Analytical Methods for Measuring QACs in Biomonitoring Studies

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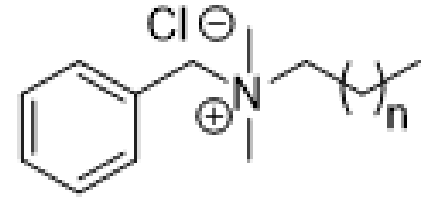
03/08/2020

Quaternary Ammonium Compounds



Benzalkonium chloride (BACs)

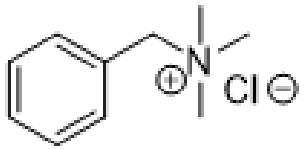
Disinfectants, pesticides, preservatives

Regularly used in a variety settings [1,2]

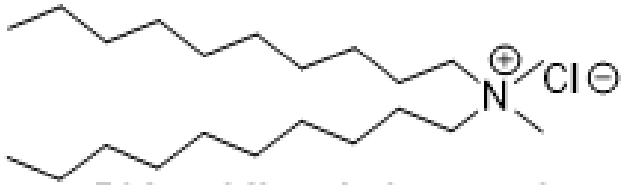
Cleaning products, eye drops, utensils, laundry detergent, milking equipment [1,2]

Dermal (eye), ingestion, inhalation exposure routes

Cetrimonium chloride



Benzotrimethylammonium chloride

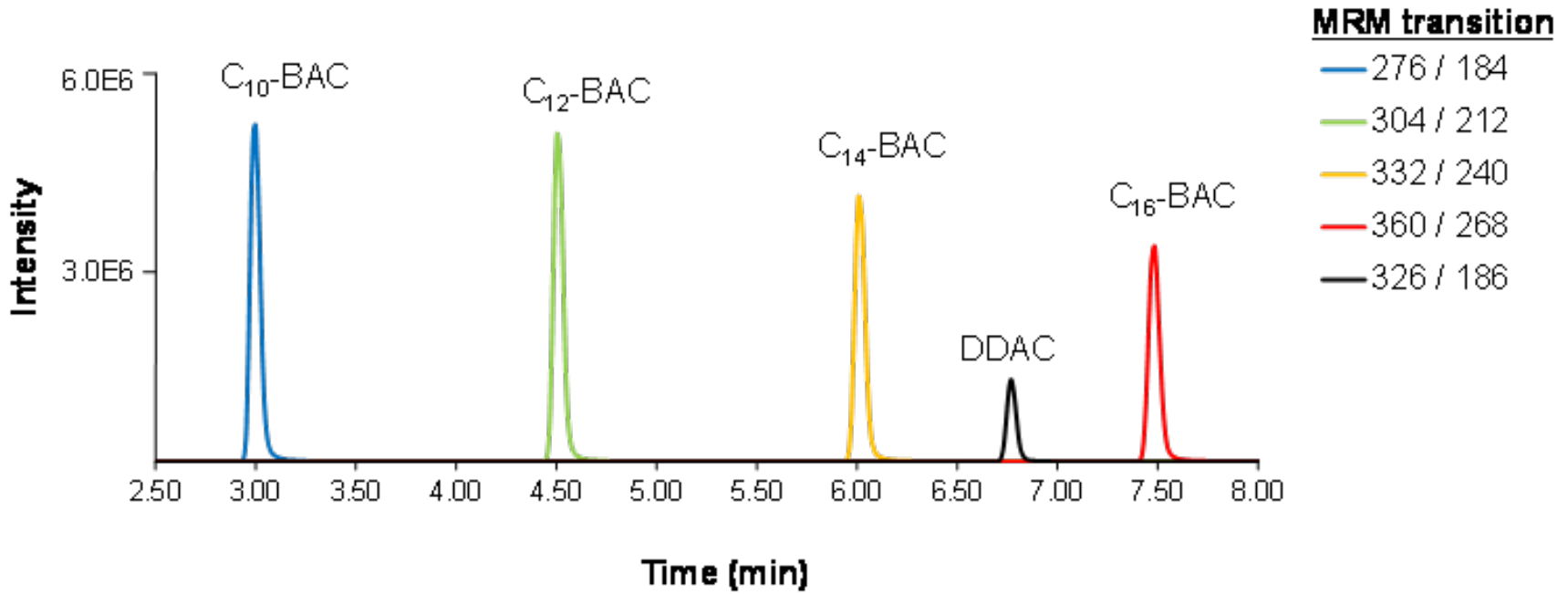


Didecyldimethylammonium chloride (DDAC)

No Public Data On QAC Exposure Levels In Humans!

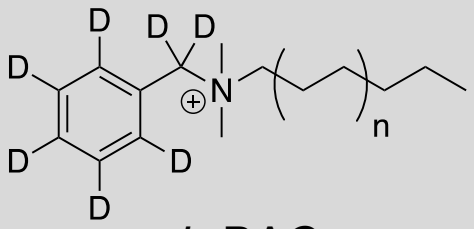
1) Hrubec, T., *et al* (2017) *Birth Defects Research*
2) US EPA (2006) RED for ADBAC, EPA739-R-06-009

Quantitation of QACs by Liquid Chromatography-Tandem Mass Spectrometry



LC Conditions:
Mobile phase: gradient of **Solvent A (**water, 0.1% formic acid, 2 mM ammonium formate) and **Solvent B** (acetonitrile)
UPLC column: Thermo Hypersil GOLD C_{18} (100 x 2.1 mm, 1.9 μm)
Run time: < 8 min

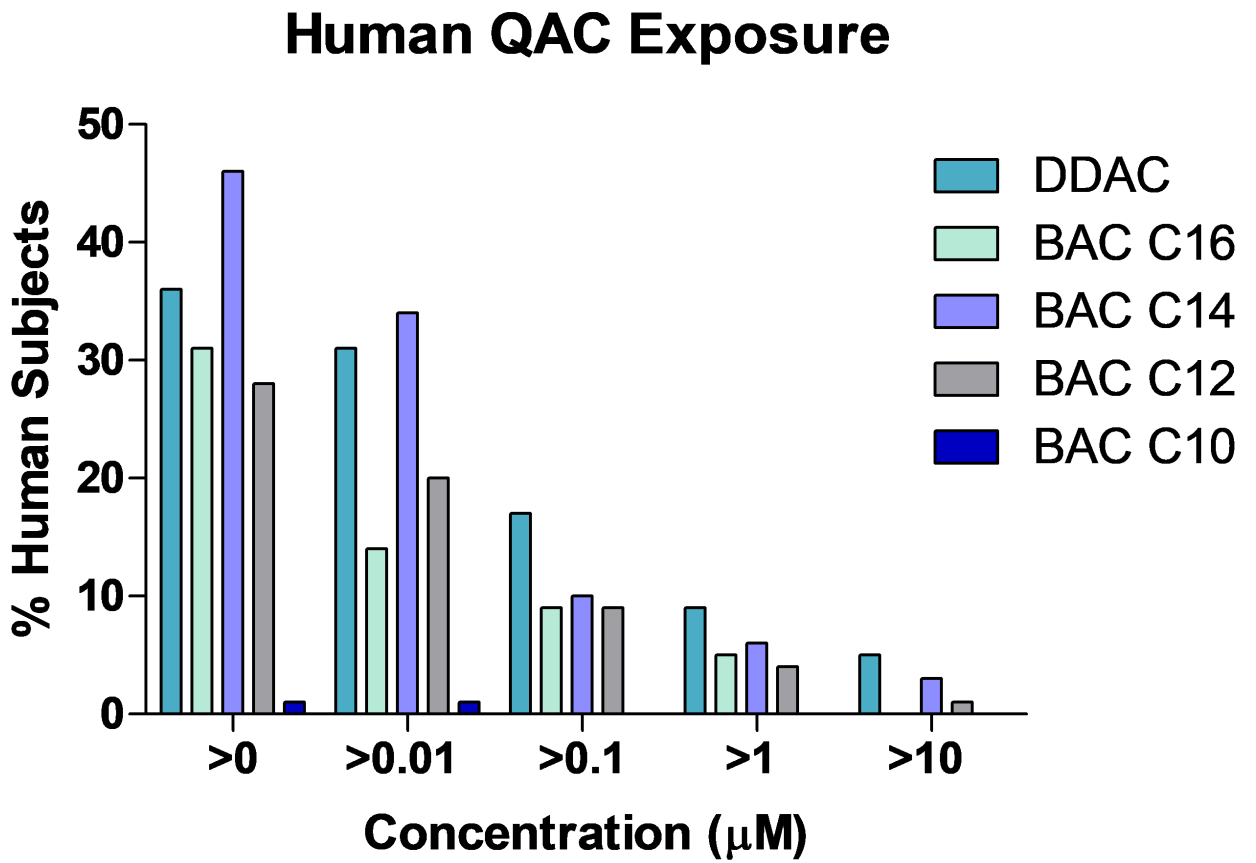
Internal Standards:



d_7-BACs

Exposure Levels in 100 Random Human Plasma Samples

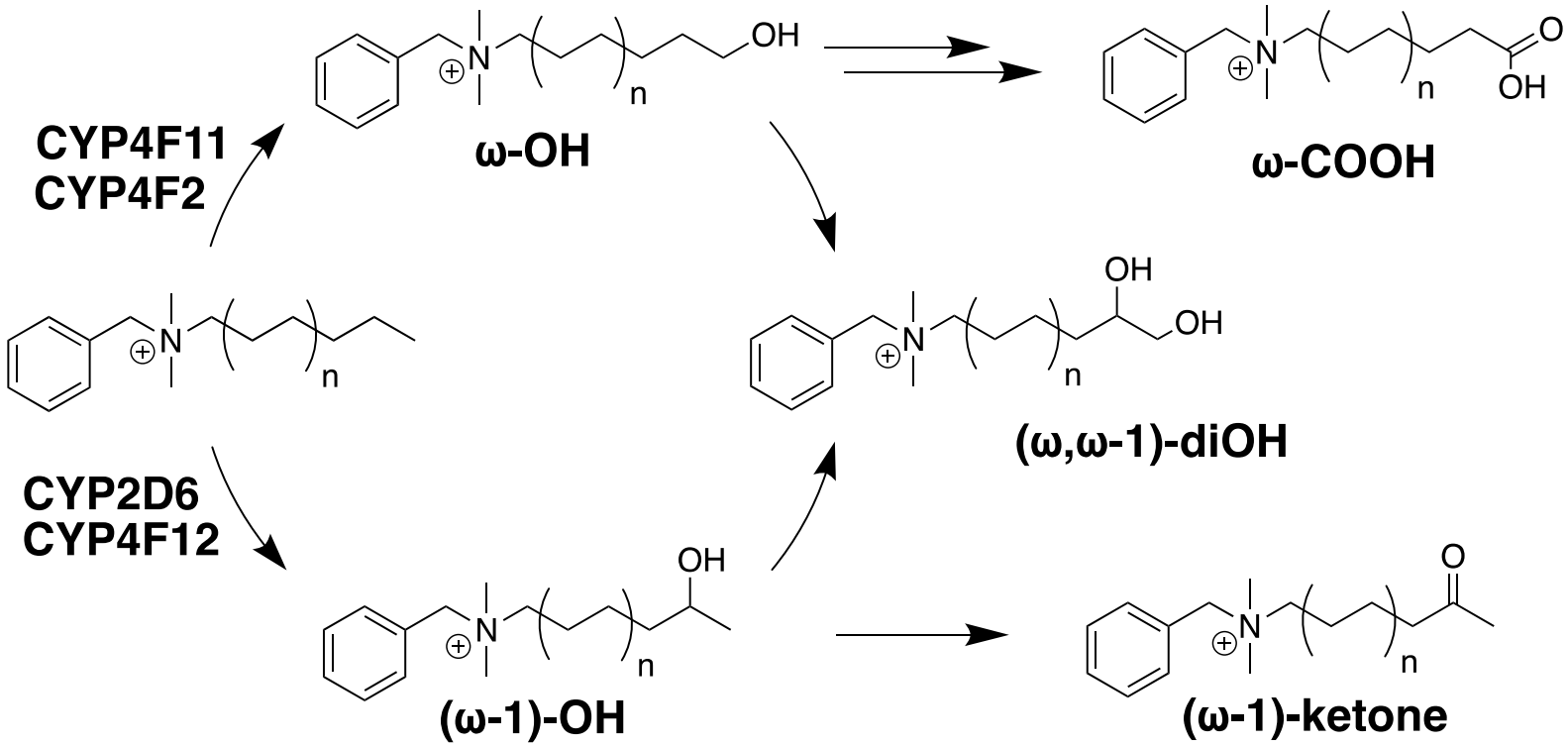
Random human plasma obtained from BioIVT



QACs Are Metabolized by Human Cytochrome P450s

Using benzalkonium chlorides (BACs) as examples

Primary Products Undergo Further Metabolism

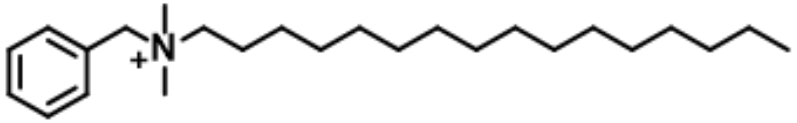


Seguin, R. P., et al. (2019) *Chem. Res. Toxicol.*, *32*(12), 2466–2478.

Further metabolism by mitochondrial β-oxidation

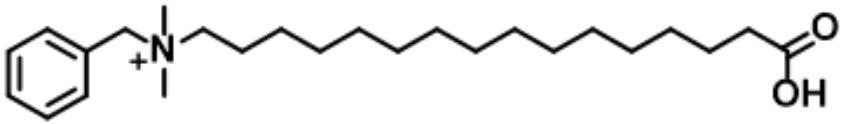
(primary human hepatocytes)

C_{16}-BAC



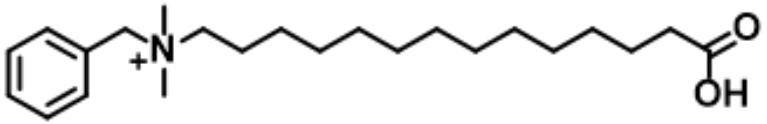
CYP4F ω-oxidation

ω-COOH
C_{16}-BAC



β-oxidation

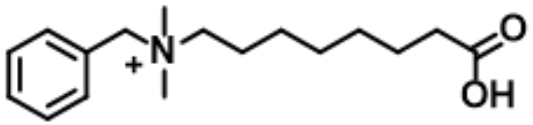
ω-COOH
C_{14}-BAC



+acetyl-CoA

Successive β-oxidation

ω-COOH
C_8-BAC



+acetyl-CoA x 3

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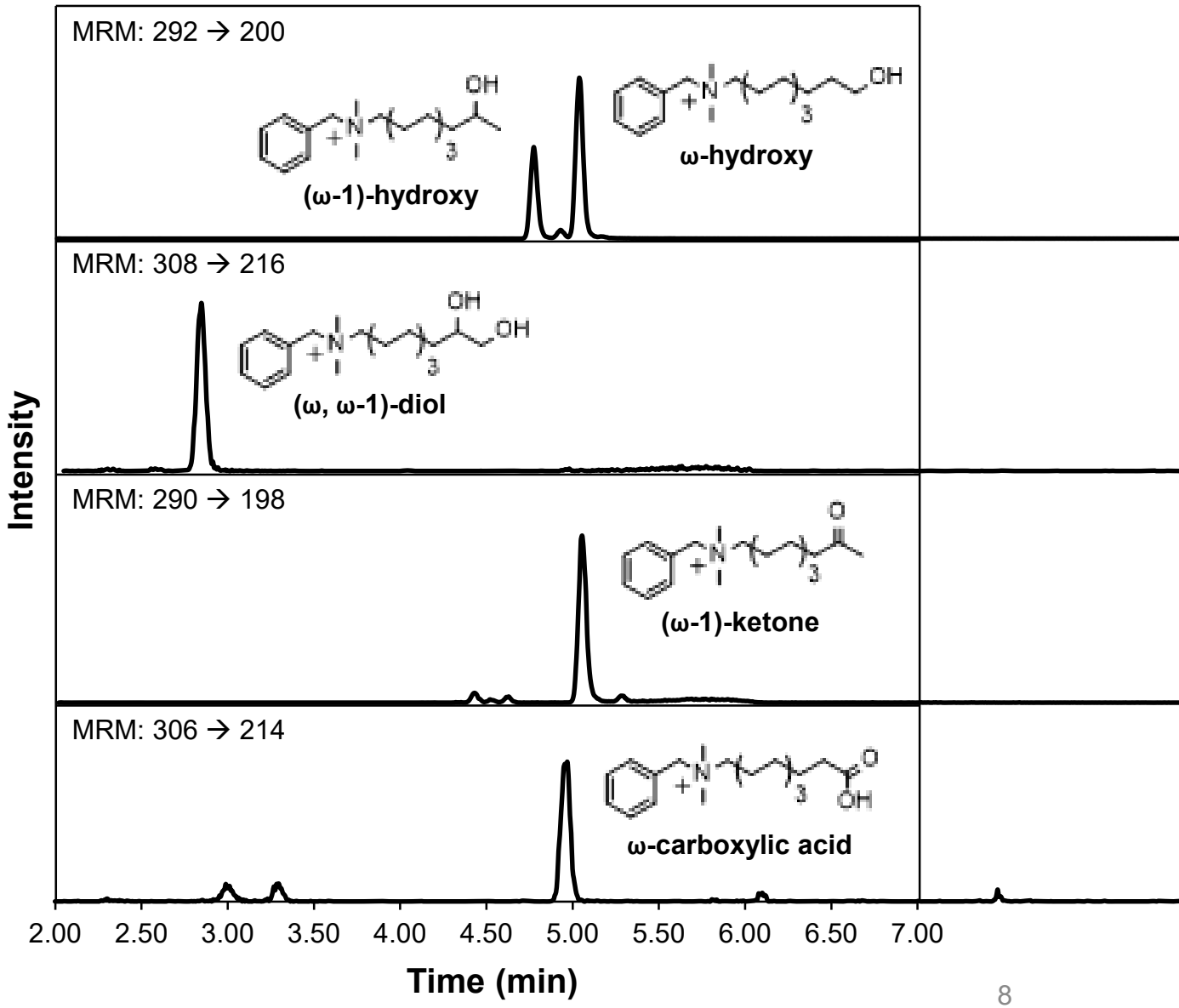
Metabolites Can Be Quantified by LC-MS/MS

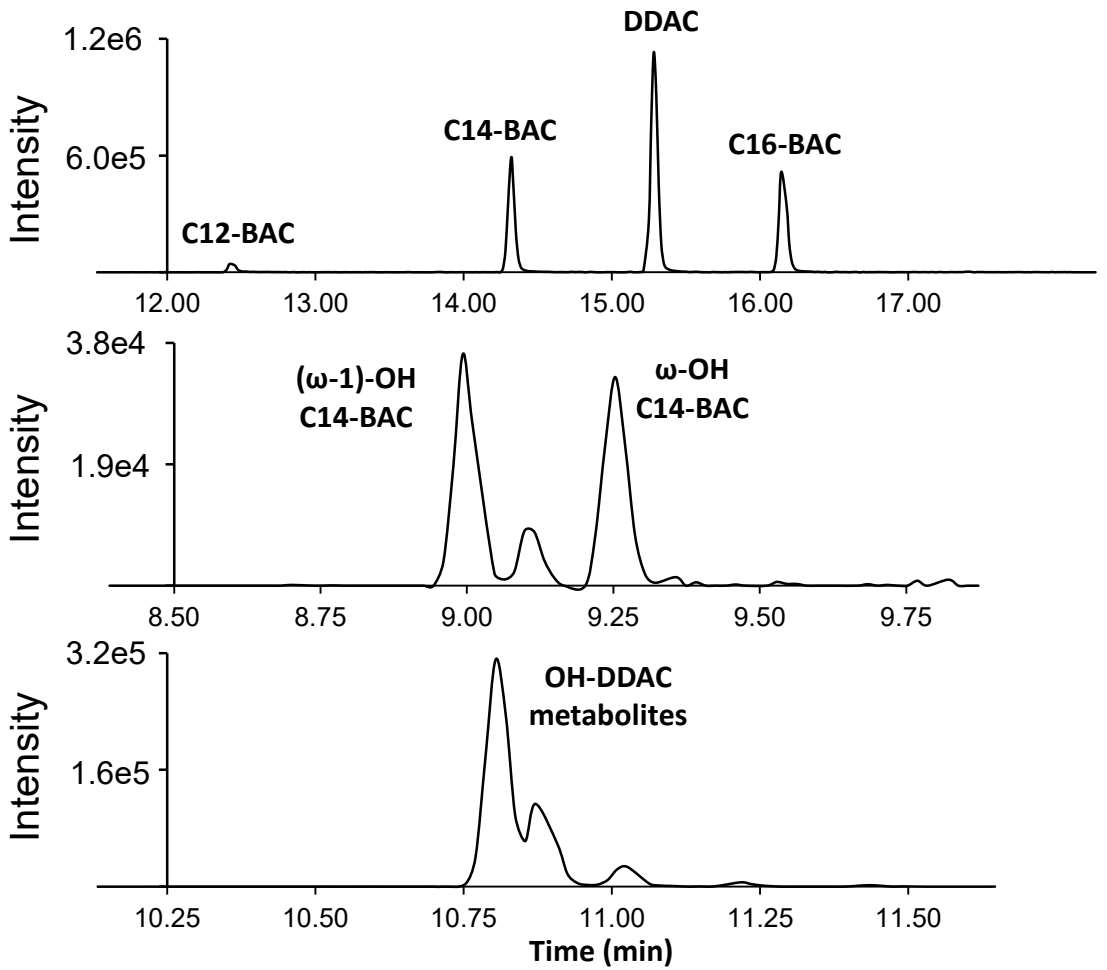
Metabolites of C_{10}-BAC



Seguin, R. P., et al. (2019) *Chem. Res. Toxicol.*, *32*(12), 2466–2478.

QACs and Their Metabolites Detected in Kidney Tissue from Mice Exposed to QACs Via Diet

120 μg/g/day QAC (40%BAC and 60%DDAC) for two weeks following Melin et al. *Reprod. Toxicol*. 2014.



Seguin, R. P., et al. (2019) *Chem. Res. Toxicol.*, *32*(12), 2466–2478.

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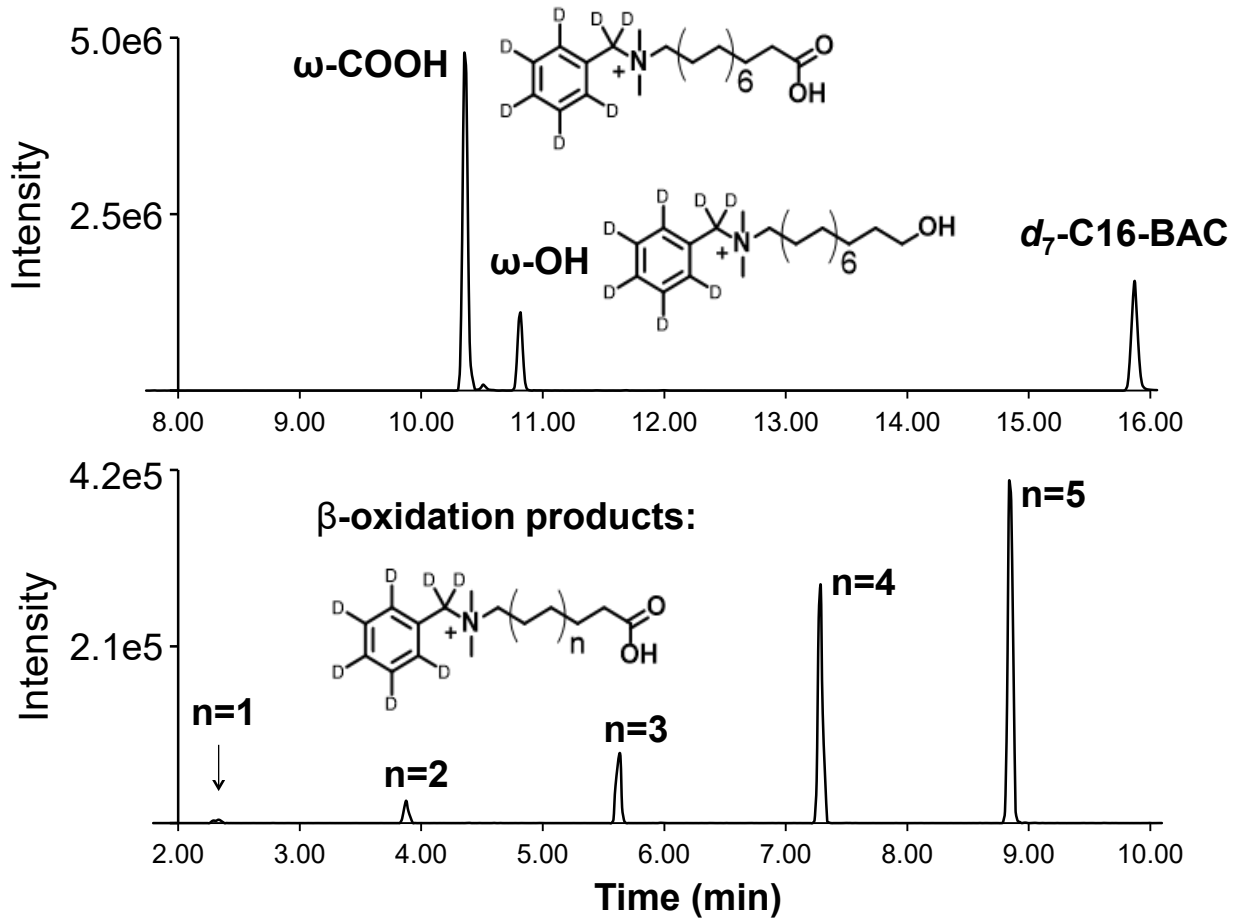
Metabolites of d_7-C16-BAC in Mouse Feces

120 μg/g/day d_7-C16-BAC for one week



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Summary of Limits of Detection

Analyte	C10-BAC	C12-BAC	C14-BAC	C16-BAC	DDAC	Carboxylic acid-BACs
LOD (nM)	0.045	0.32	0.18	0.26	0.19	0.1
LOD (ng/L)	12	97	60	94	62	20-30

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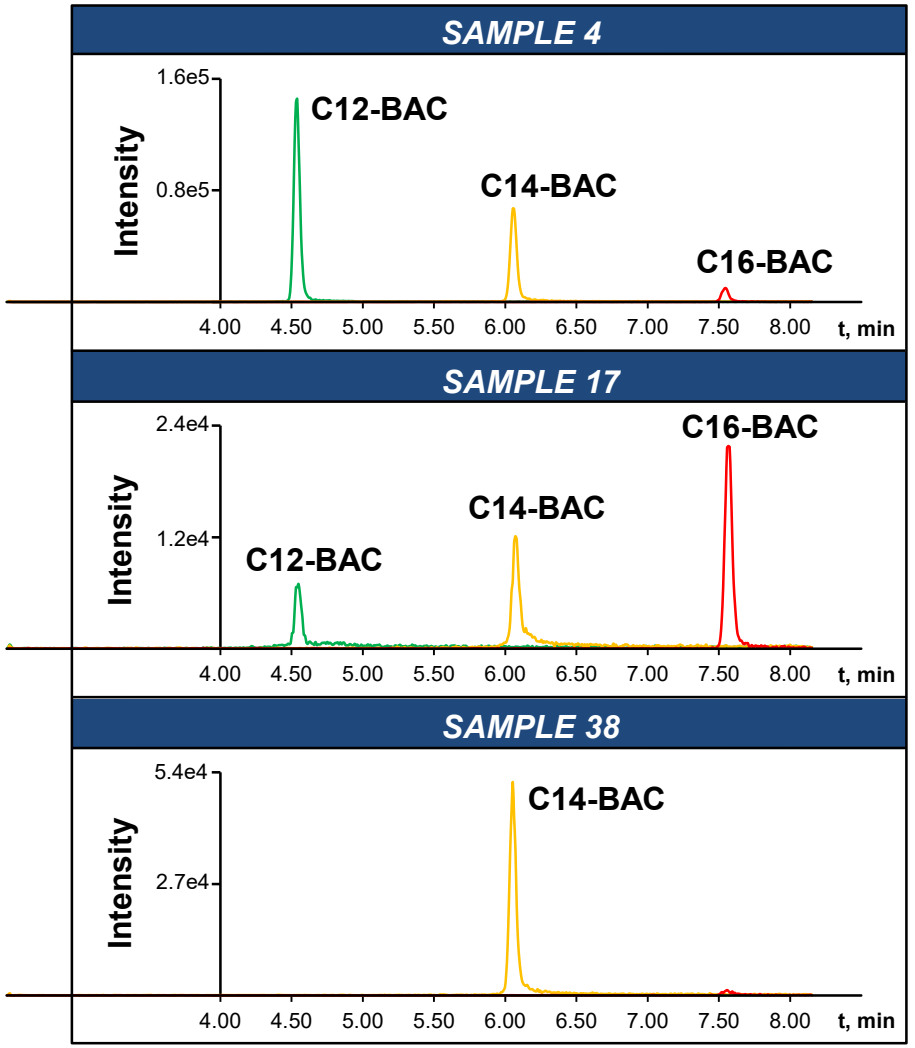
Analysis of QACs and Metabolites in Human Samples

Human Blood Samples

- Collaboration with Terry Hrubec, Virginia Tech
- Blood samples collected from participants recruited from Blacksburg, VA
- 18 years or older
- 43 samples collected
- Sample processing:
  - Spike in d_7-BAC internal standards →
  - Folch extraction (chloroform:methanol = 2:1) →
  - Reconstitute in LC solvent →
  - LC-MS/MS analysis

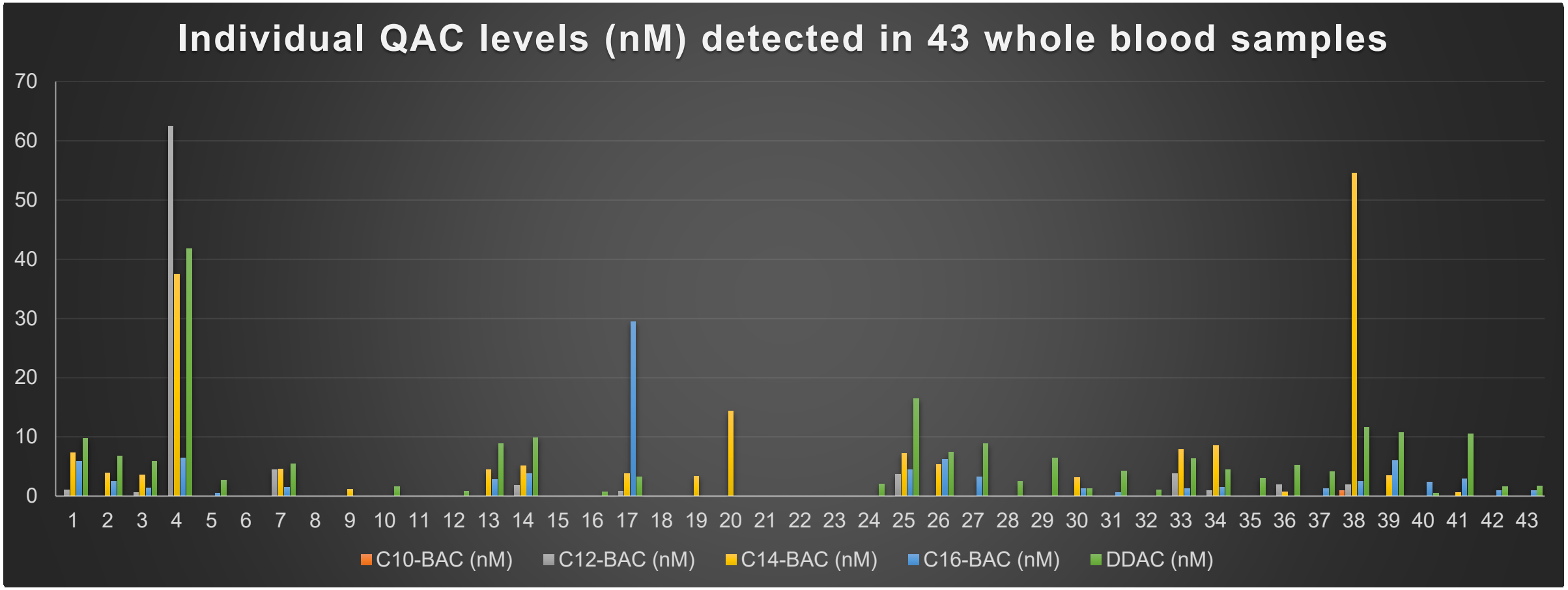
Hrubec, T. et al., *medRxiv*, 2020.

LC-MS/MS Chromatograms of QACs Observed in Human Blood



C12-BAC (MRM: 304 → 212)
C14-BAC (MRM: 332 → 240)
C16-BAC (MRM: 360 → 268)

Distribution of QAC Levels in Human Blood Samples



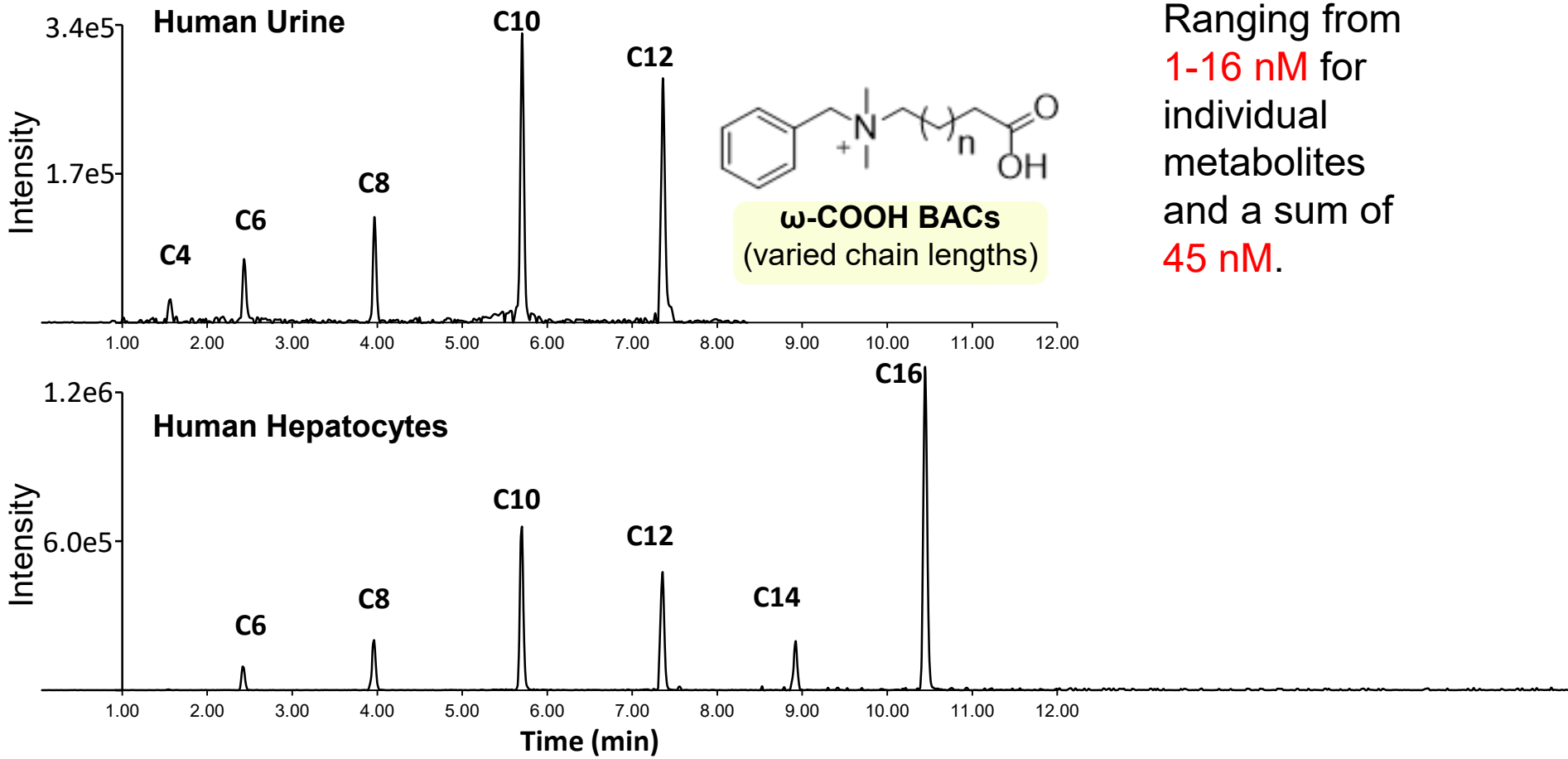
Human Urine Sample

From Biomonitoring California Inter-program Pilot Project

Urine Sample Processing

BAC Carboxylic Acid Metabolites Observed in Human Urine

A human urine sample collected by Biomonitoring California

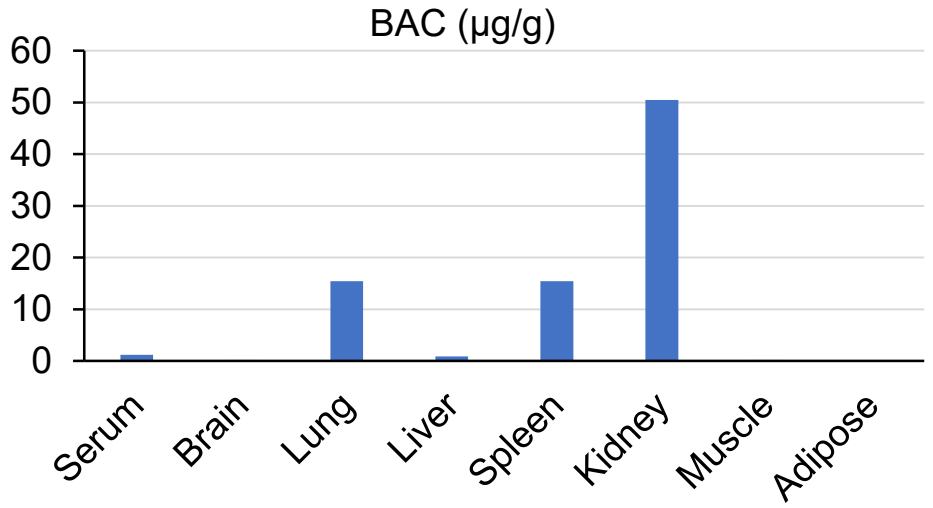


Ranging from 1-16 nM for individual metabolites and a sum of 45 nM.

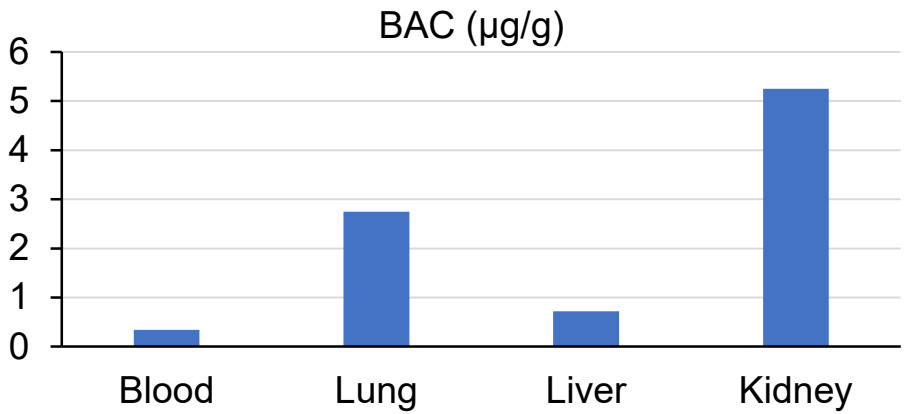
QAC Distribution Data from the Literature

QAC Distribution in Rats After IV or Oral Intake

- *7 µg/g IV injection*
- *Rats sacrificed at 30 min*



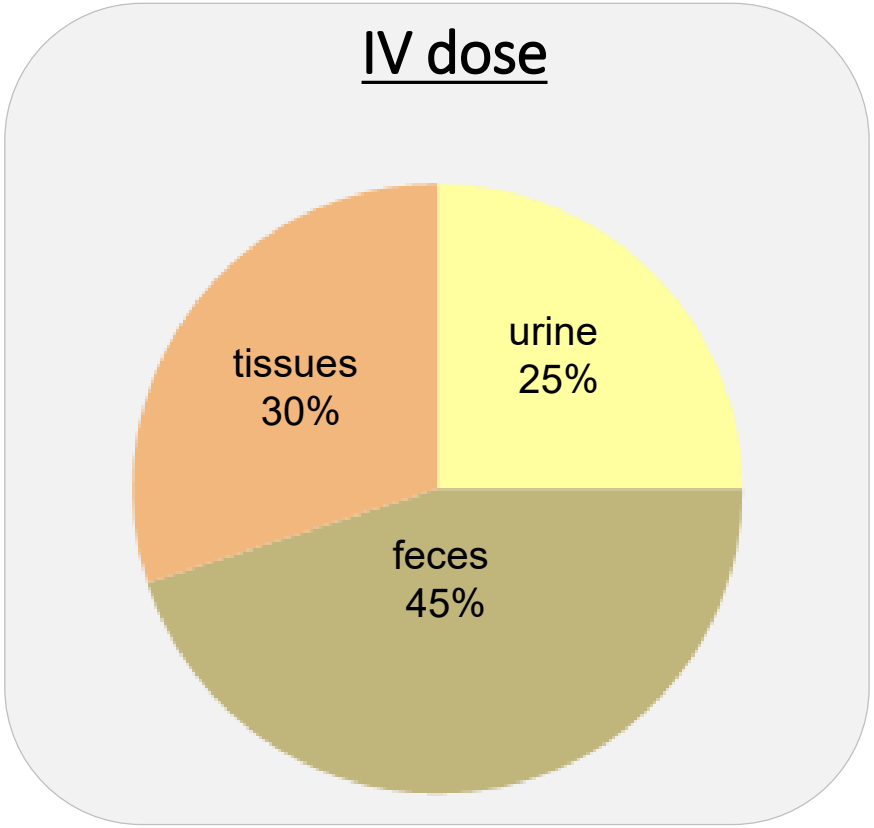
- *150 µg/g oral intake*
- *Rats sacrificed at 24 hr*

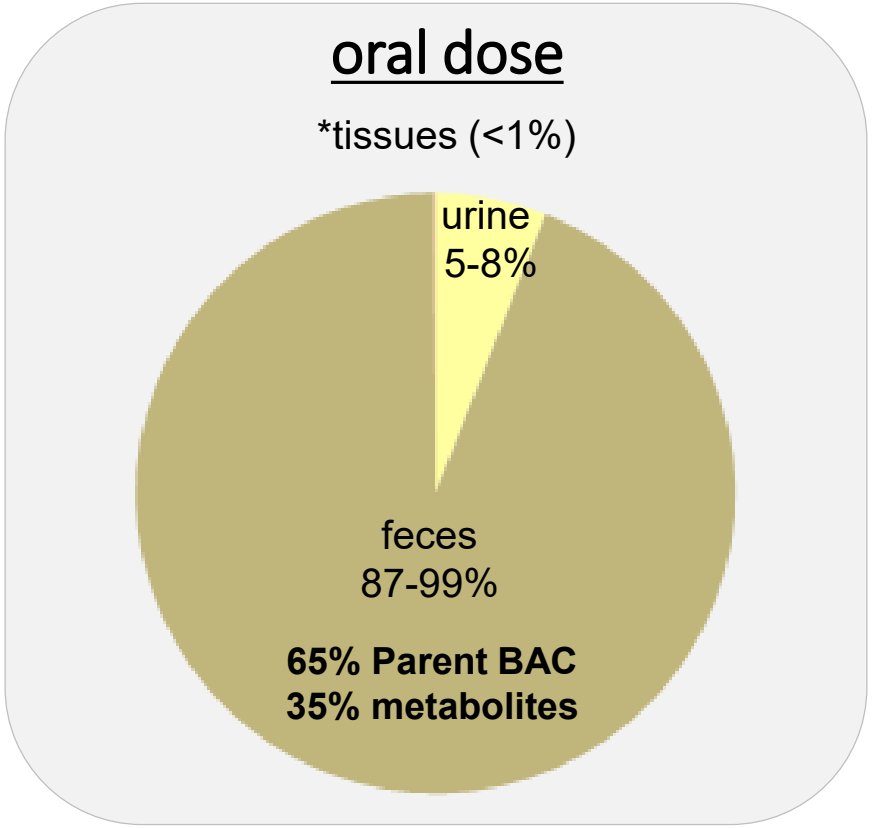


Xue, Y., et al. *Legal Med* (2002) 4, 232.
Xue, Y., et al., *J. Chromatogr. B* (2004) 811, 53.

^{14}C-BAC Recovery After Dosing Rats

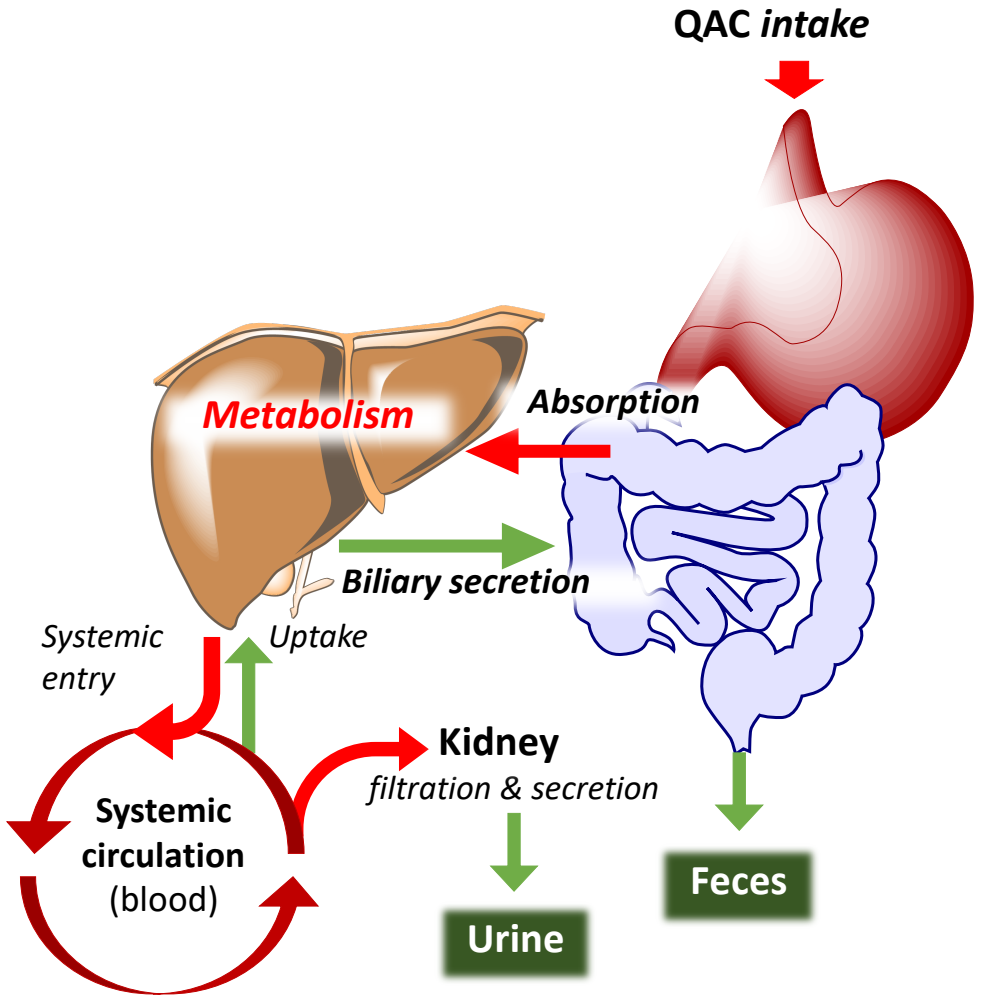
- *Single IV or oral dose (10 mg/kg) of radiolabeled BAC*
- *Feces, urine, and tissues were collected*





FDA document: FDA-2016-N-0124-0001 (https://www.regulations.gov/docket/FDA-2016-N-0124)
Luz, A., et al., *Regul Toxicol Pharm* 116, 104717 (2020).

Summary of Routes of QAC Distribution



Summary

- QACs can be metabolized by human cytochromes P450.
- QACs and metabolites can be quantified by LC-MS/MS in various biological samples after QAC exposure.
- QACs are observed in human blood and urine samples.
- Fecal, urine, and/or blood samples should serve as good biomonitoring samples.

Acknowledgements

Hideaki Tomita

– **Josi Herron**, Libin, Amy Li, Quynh Do, Emily Pruitt, **Vanessa Lopez**, Jonathan Palmer, **Ryan Seguin**, Dylan Ross, Rutan Zhang

- Colleagues in Dept. of Med. Chem. and School of Pharmacy
- School of Pharmacy Mass Spectrometry Center (Dale Whittington, Scott Edgar)
- **Funding:** NIH (R01HD092659, R01AI136979), SOP Innovation Fund, UW Royalty Research Fund

Eunice Kennedy Shriver NICHD National Institute of Child Health & Human Development

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