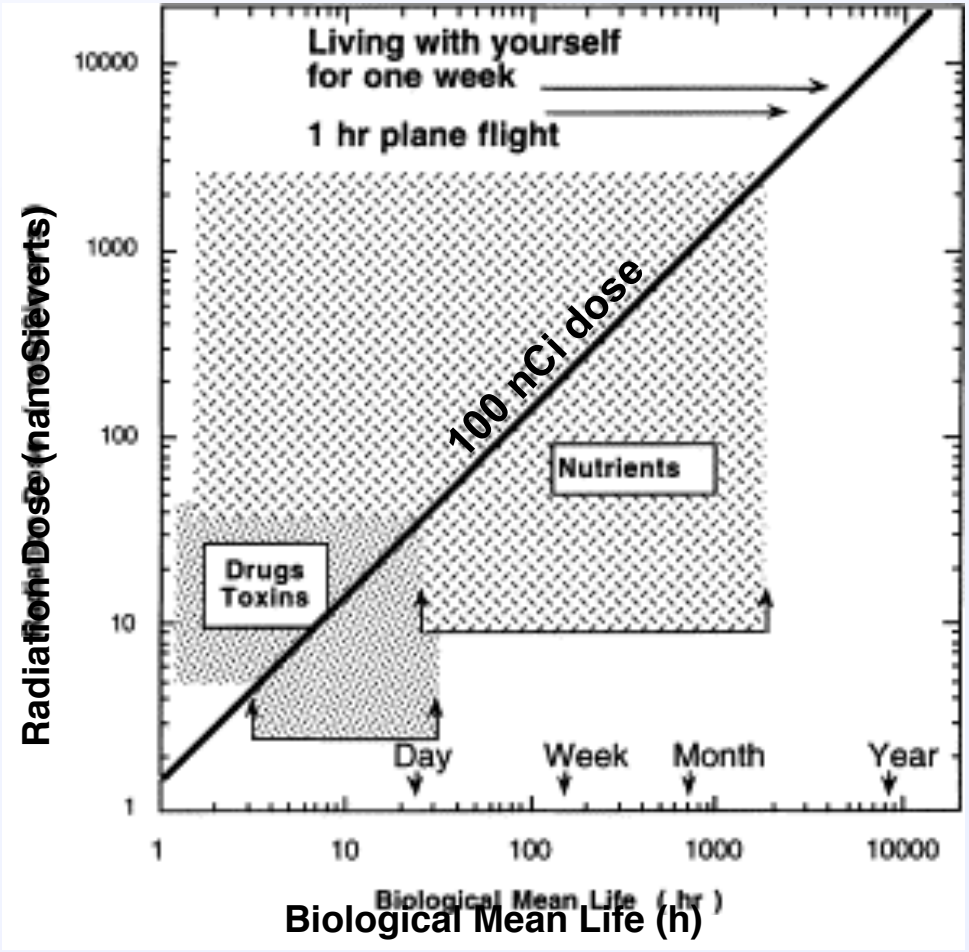
Tissue	Amount
Plasma	25 µl
RBCs	6 µl
Blood	10 µl
Urine	200 µl
Feces	10 mg
Cells	5 mg
HPLC fractions	Add carrier

Sample prep. process destroys chemical information

- Small blood volume allows frequent sampling and high density data
- Proteins and DNA are about 35% carbon by mass
- Add carrier carbon when needed
- Carrier carbon must be non-volatile, compatible with samples, nonreactive, high in carbon, easily added with high precision. We use tributyrin.

AMS minimizes radiation risk and often avoids radioactive waste

Radiation Dose Equivalent for 70 kg Human Exposed to ^{14}C



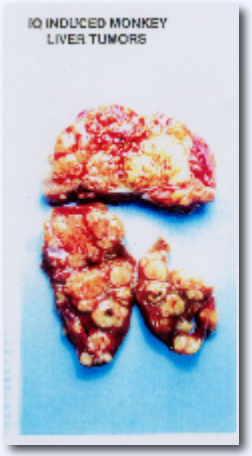
- Radiation doses can be expressed in hours of plane flight or bananas consumed.
- Typically no samples are radioactive
- Low dose leads to a reduction in radiological waste disposal fees:
- § 20.2005 Disposal of specific wastes.
  - A licensee may dispose of the following licensed material as if it were not radioactive:
  - 0.05 microcurie or less of ^{3}H or ^{14}C per gram of animal tissue, averaged over the weight of the entire animal.

To Determine Risk, We Often Test Chemicals at Very High Doses That Do Not Reflect Actual Human Exposure

We test unrealistically high levels of chemicals to be able to see cancer in a short period of time

X 10,000/day

=



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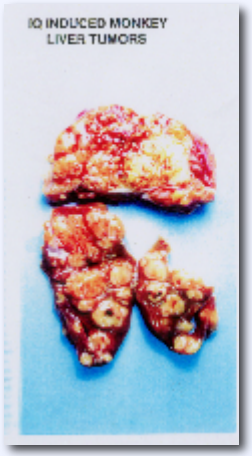
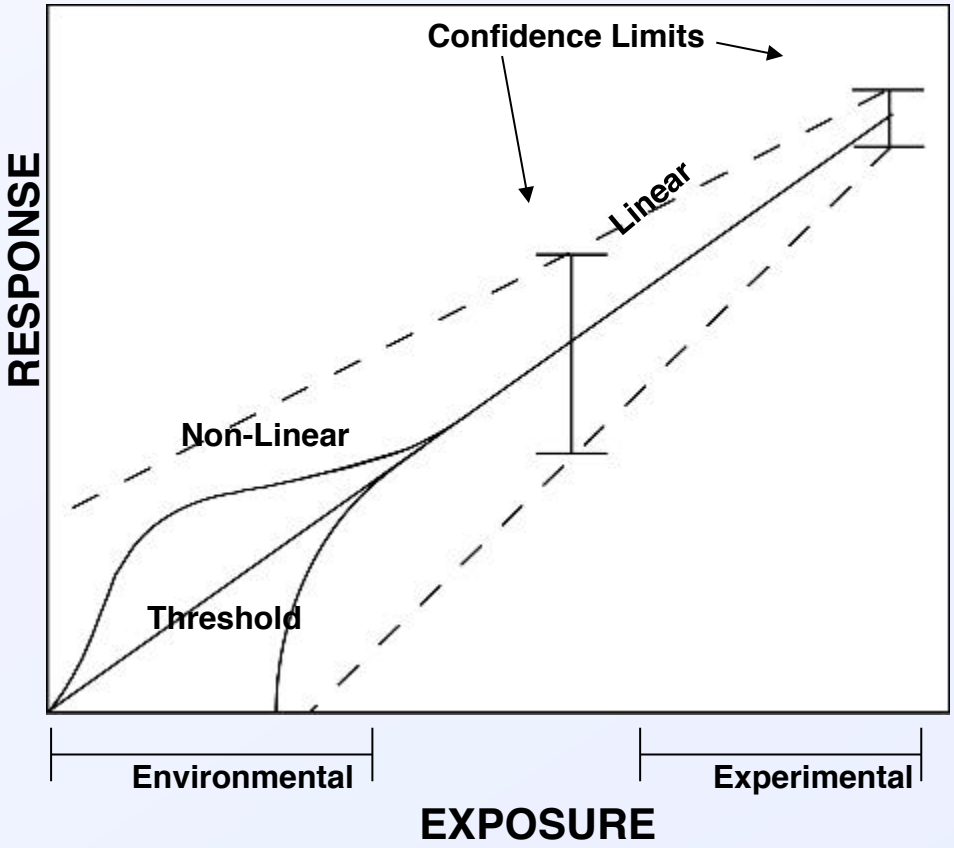
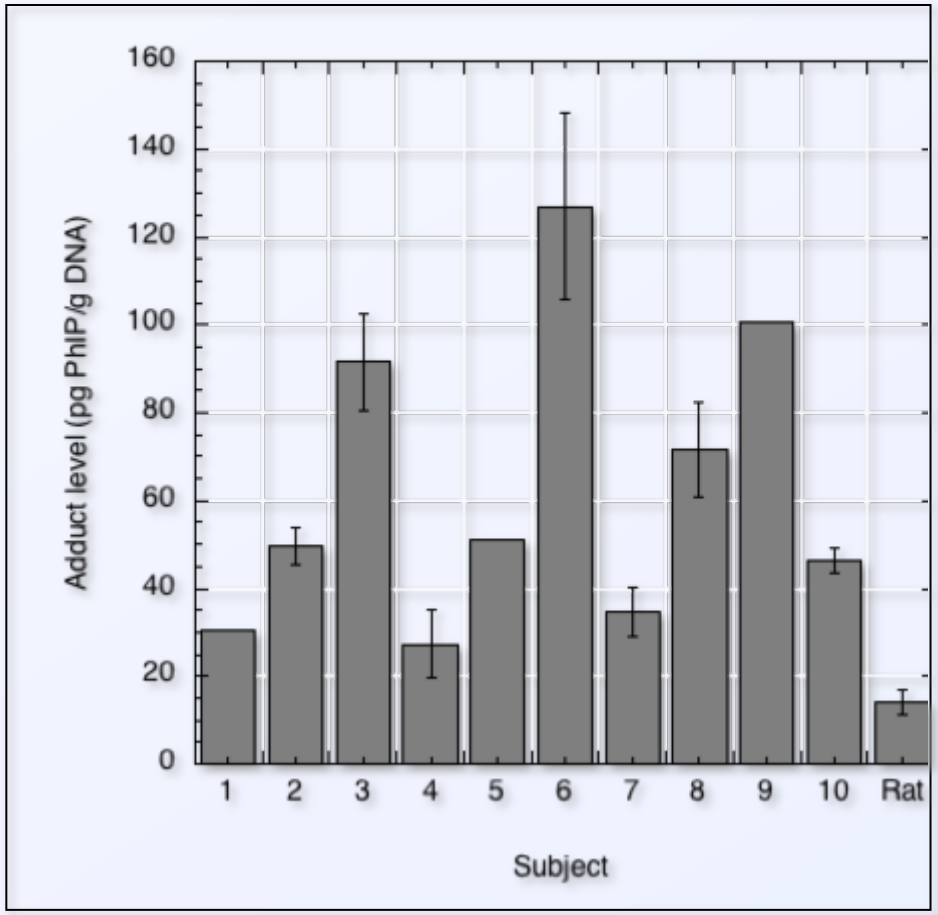




AMS enables studies at realistic environmental or occupational exposures

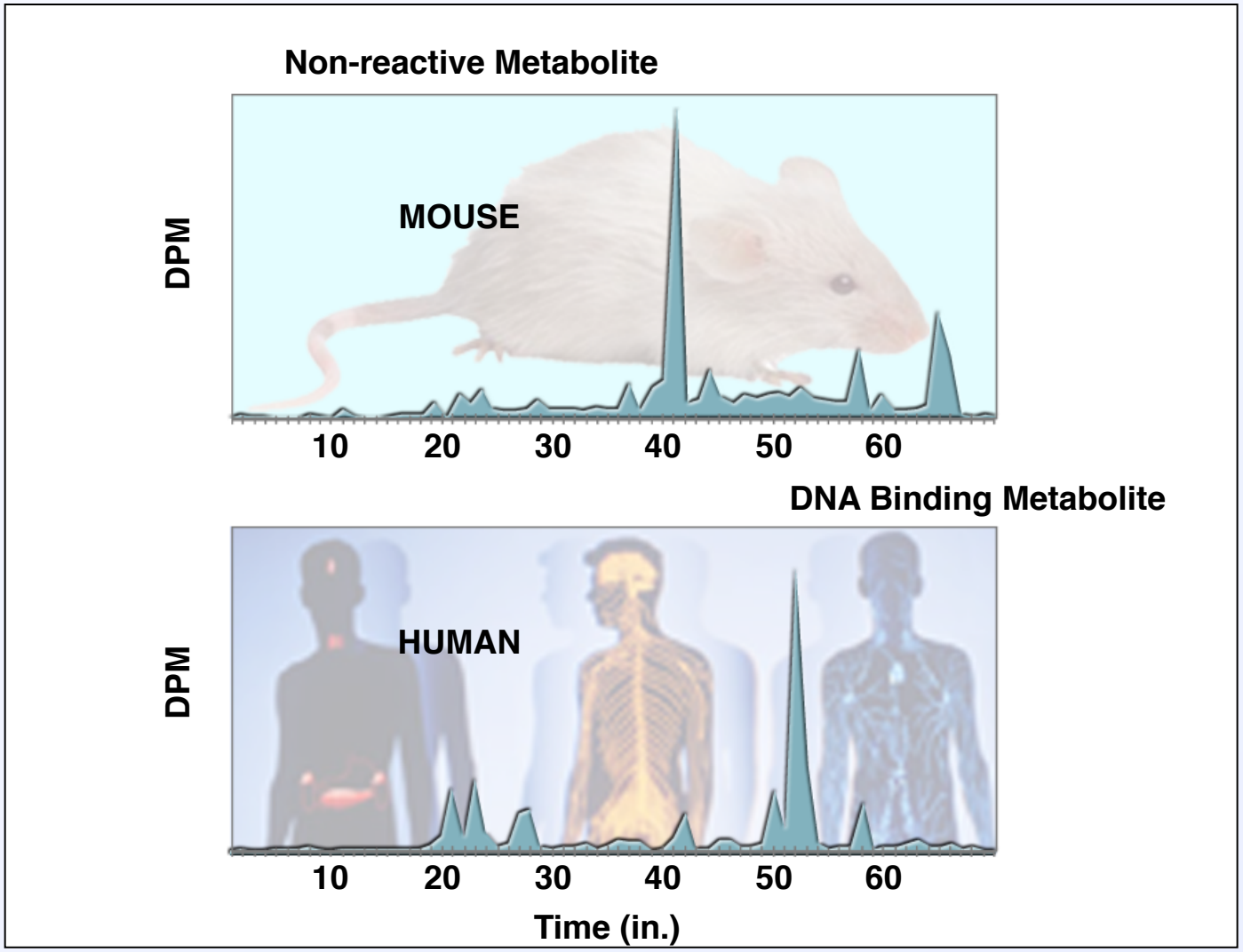
Does Food Carcinogen PhIP Bind to DNA in People and How Does the Binding Compare to Animals?



- **Humans form almost 10-times more DNA adducts for the same relevant PhIP consumption as rodents**
- **This may mean the people are more sensitive to the effects of PhIP than rodents**

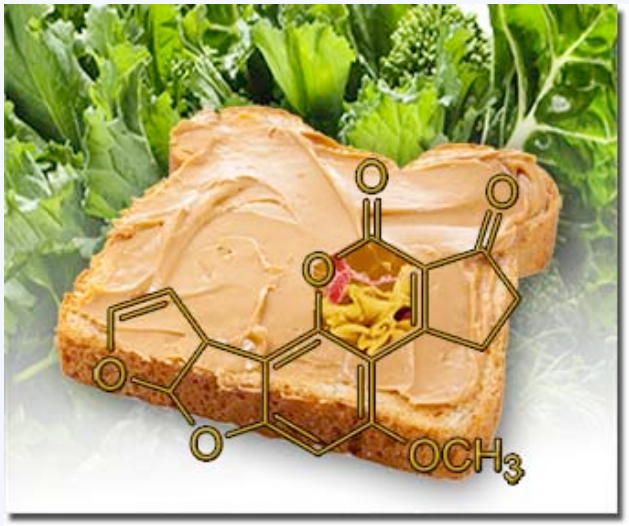
Turteltaub, et al. Cancer Letters. (1999) 143: 149-155.

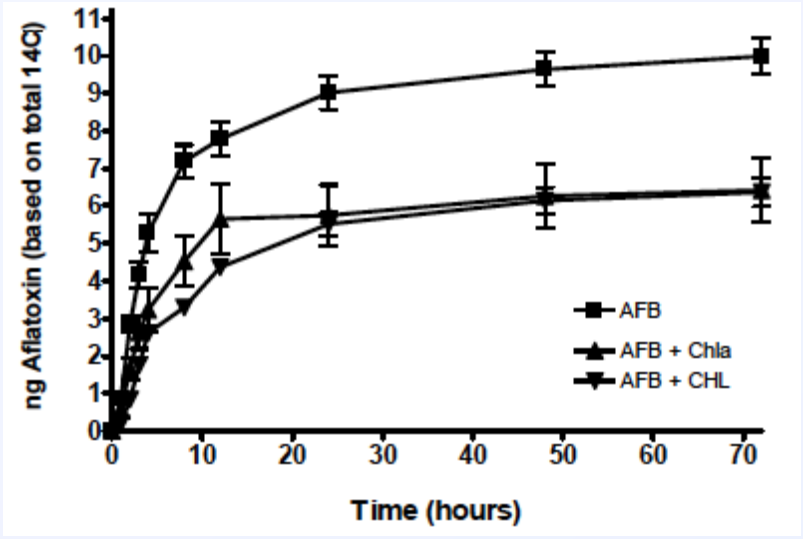
Why Do People Form More DNA Damage?
People Metabolize the Compound Differently!



Dingley, et al. Cancer Epidemiol. & Biomarkers. (1999) 8: 507.

Chlorophyll and chlorophyllin co-consumption limit the bioavailability of ingested aflatoxin in humans

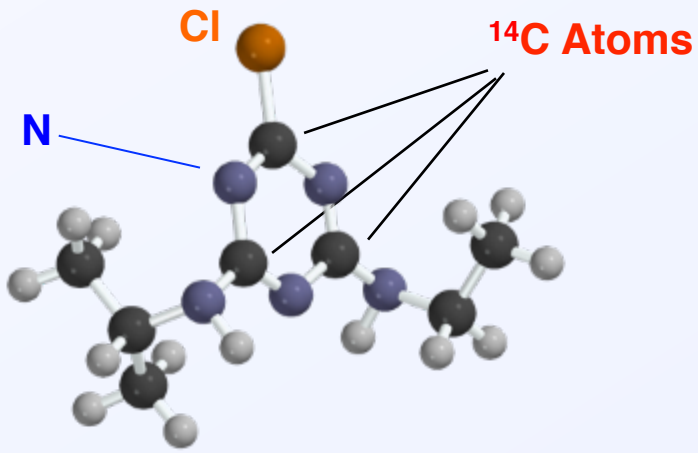




- Aflatoxin (AFB1) – a potent, naturally occurring carcinogenic mycotoxin associated with mold-foods prone to aflatoxin contamination include corn, cottonseed, peanuts and milk.
- Chlorophyll (Chla) and chlorophyllin (CHL) reduce AFB1 bioavailability in animal models.
- Four volunteers ingested a 5 nCi dose of ^{14}C- aflatoxin (a fraction of that found in a peanut butter sandwich). Concentration of ^{14}C in blood and urine measured by AMS.
- Subsequently, the volunteers were given 150 mg of Chla or CHL concomitantly with the same dose of ^{14}C- aflatoxin and measurement protocol repeated
- Chla and CHL treatment each significantly impeded AFB1 absorption

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

Determine biomarkers of dermal exposure to Atrazine in urine

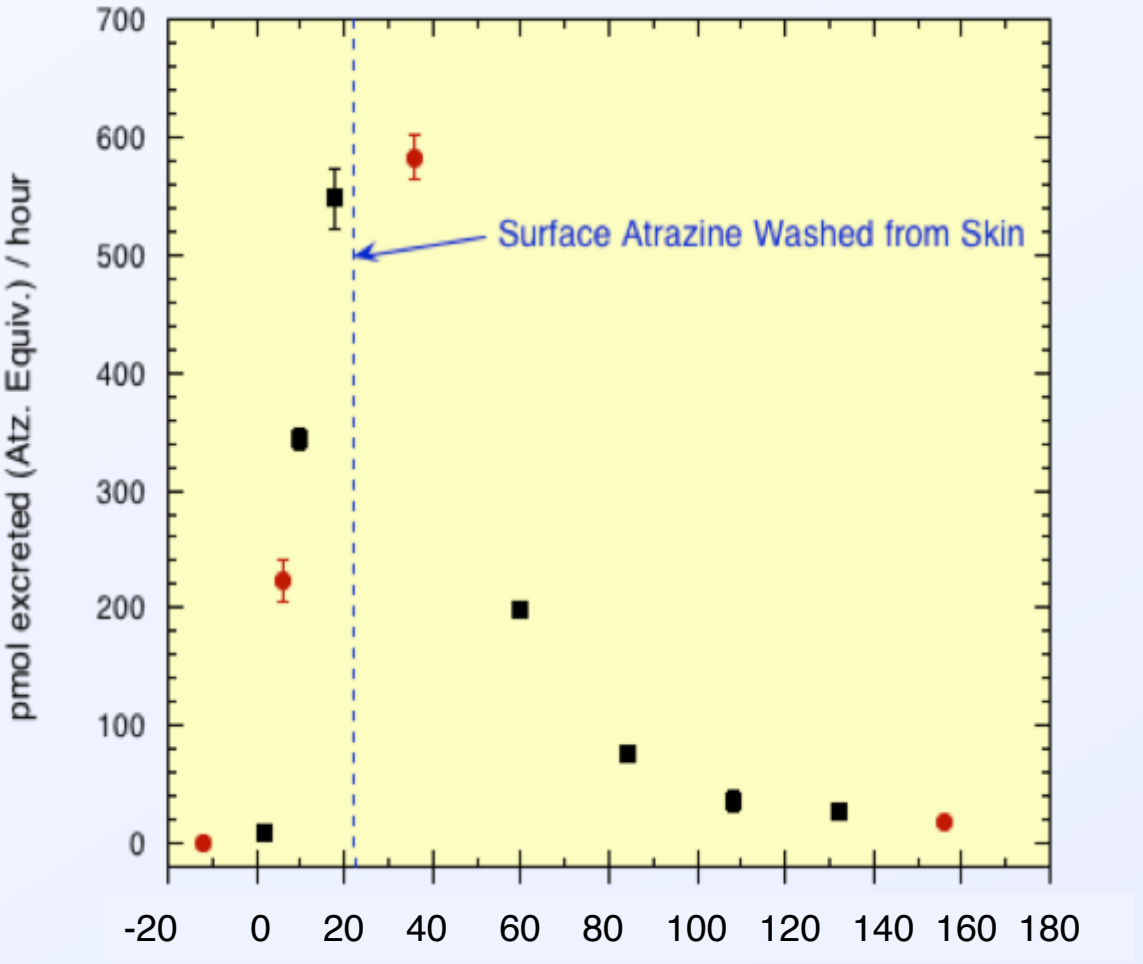


- ^{14}C-Atrazine applied to skin for 24 h (0.167 mg, 6.45 μCi)
- Urine collected predose and postdose for 7 d
- Separate metabolites by HPLC to determine distribution
- Quantify by AMS

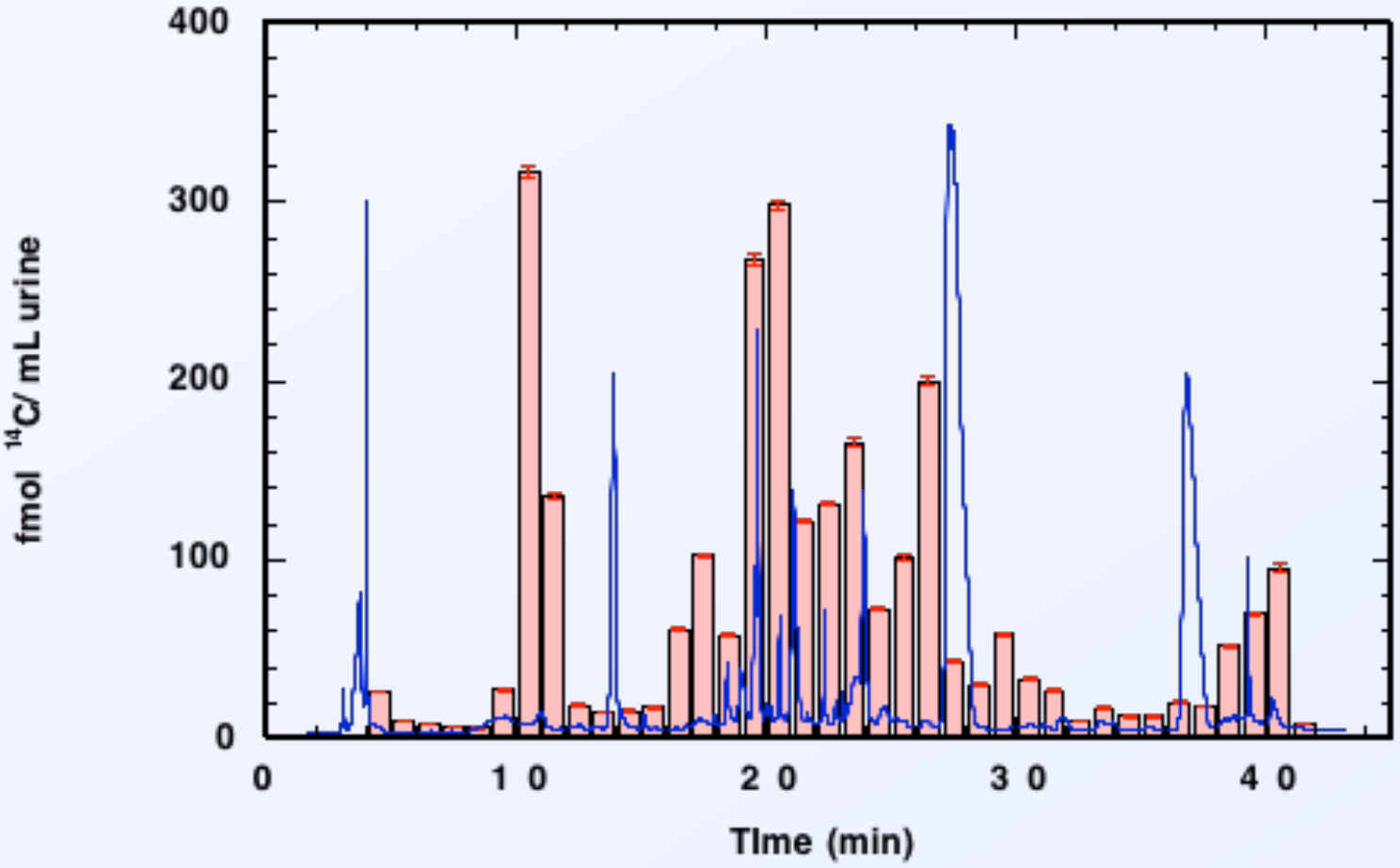
Gilman et al., Anal. Chem. (1998) 70:3463, Buchholz et al., (1999) 71:3519

Selection of time points for metabolite analysis based on excretion rate of Atrazine Equivalents

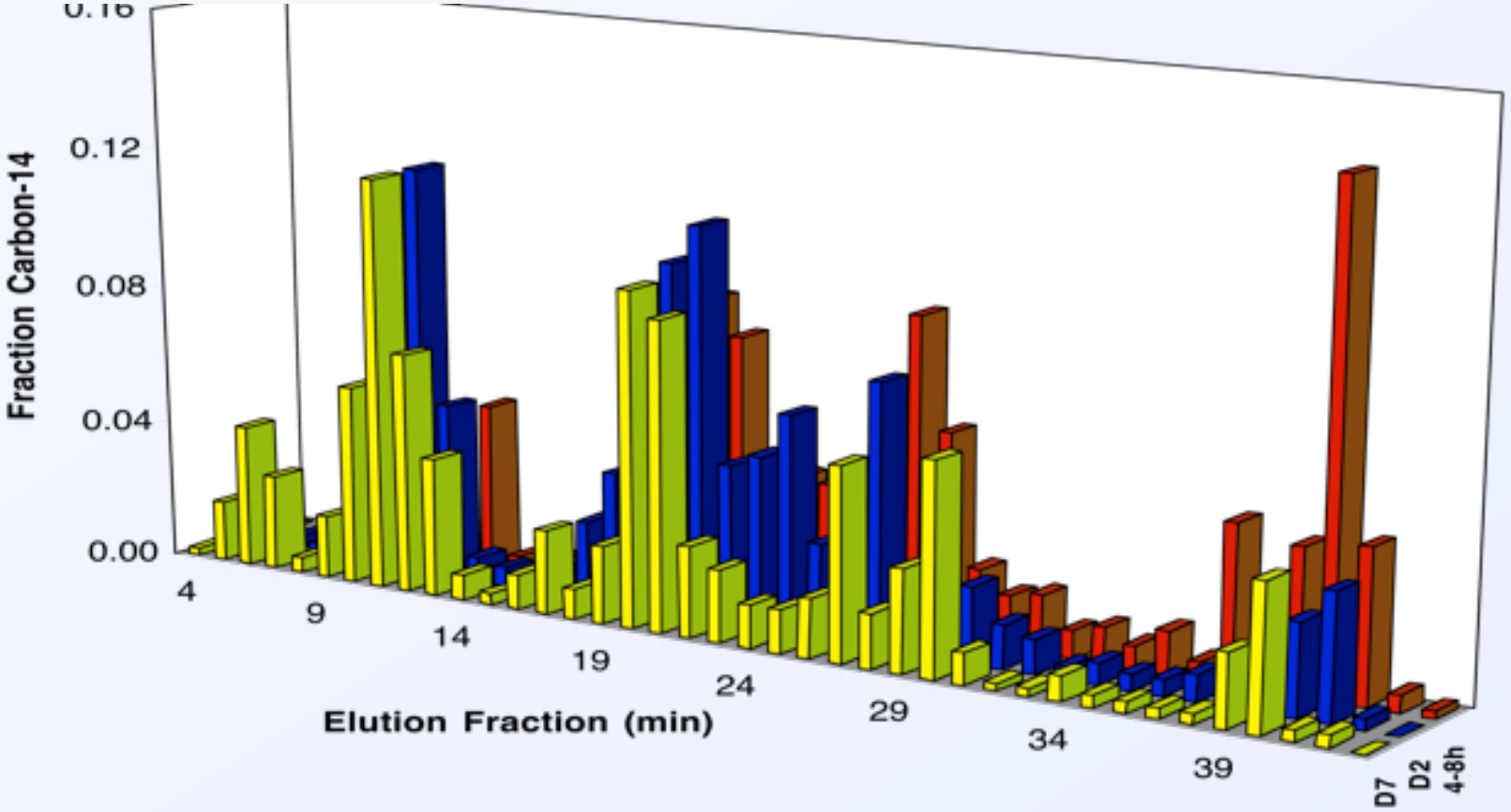
- **Subject 2**
- **Samples selected for HPLC separation based on excretion rate dose time line**
  - **Predose**
  - **4 - 8 h**
  - **24 - 48 h**
  - **144 - 168 h**



Metabolite separation on day 2 for subject 2, UV & AMS



Metabolite profiles shift during and after exposure



Metabolite profile shifts with time

Mercapturate metabolites dominate early time points

No labeled parent compound seen in any sample

Human Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) is common

- PAHs are combustion products
  - Small PAHs often inhaled (napthalene, pyrene)
  - Large PAHs ingested in foods (≥ 5 rings,benzo[*a*]pyrene (BaP))
- EPA priority pollutants list contains 16 PAHs
  - Dibenzo-[*def,p*]chrysene (DBC) is not listed
  - DBC is IARC class 2a probable human carcinogen
  - Metabolically activated DBC forms DNA adducts
- Human exposure study with 29 ng DBC (5 nCi ^{14}C)
  - Equivalent to the BaP content of a 5.2 oz. serving of smoked meat at the European Union maximum legal limit
  - 28% of the average daily dietary PAH intake
  - Radiation dose = 5 bananas (^{40}K)

Compare Pharmacokinetics of DBC in humans and rats

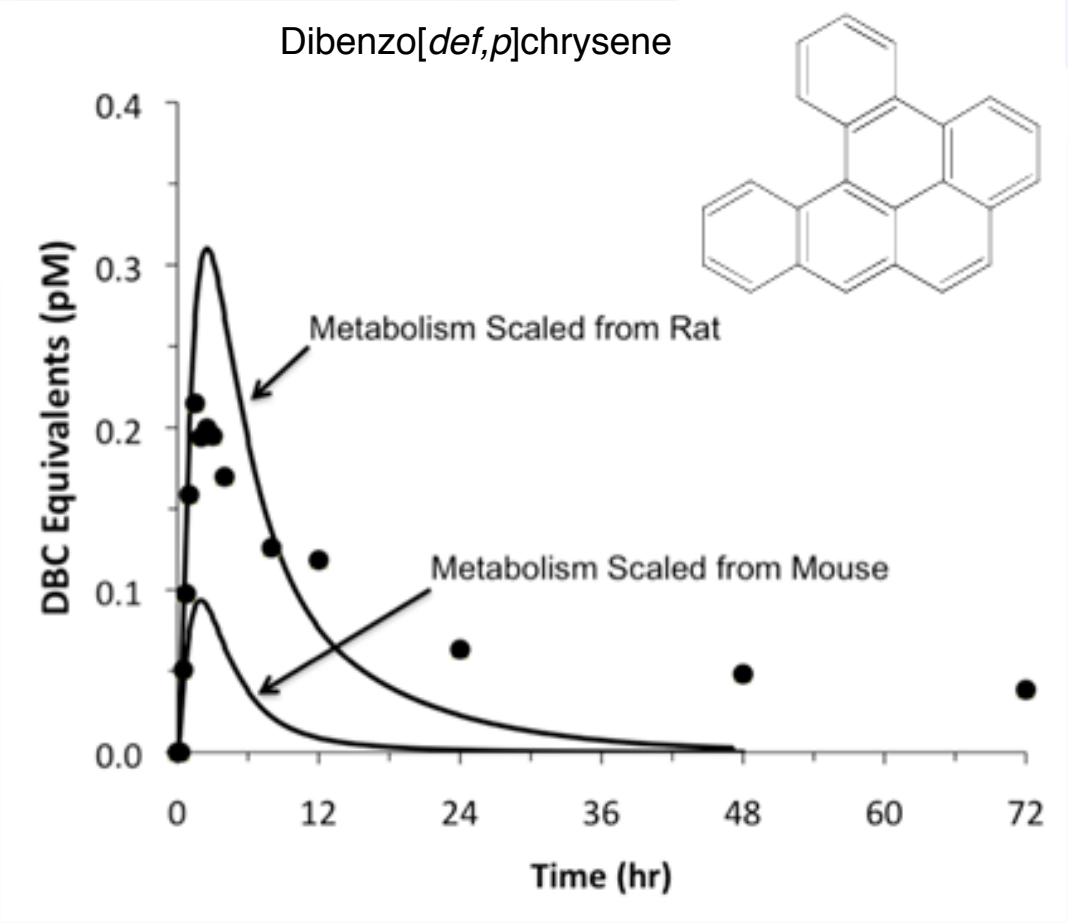


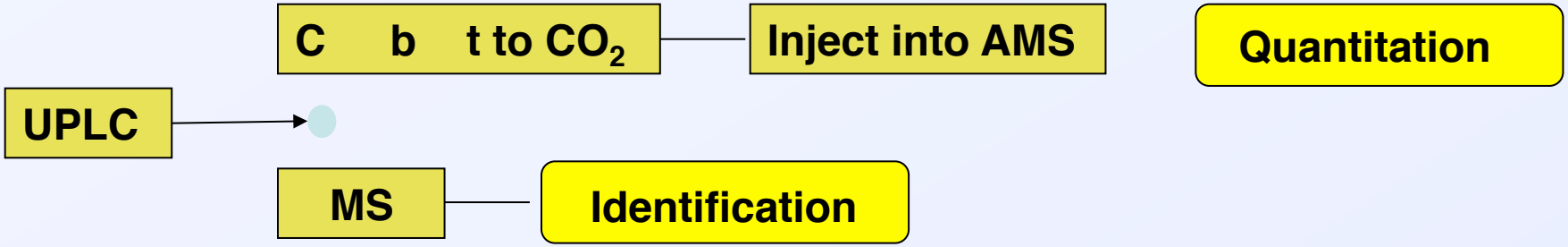
Figure 10. Rate of ^{14}C-DBC Absorption and Excretion in Humans: Comparison to PBPK Model Prediction Generated in Rodents

- Average of 6 humans
- Solid curves are PBPK models of rat and mouse with metabolic rates scaled
- Enables comparison of human and animal models

Madeen et al, (2015) *Chem. Res. Toxicol.* 28:126-134.

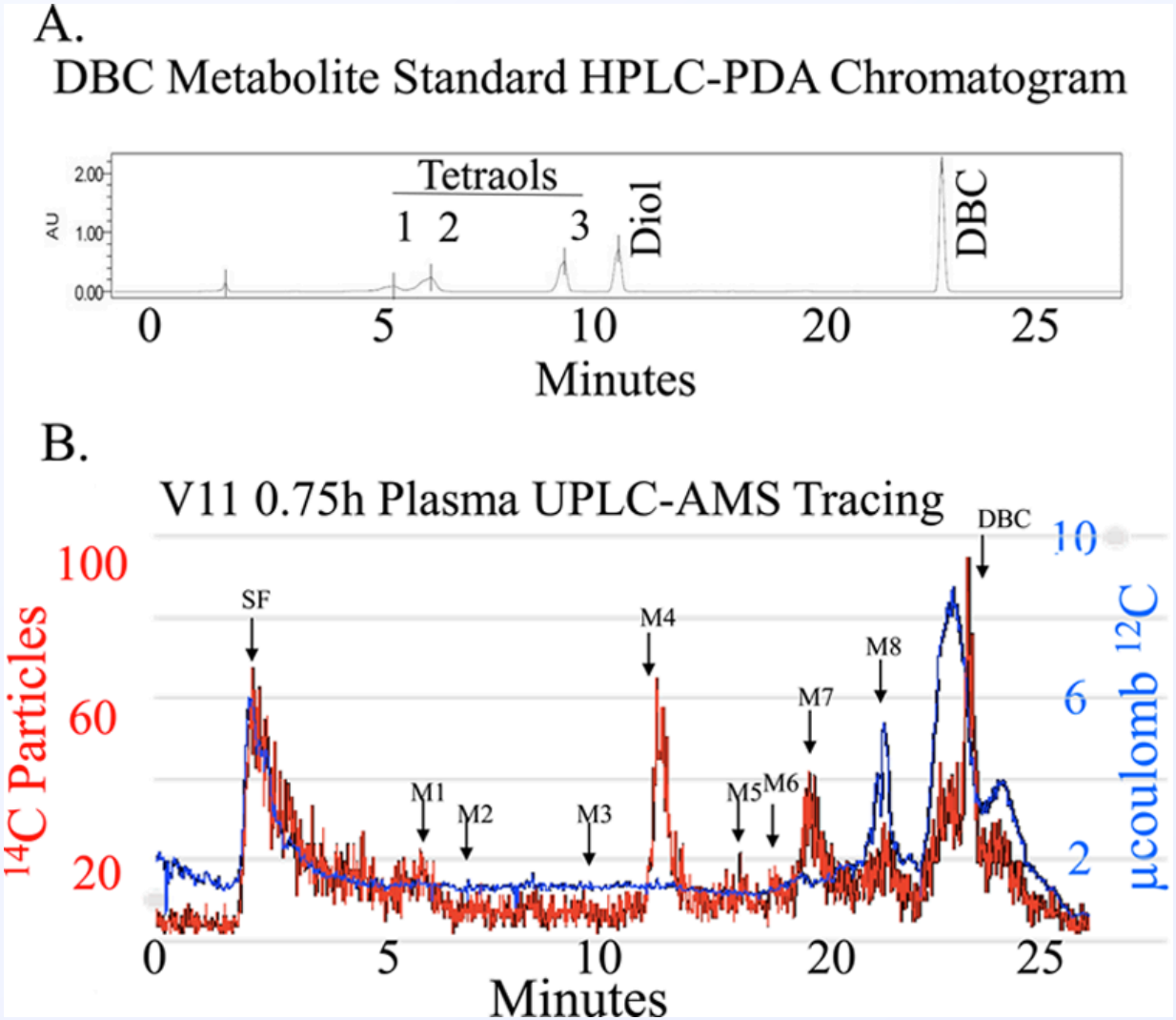
Coupled UPLC-AMS-MS System Enables Direct Measurement without Conversion to Graphite

- Identify and quantify metabolites simultaneously
- AMS is the detector
- Addition of a flow splitter and MS allows UPLC-MS-AMS
- Analyses as fast as UPLC



Thomas et al, (2013) *Anal Chem* 85: 3644-50. Ognibene et al, (2015) *Nucl Instr. & Meth. B* 361:173-7.

Identify metabolites of DBC with Coupled UPLC-AMS System

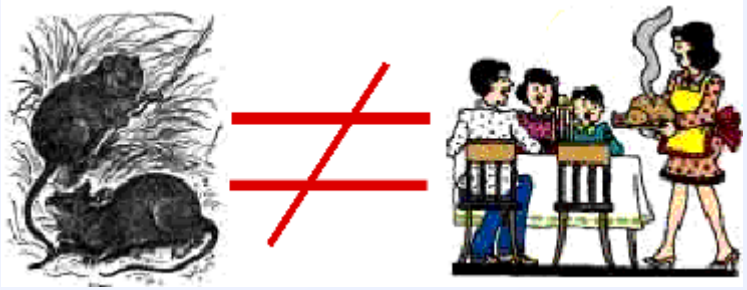


- Identify metabolites by co-elution with standards
- M1-M3 DBC-Tetraols, M4 DBC-Diol, DBC
- M5-M8 unidentified
- Analyses as fast as UPLC

Madeen et al, (2016) *Chem. Res. Toxicol.* 29:1641-1650.

AMS Enables Human ADME, PK, and Biomarker Studies Using Environmentally Relevant Exposures

- Extreme sensitivity and specificity enable human studies
  - No radiological hazard above accepted risks
  - No additional chemical hazards
- Best to study humans using humans
- Follows ALARA principles (As Low As Reasonably Achievable)
- New liquid sample interface streamlines analyses



Automation and Simplified Processing Remain Technology Goals

- Liquid sample interface with gas injection eliminates graphite processing
- UPLC-AMS makes large scale LC viable
- Recent LASER based detection of $^{14}CO_2$ is the next step. US Patent 9,645,077 B2 issued May 9, 2017

Collaborators, Contributors & Funding

- **LLNL**
  - Kurt Haack
  - Ted Ognibene
  - Ken Turteltaub
  - Mike Malfatti
  - Heather Enright
  - Ben Stewart
  - Graham Bench
- **Oregon State SRP**
  - Erin Madeen
  - Dave Williams
  - Richard Corley (PNNL)
  - Carole Jubert
  - Rod Dashwood
  - George Bailey
- **UC Davis SRP**
  - Bruce Hammock
  - Shirley Gee
  - Alan Buckpitt
  - Laura Van Winkle
  - Sarah Carratt
  - Ki Chang Ahn
  - Guomin Shan
  - Doug Gilman
- **Others**
  - Howard Maibach, UCSF
  - John Casida, UCB
  - Dave Eastmond, UCR
  - Mike Rust, UCR
  - Linda Hooper-Bui, UCR

Grants

P41RR013461

P41GM103483

P42ES016465

P42ES004699

http://bioams.llnl.gov/