Reviewing QACs for Biomonitoring: Metabolism, Analytical Considerations, and Effects on Cholesterol Homeostasis

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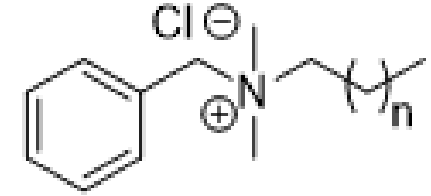
Quaternary Ammonium Compounds



Benzalkonium chloride (BACs)

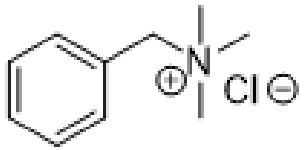
Disinfectants, pesticides, preservatives

Regularly used in a variety settings [1,2]

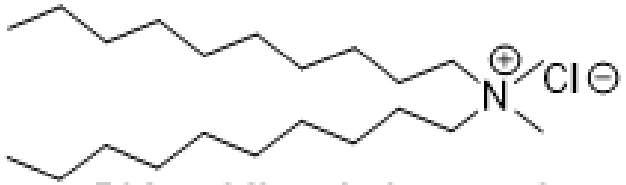
Cleaning products, eye drops, utensils, laundry detergent, milking equipment [1,2]

Dermal (eye), ingestion, inhalation exposure routes

Cetrimonium chloride



Benzotrimethylammonium chloride

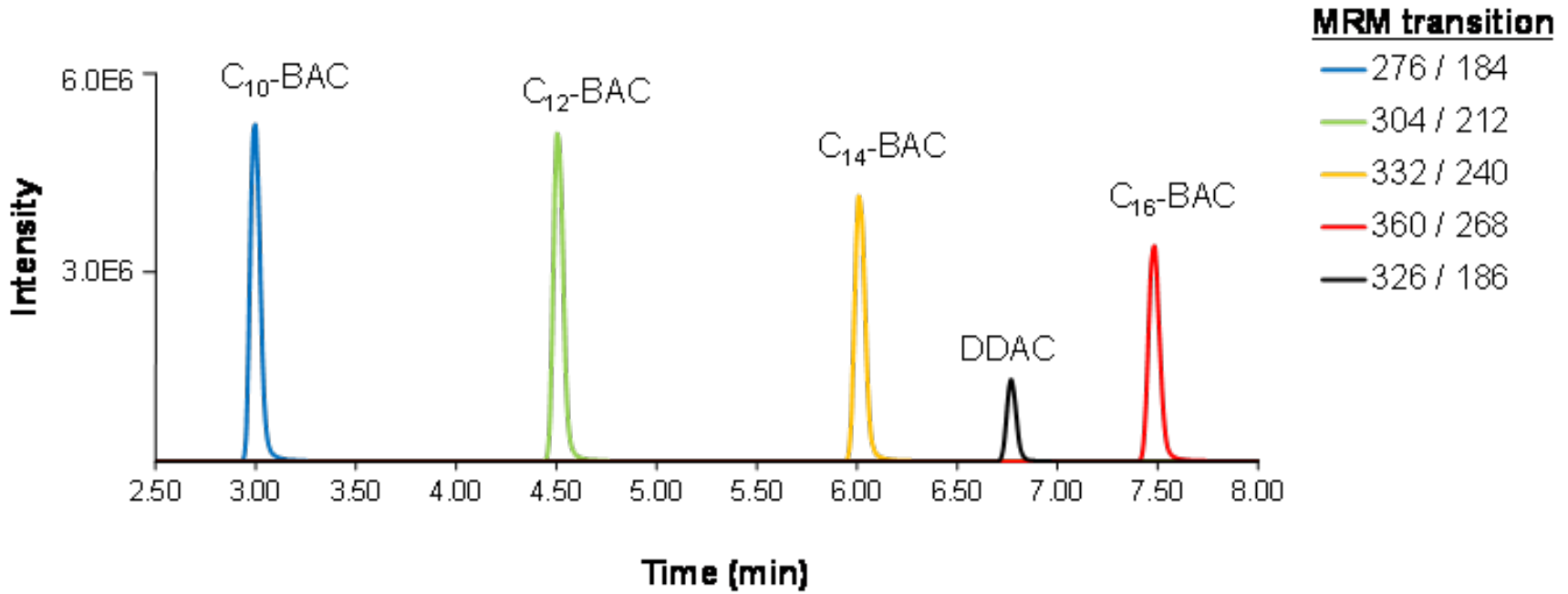


Didecyldimethylammonium chloride (DDAC)

No Public Data On QAC Exposure Levels In Humans!

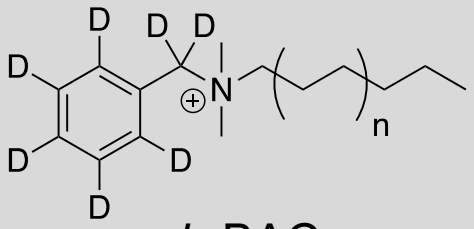
1) Hrubec, T., *et al* (2017) *Birth Defects Research*
2) US EPA (2006) RED for ADBAC, EPA739-R-06-009

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

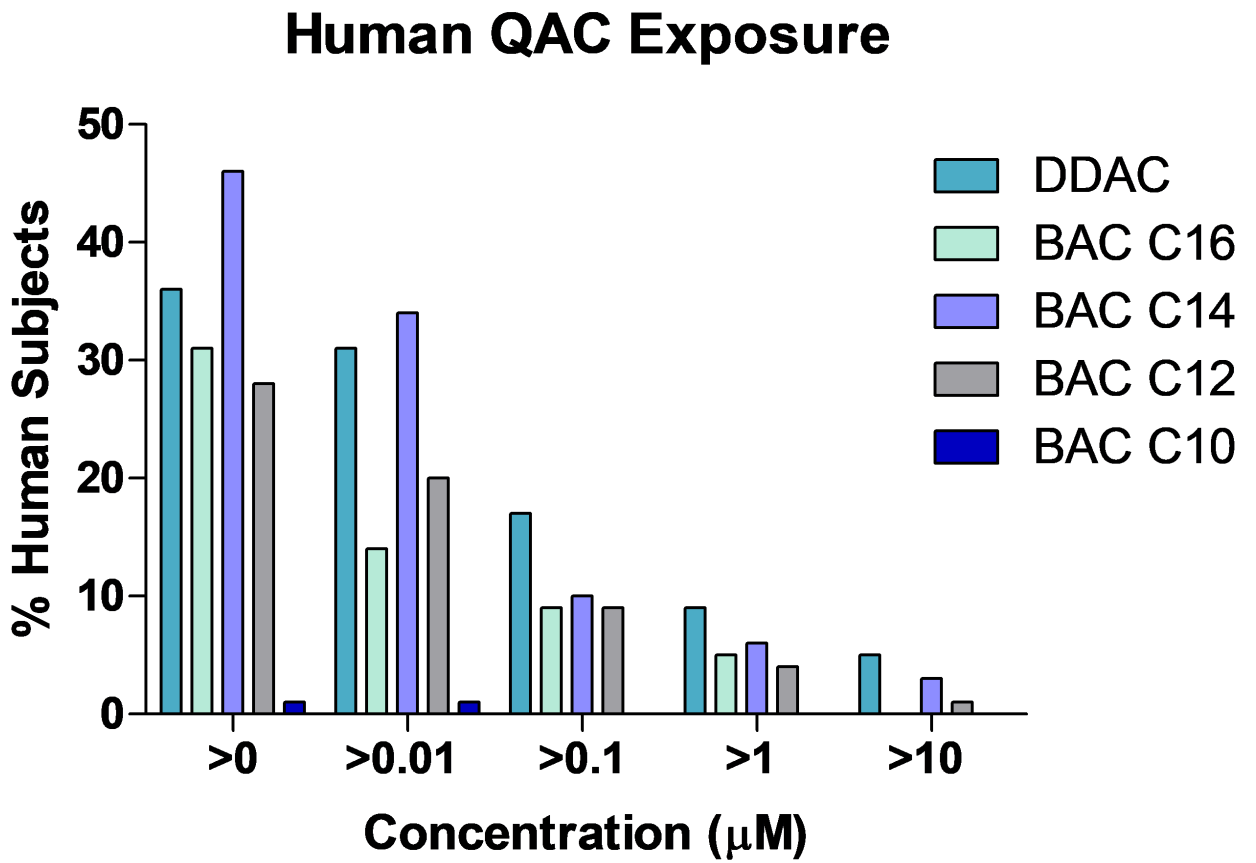
Internal Standards:



d_7-BACs

Exposure Levels in 100 Random Human Plasma Samples

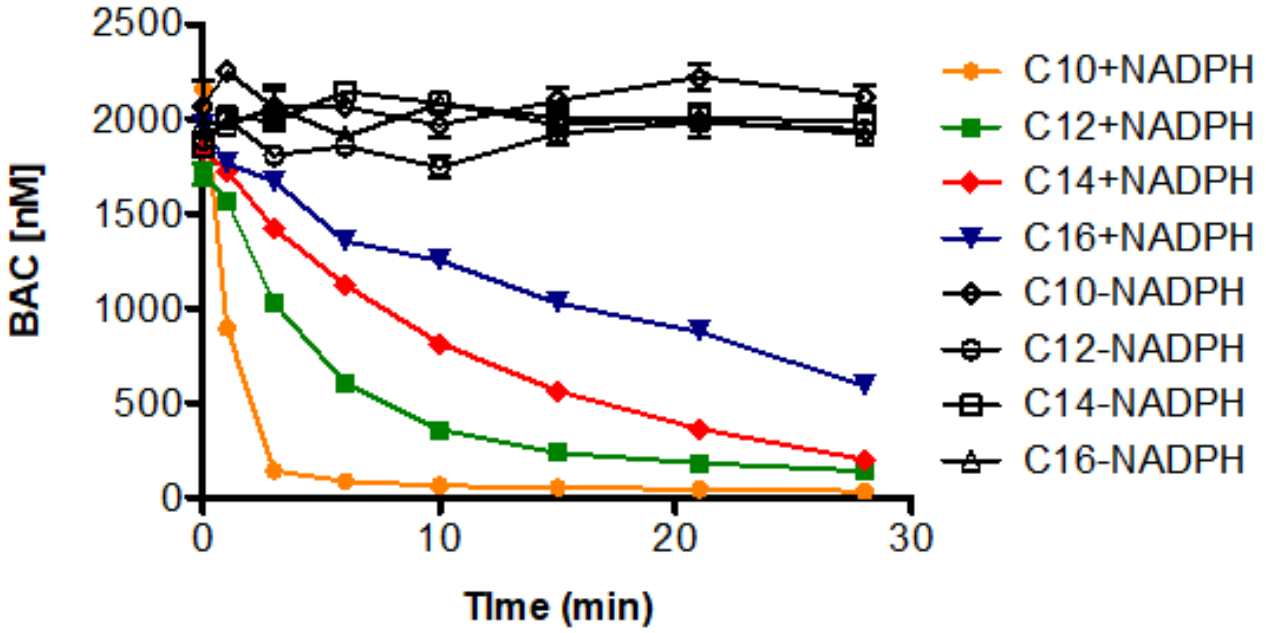
Random human plasma obtained from BioIVT



Can Human Body Metabolize QACs?

Using benzalkonium chlorides (BACs) as examples.

Metabolism by Human Liver Microsomes

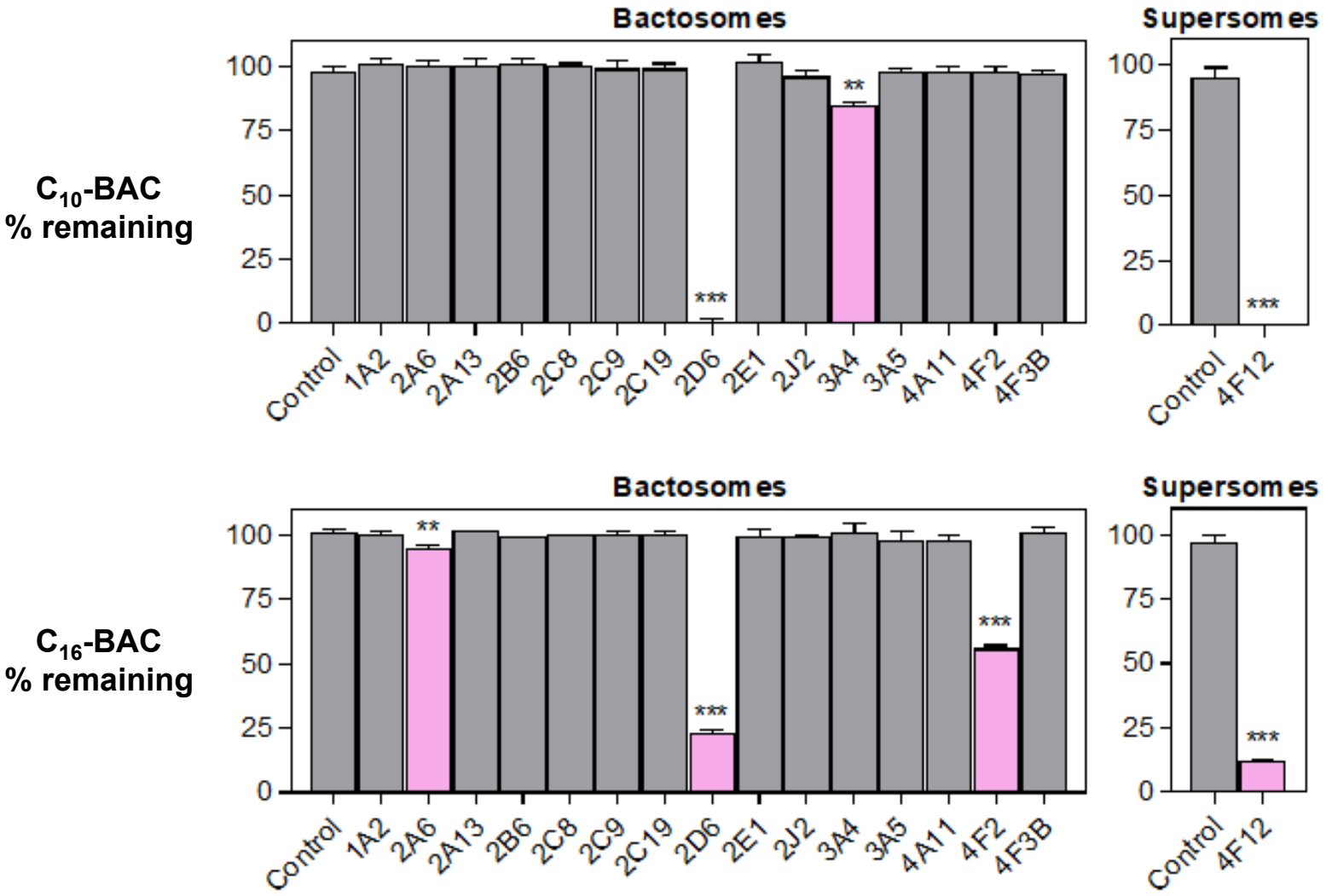


BACs	Half-Life (minutes)
C10	1
C12	3
C14	7
C16	15

Metabolism is cofactor NADPH-dependent → cytochrome P450 involvement

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

Screening Recombinant CYPs Identifies Responsible CYP Isoforms



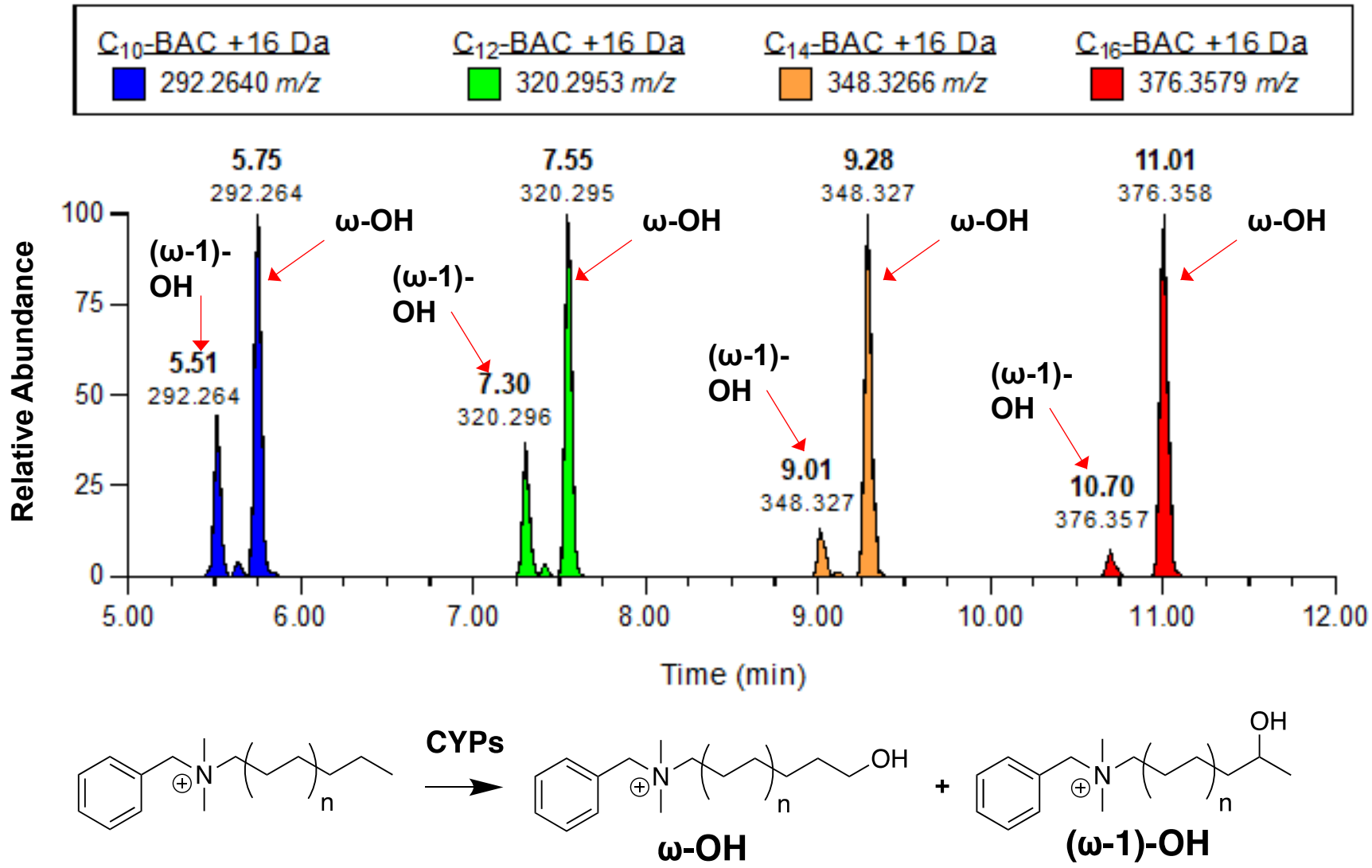
CYPs **2D6**, **4F2**, and **4F12** are major metabolizing enzymes!

These enzymes are highly polymorphic in humans!

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

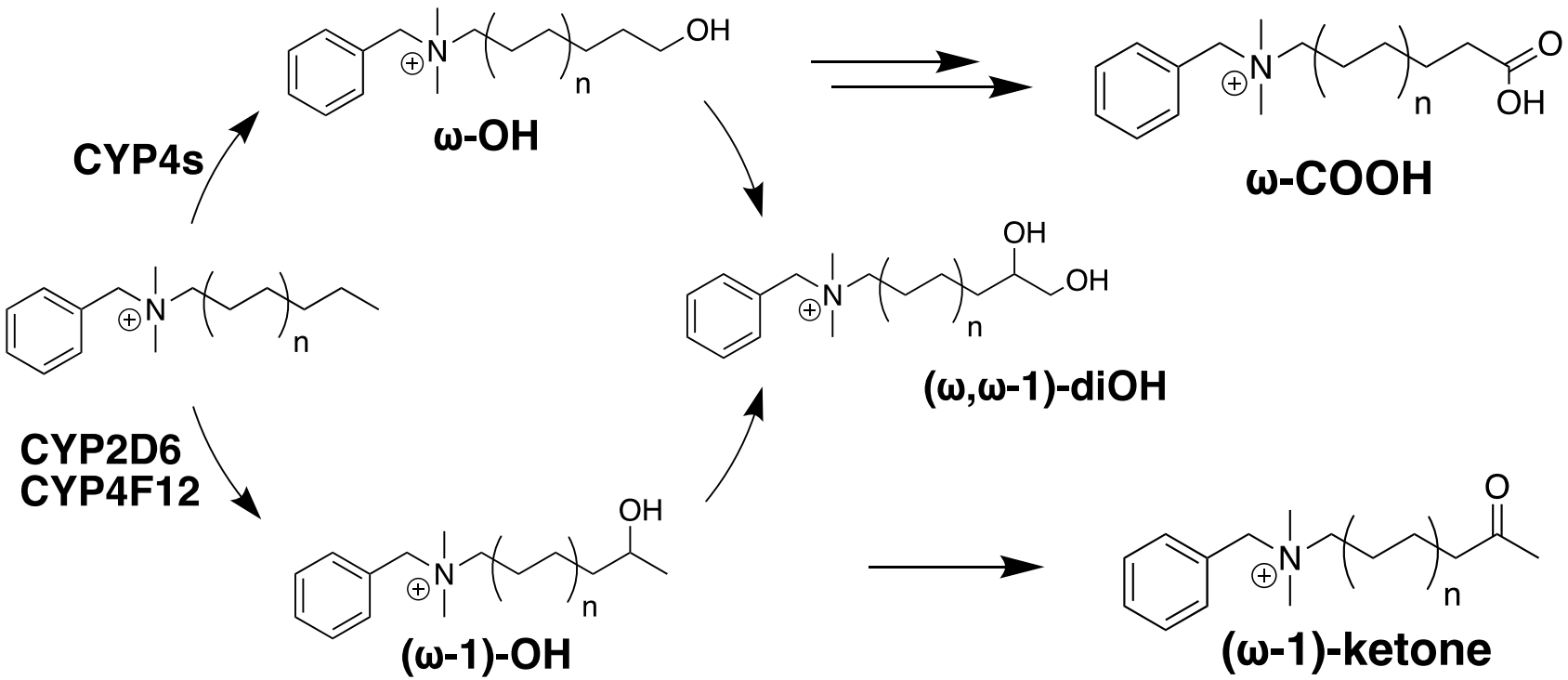
What Metabolites are Formed from BACs?

Primary products: ω- and (ω-1)-hydroxylation



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

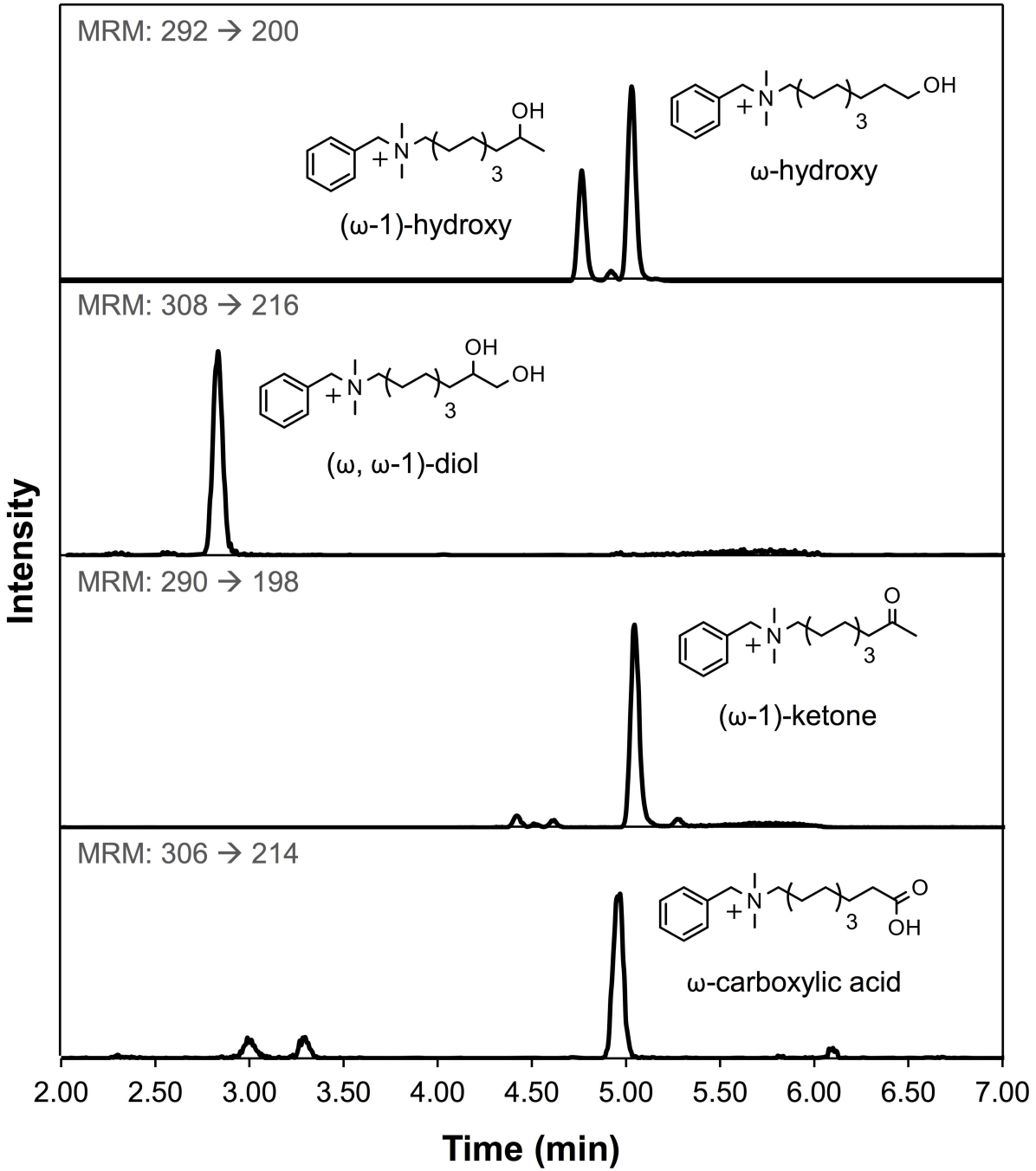
Primary Products Undergo Further Metabolism



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

Metabolites Can Be Quantified by LC-MS/MS

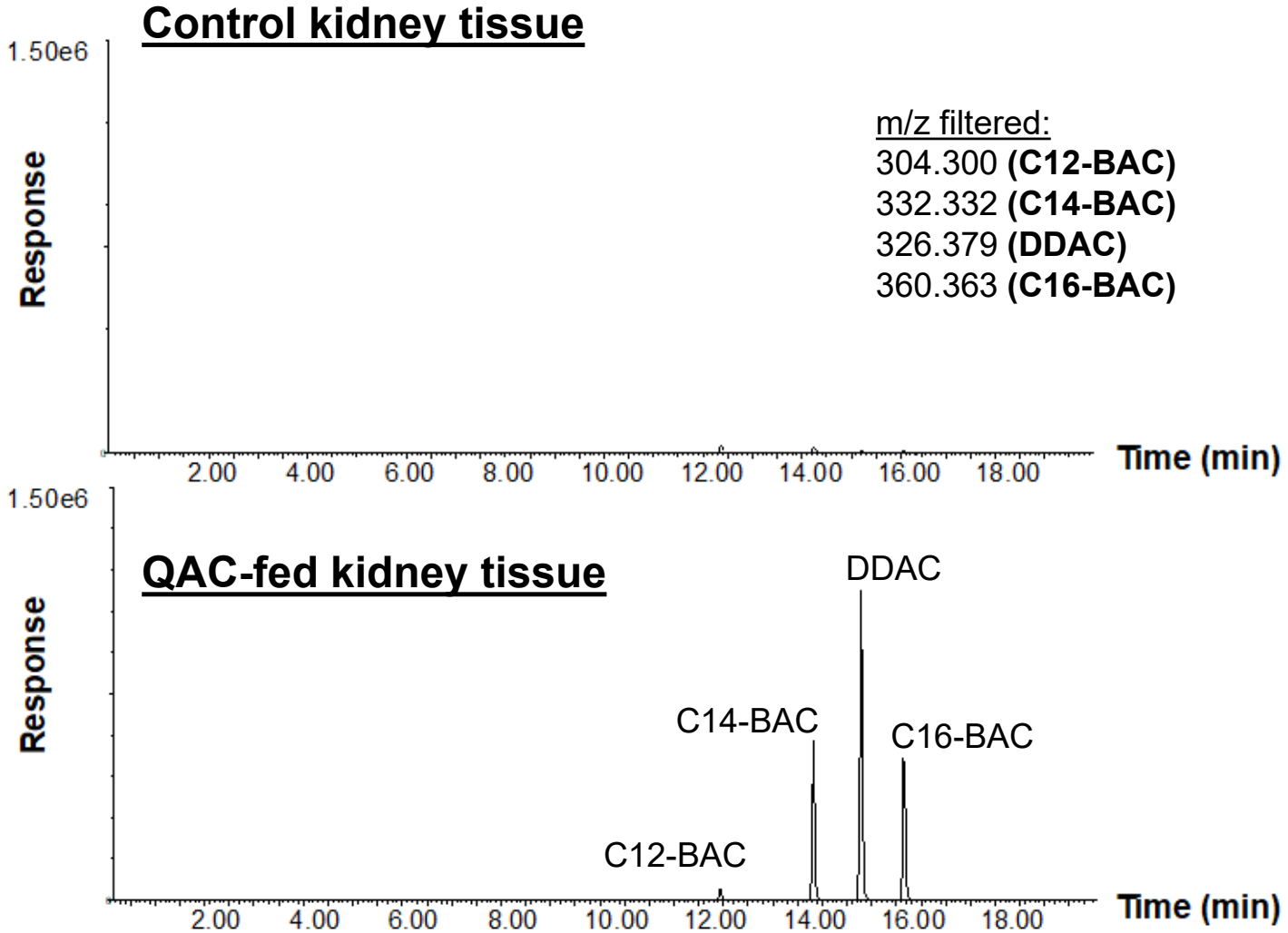
Metabolites of C_{10}-BAC



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

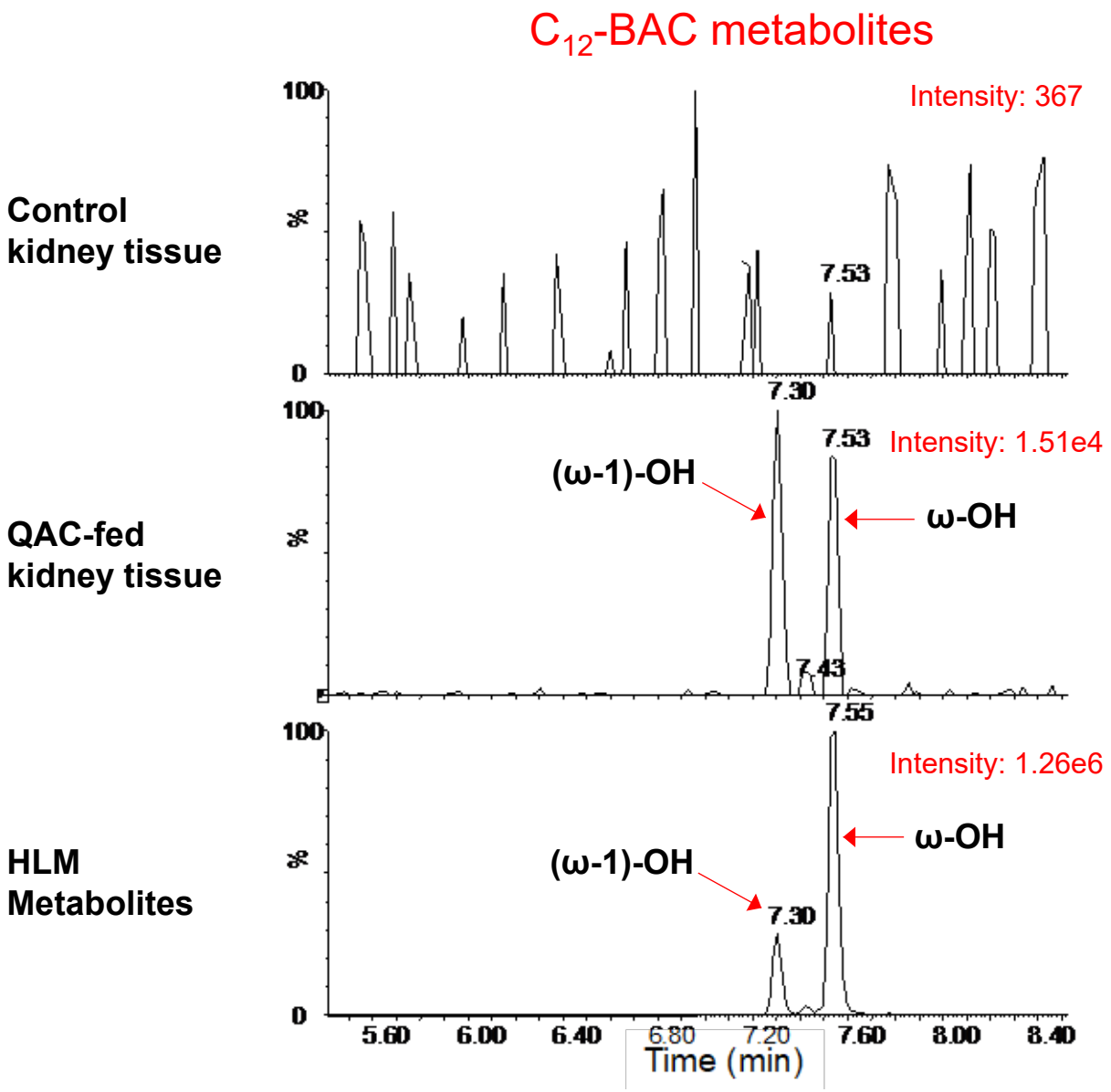
QACs Detected in Kidney Tissue from Mice Exposed to QACs Via Diet

120 mg/kg/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.***

QAC Metabolites are Observed in Mice Fed a QAC Diet



Metabolites of BAC-C14 and C16 are also observed!

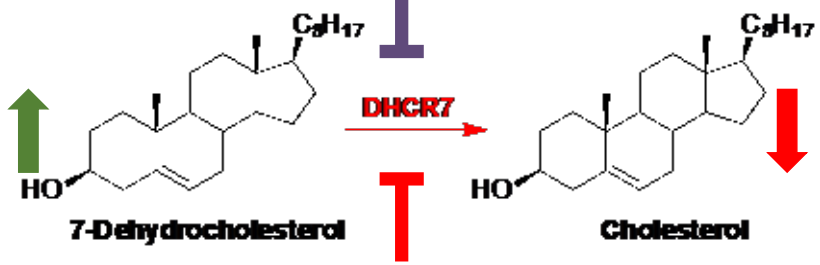
Ryan Seguin

Effect of Benzalkonium Chlorides on Cholesterol and Lipid Homeostasis: Why Are We Interested in It?

Why Do We Study Benzalkonium Chlorides (BACs)?

- 1 in 10,000 to 60,000
- Congenital malformation, mental retardation, autistic behavior
- Carrier frequency: 1 in 30

Mutations ⇒ **Smith-Lemli-Opitz syndrome (SLOS)**



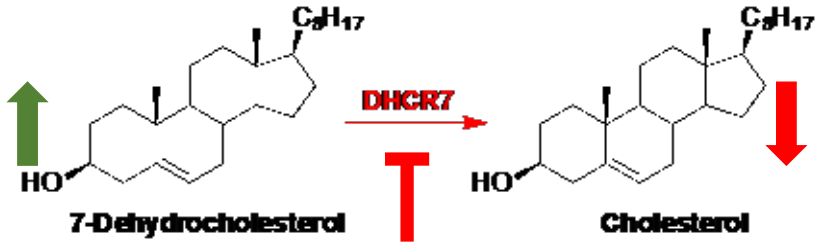
Inhibition by drugs or environmental molecules ⇒ **Neurodevelopmental Defects**

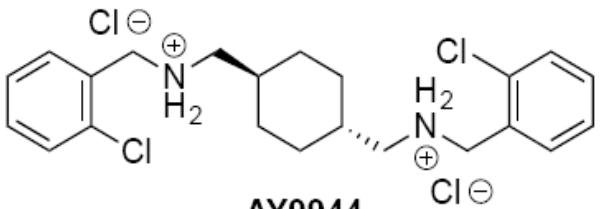
Small molecule inhibitors of DHCR7:

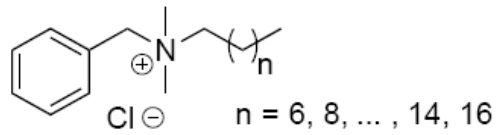
- Pharmaceuticals: breast cancer, antipsychotics
- **Disinfectants: benzalkonium chlorides**

Porter, F. D.; Herman, G. E. *J. Lipid. Res.* **2011**, *52*, 6.
Canfrán-Duque et al. *J. Lipid Res.* 2013
Korade, Z. et al. *J. Med. Chem.* 2016
Herron, J. et al. *Tox. Sci.* **2016**, *151*, 261.

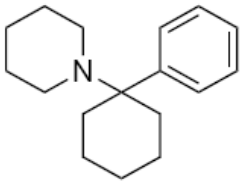
Benzalkonium Chlorides Are Structurally Similar to Known Inhibitor of DHCR7, AY9944



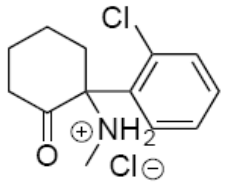




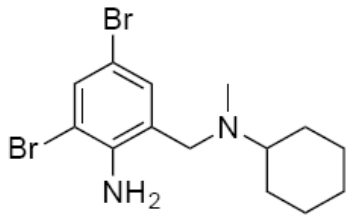
Benzalkonium chloride (BAC)
72.9%



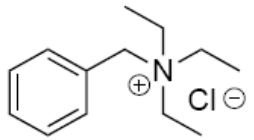
Phencyclidine
57.4%



Ketamine
51.3%



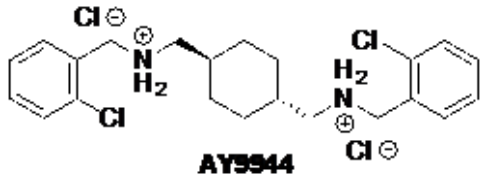
Bromhexine
48.1%



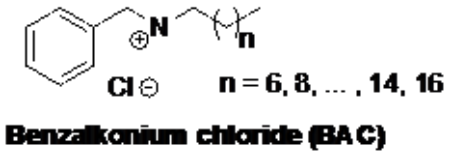
Benzyltriethylammonium chloride, 46.5%

"%" indicates similarity score.

Benzalkonium Chlorides Inhibit DHCR7 in a Chain Length-Dependent Manner

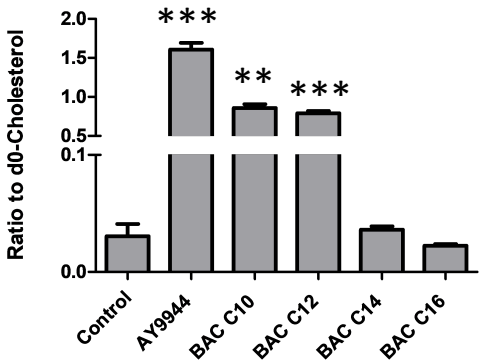


Known inhibitor

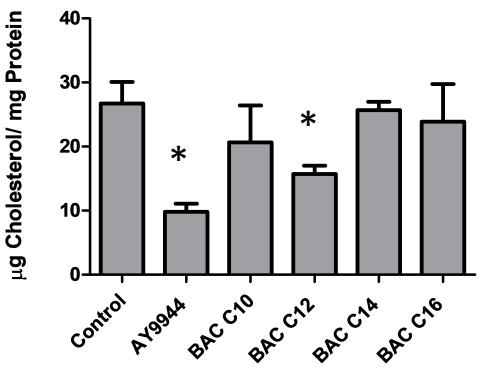


BAC: highest similarity to AY9944 (72.9%)
among molecules in Tox21

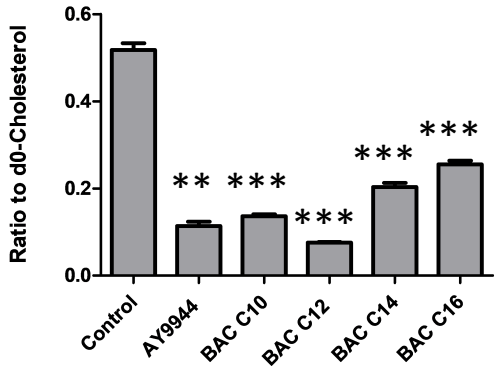
7-Dehydrocholesterol



Cholesterol



Desmosterol

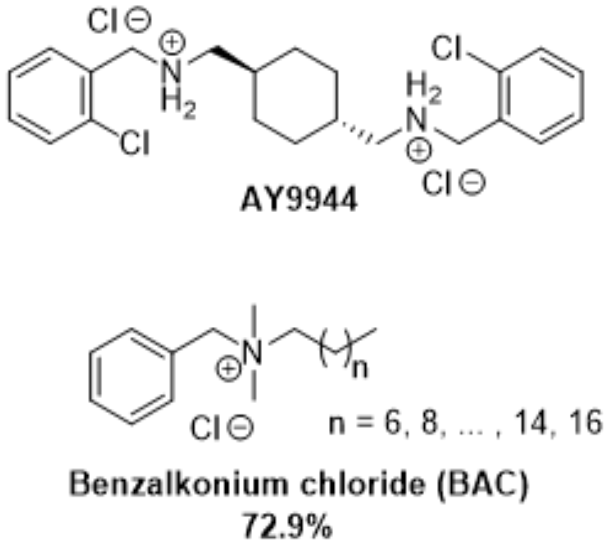


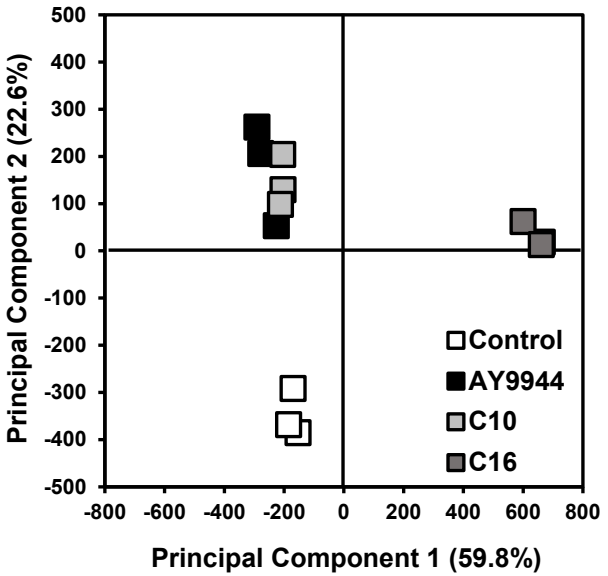
*, $p < 0.05$
**, $p < 0.005$
***, $p < 0.0005$

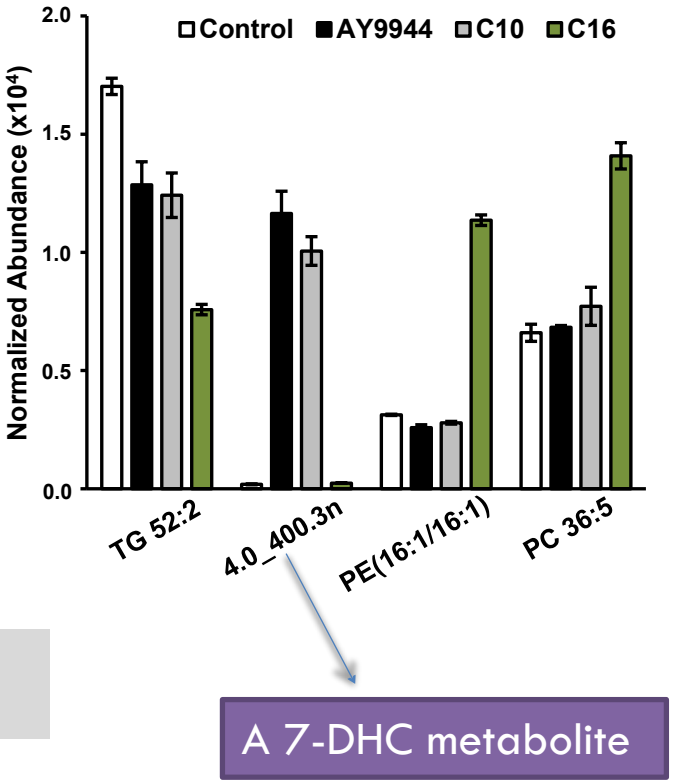
Neuro2a cells were exposed to each compound at 100 nM for 2 days

Herron et al. *Tox. Sci*. 2016

BACs Alter Lipid Homeostasis in Neuronal Cells







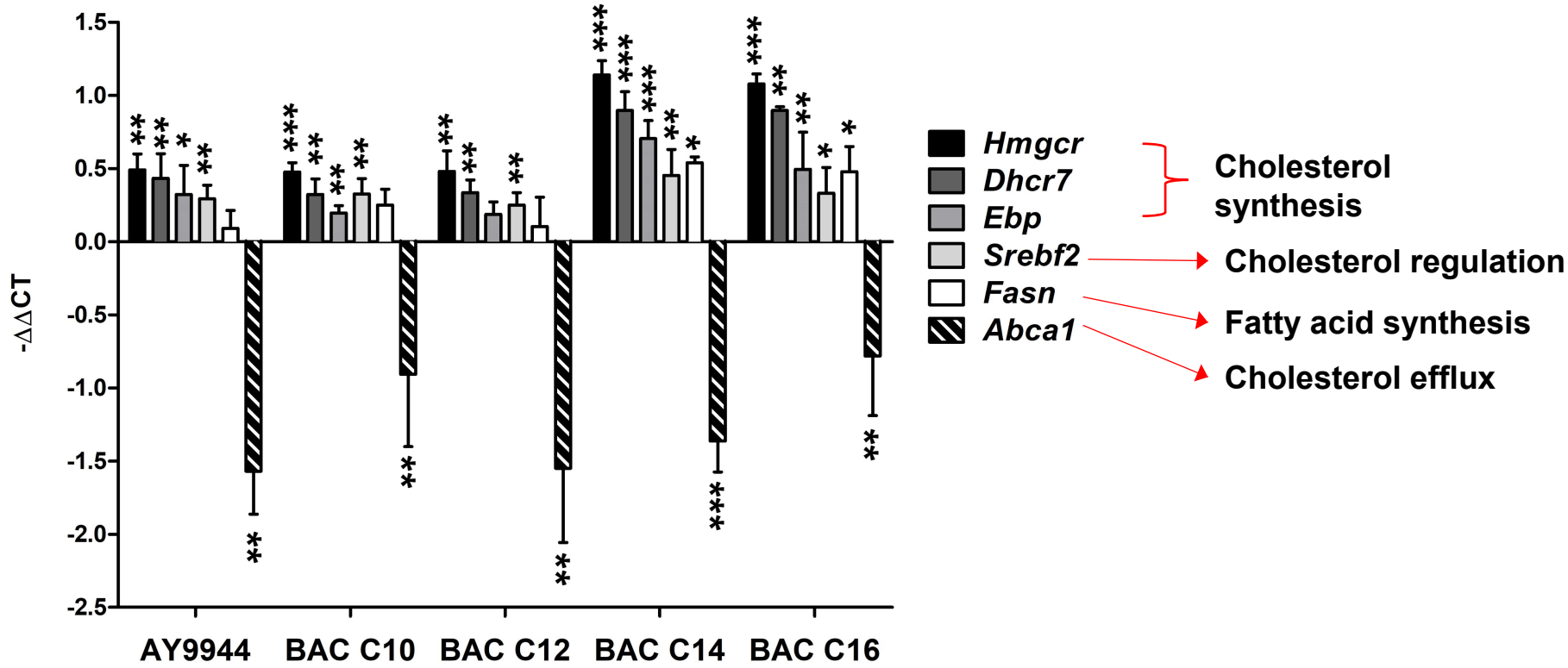
Neuro2a cells were exposed to each compound at 100 nM for 2 days

A 7-DHC metabolite

Herron, J. *et al.* (2016) *Toxicol. Sci.*

Hines, Herron, and Xu. (2017) *J. Lipid Res.*

Individual BACs Alter Gene Expression Involved in Sterol and Lipid Homeostasis

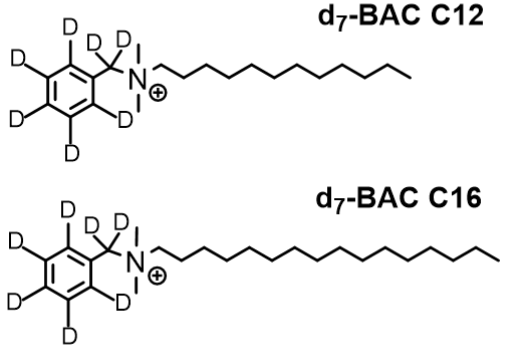


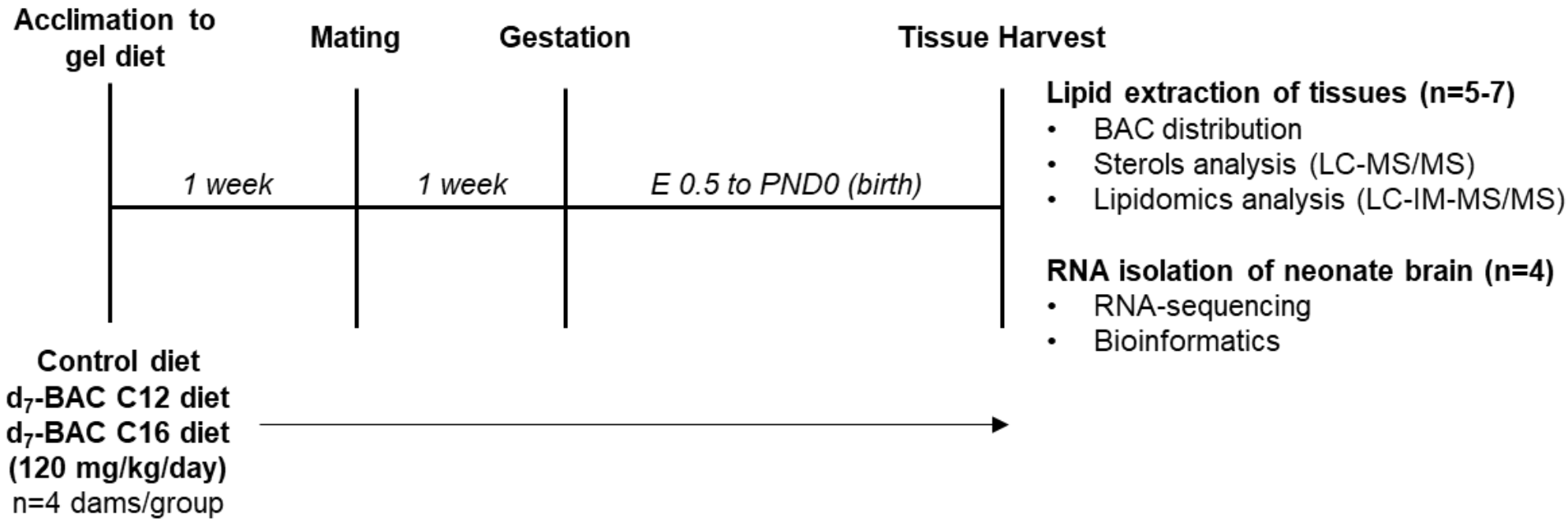
Herron, J. *et al.* (2016) *Toxicol. Sci.*

Hines, Herron, and Xu. (2017) *J. Lipid Res.*

Can BACs Alter Sterol and Lipid Homeostasis In Developmental Brain?

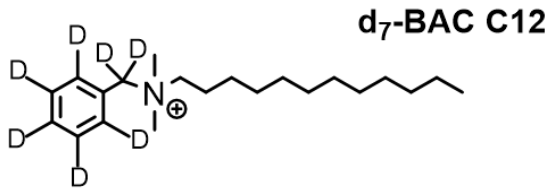
In utero Exposure to BACs – Experimental Design

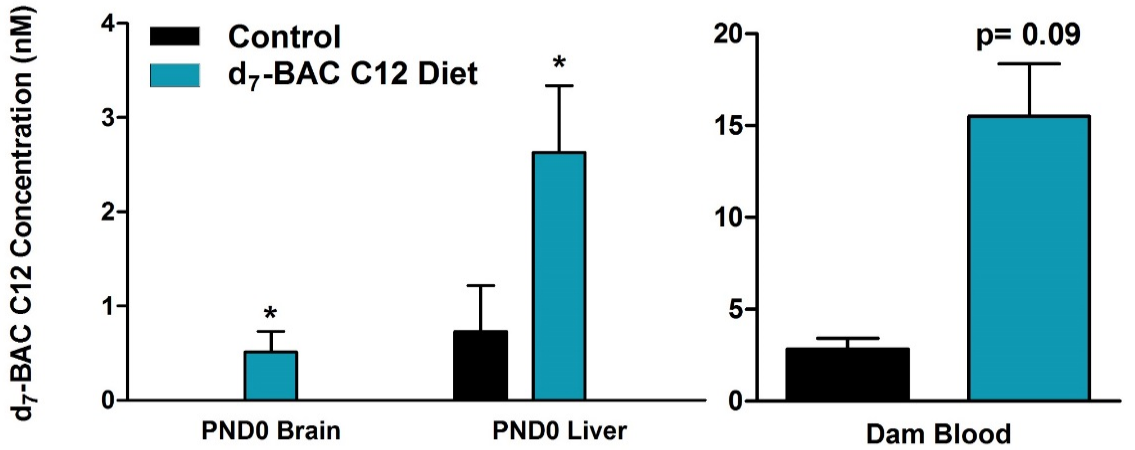


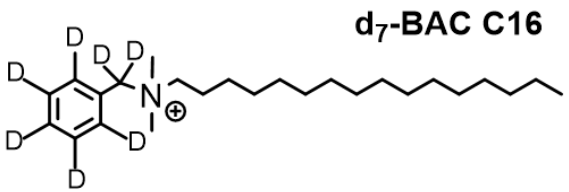


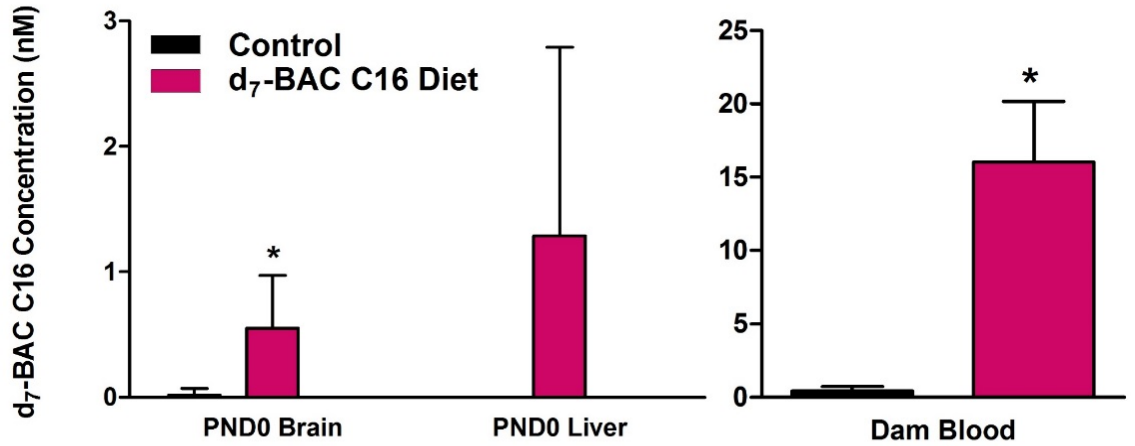
Herron, *et al.* (2019) *Toxicol. Sci.*

BACs Cross the Placenta and Enter the Developing Brain



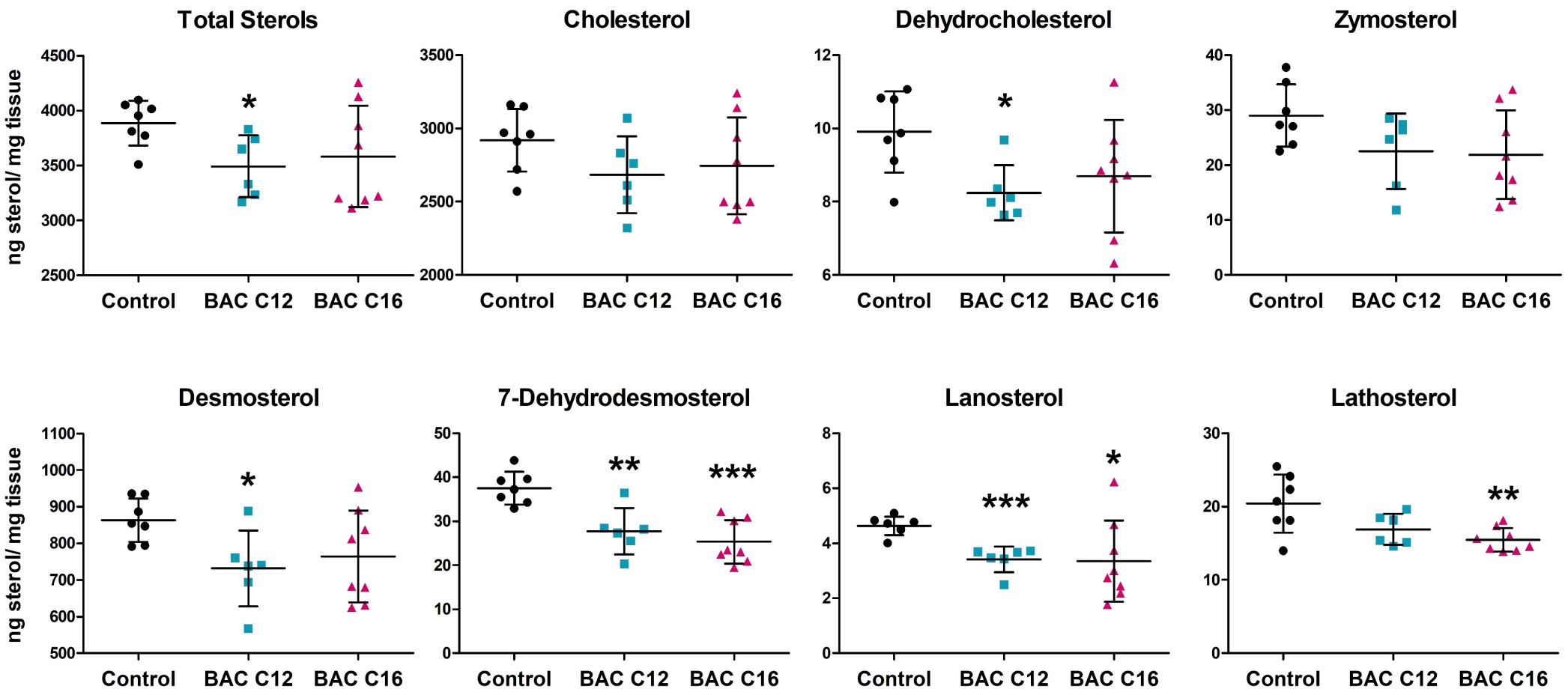






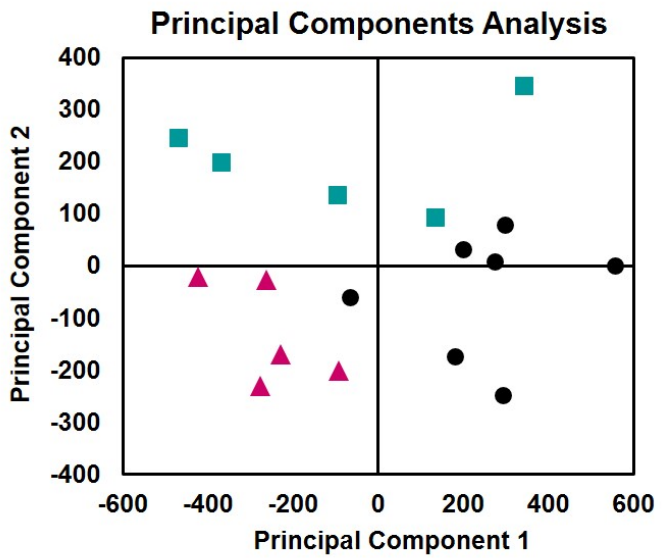
Herron, *et al.* (2019) *Toxicol. Sci.*

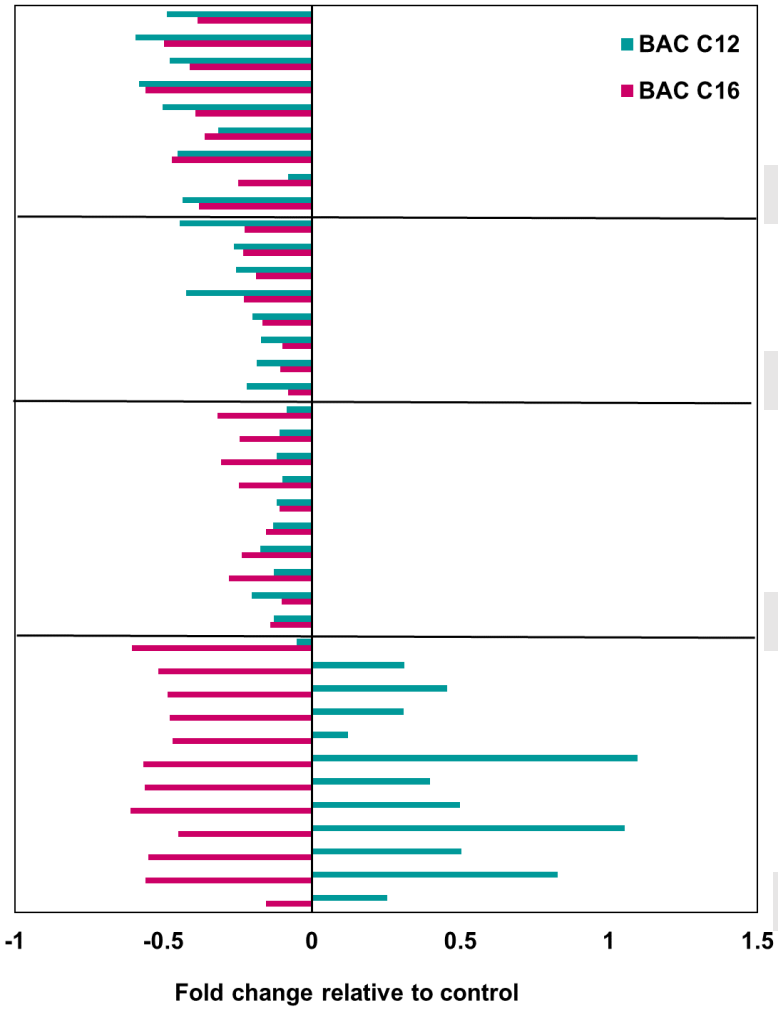
BACs Decrease Sterols in Neonatal Brains

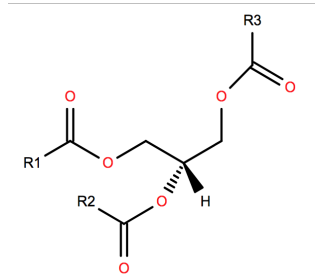


Herron, *et al.* (2019) *Toxicol. Sci.*

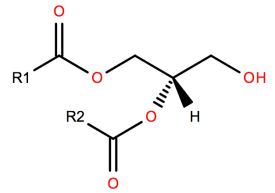
BACs Alter Lipidome of Neonatal Brains



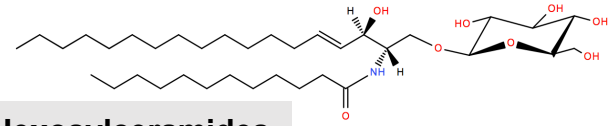




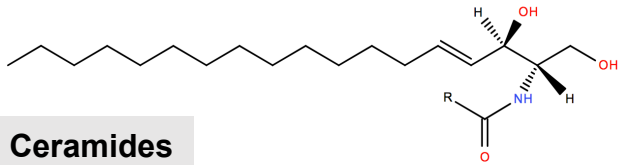
Triglycerides



Diglycerides



Hexosylceramides

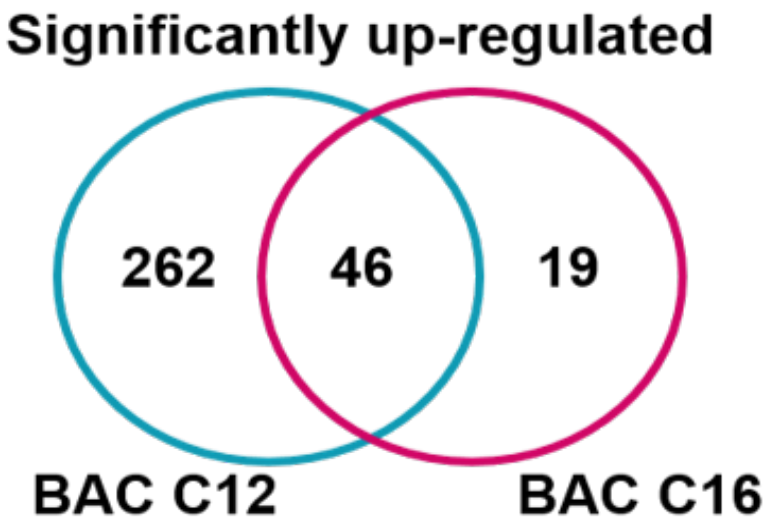


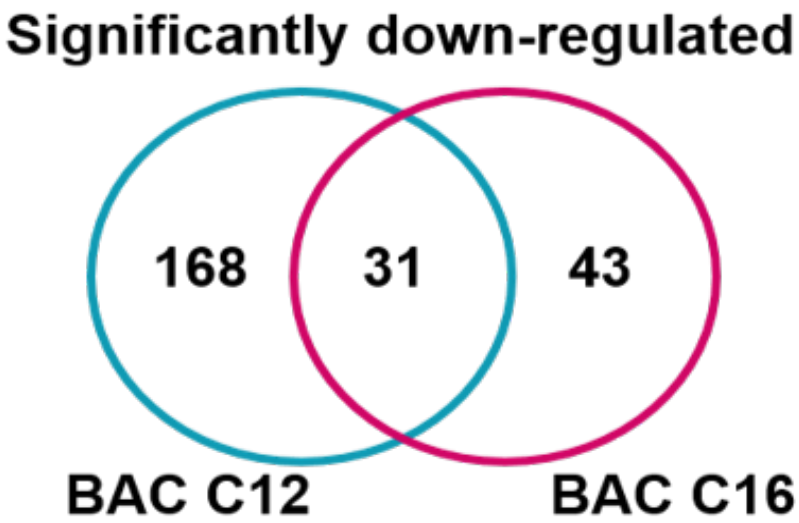
Ceramides

Herron, *et al.* (2019) *Toxicol. Sci.*

What Underlying Gene Expression Changes Are Associated with Alterations in Sterols and Lipids? RNA Sequencing

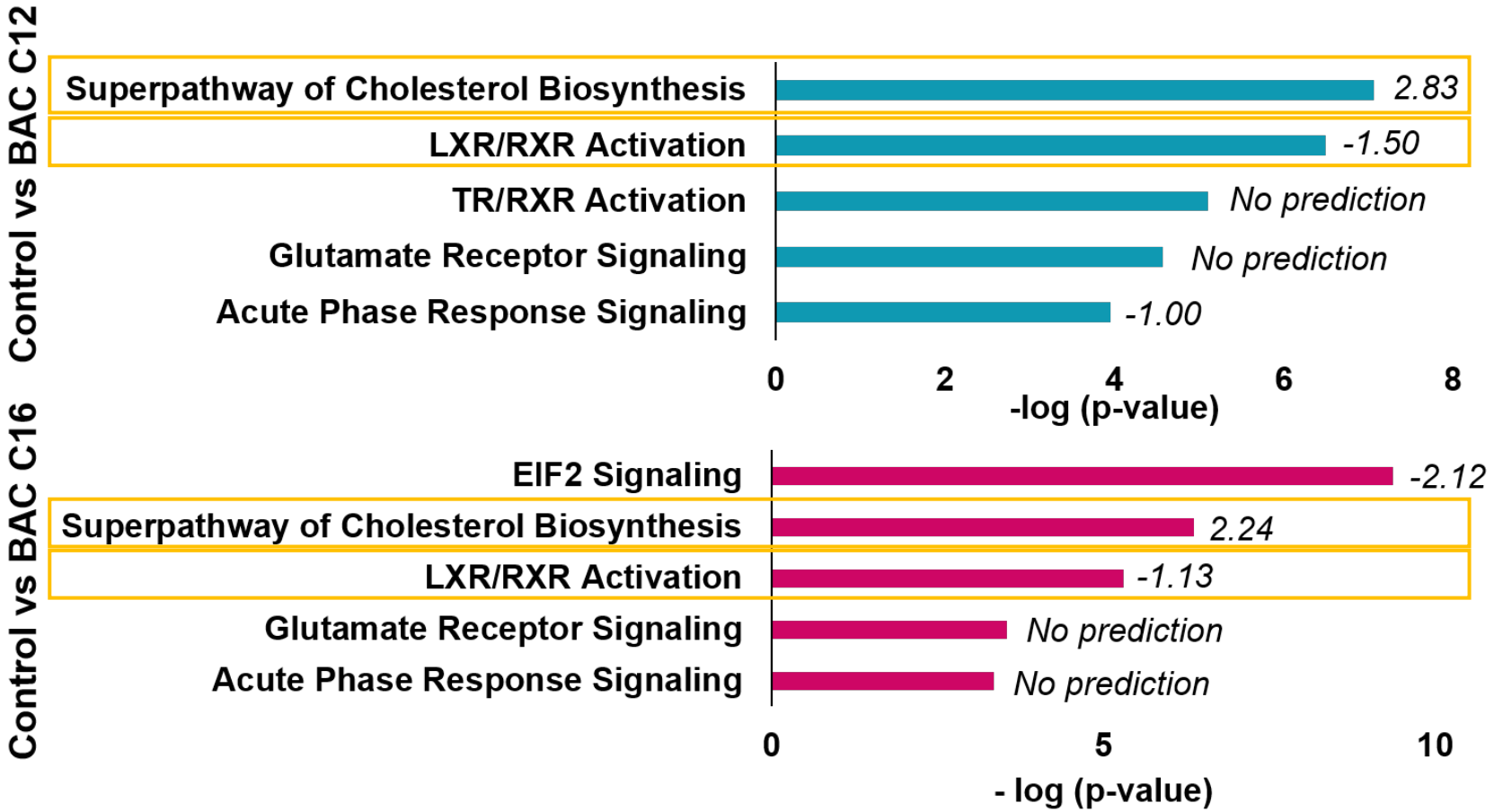
Global Gene Expression Changes in Exposed Neonatal Brains





Herron, *et al.* (2019) *Toxicol. Sci.*

Cholesterol Biosynthesis Pathway is Activated in Exposed Neonatal Brains

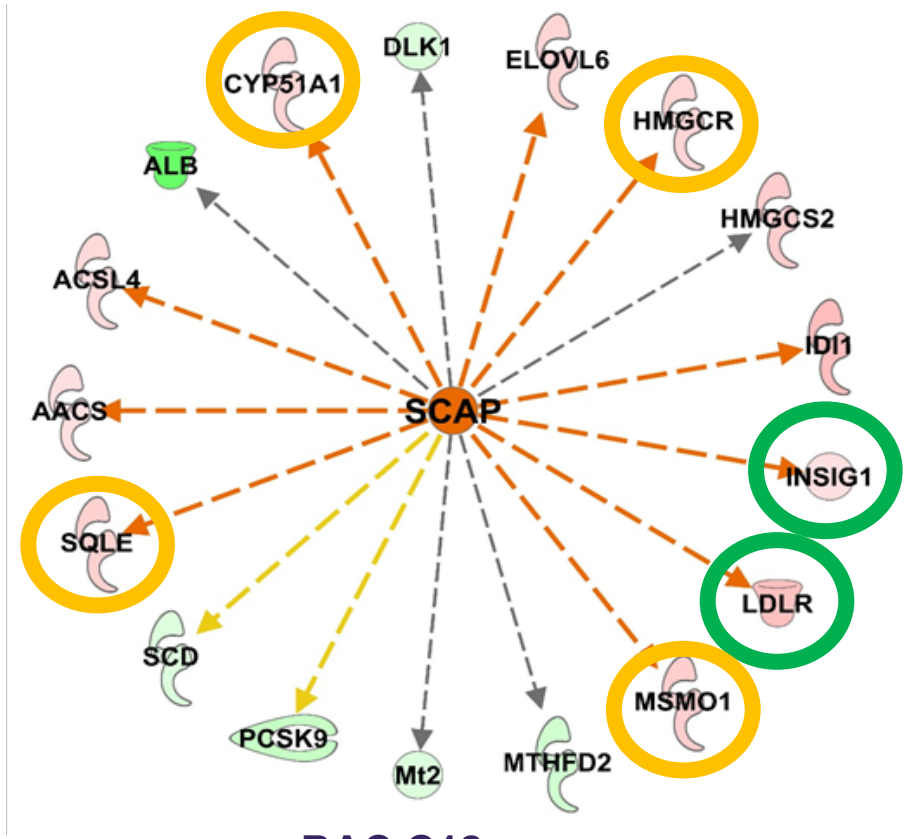


Herron, *et al.* (2019) *Toxicol. Sci.*

Upstream regulator SREBP cleavage-activating protein (SCAP) is activated in exposed neonatal brains

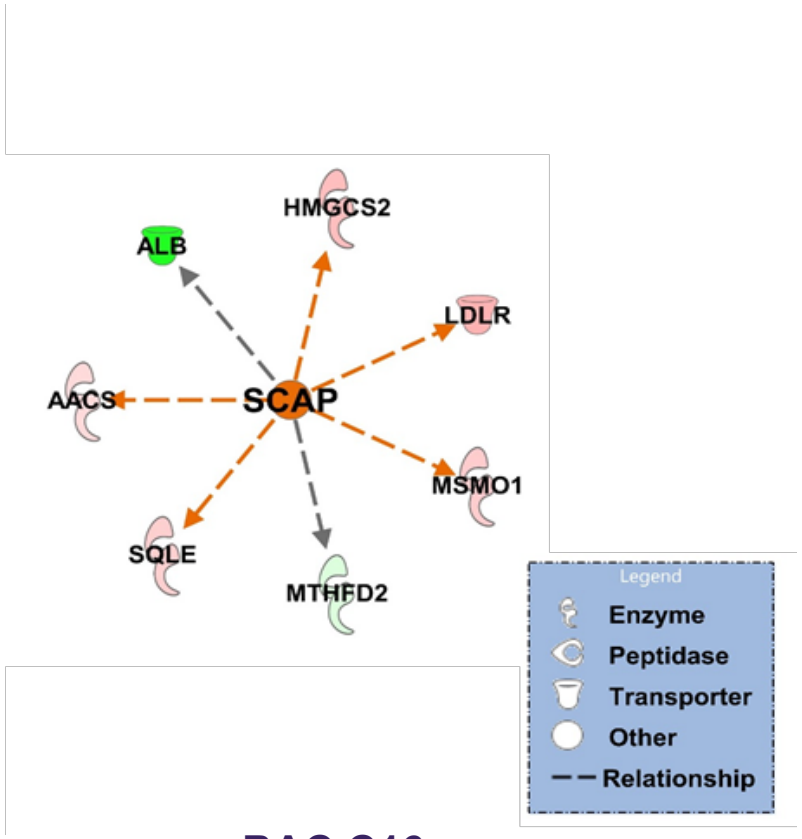
Cholesterol synthesis

Cholesterol regulation



BAC C12

Adjusted *P*-value = 2.02E-12



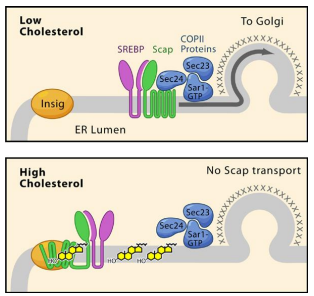
BAC C16

Adjusted *P*-value = 4.37E-07

Herron, *et al.* (2019) *Toxicol. Sci.*

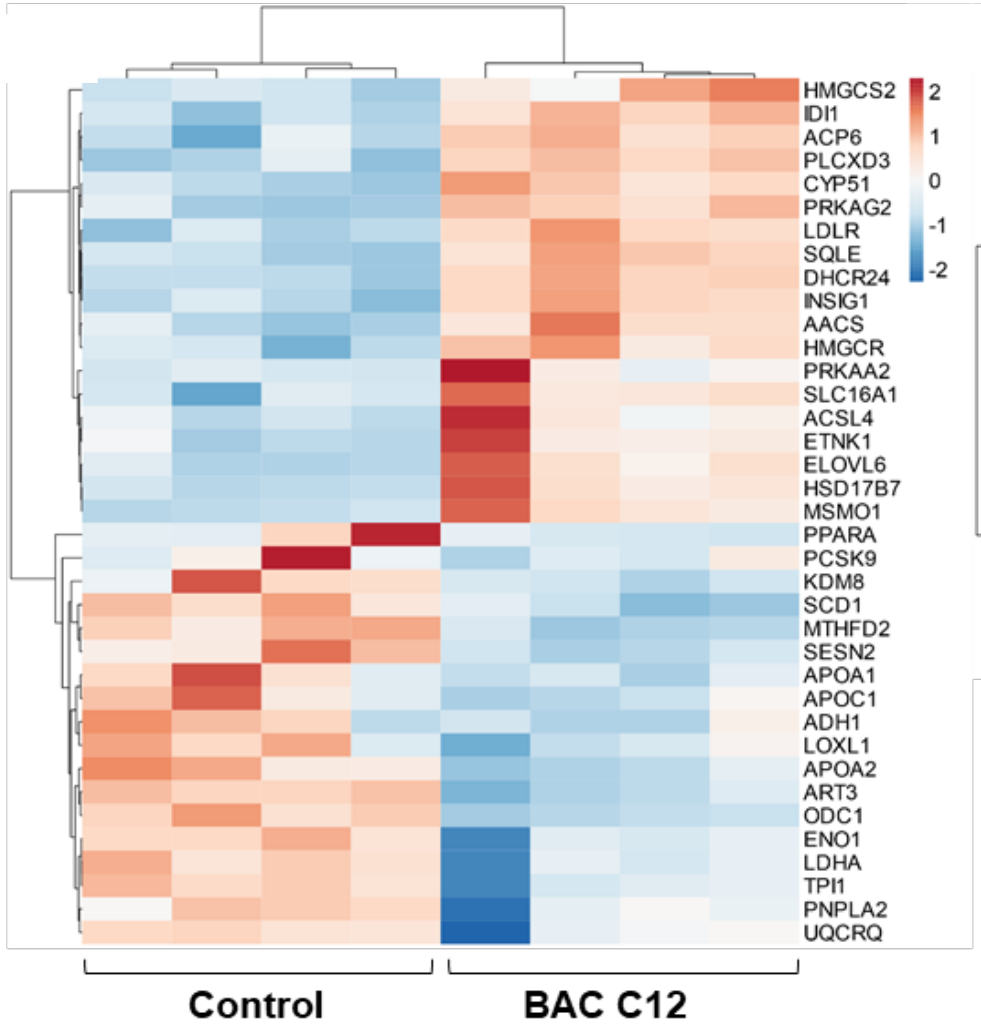
Regulation of Cholesterol Homeostasis by Scap and Insig

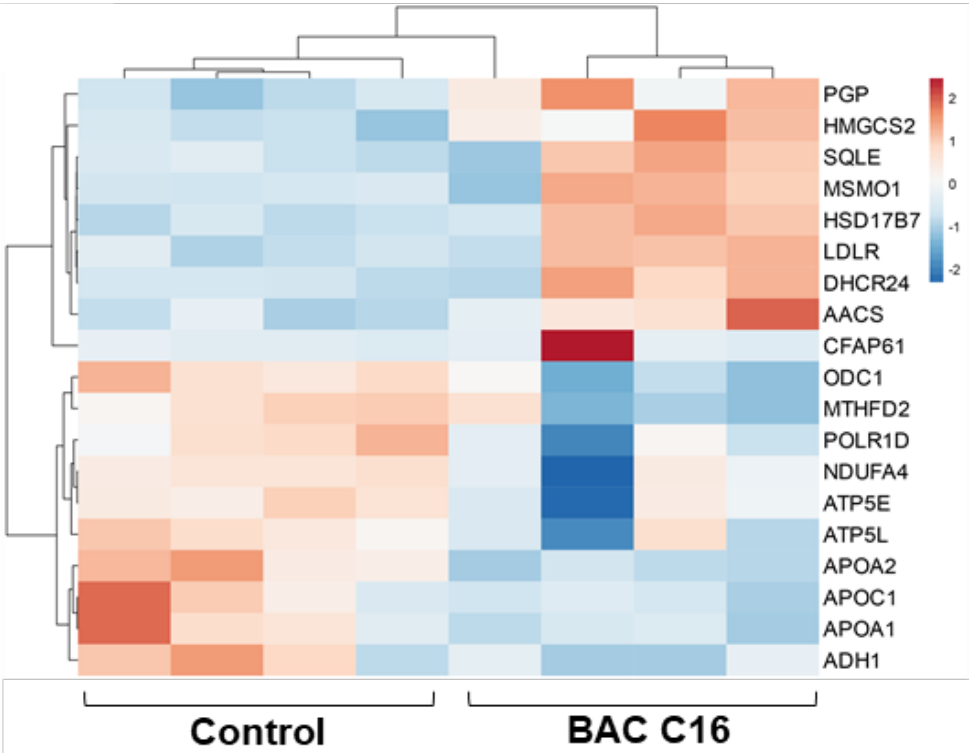
Goldstein, J. L., et al. (2006).
Cell, *124****(1), 35–46.***



Distinct Expression Patterns of Genes Involved in Sterol- And Lipid- Homeostasis

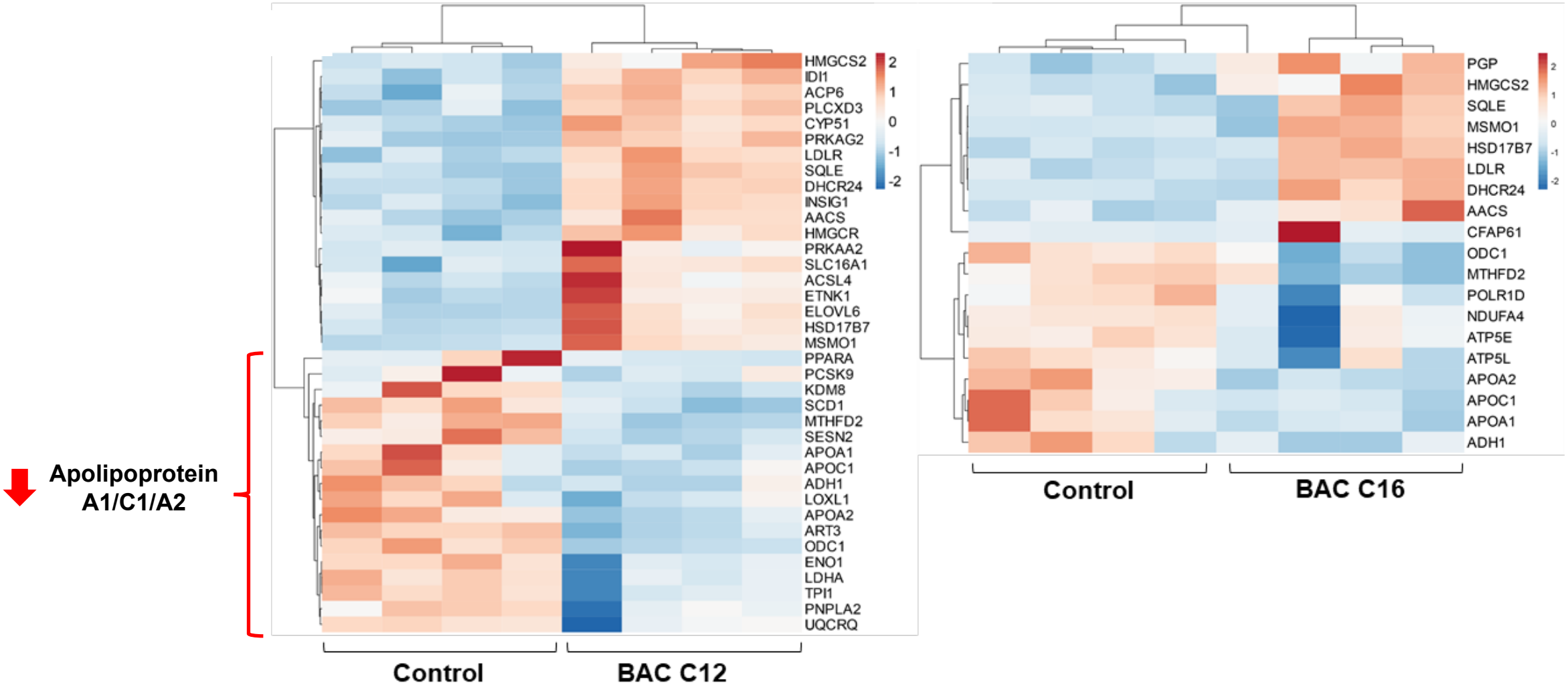
- **Cholesterol biosynthesis genes**
- **Insulin induced gene 1 (Insig1)**
- **Low density lipoprotein receptor**
- **ELOVL fatty acid elongase 6**





Herron, *et al.* (2019) *Toxicol. Sci.*

Distinct Expression Patterns of Genes Involved in Sterol- And Lipid- Homeostasis



Herron, *et al.* (2019) *Toxicol. Sci.*

Summary

- QACs are observed in random human plasma samples.
- BACs can be metabolized by human cytochromes P450.
- BACs and metabolites can be quantified by LC-MS/MS and both should be used for biomonitoring for complete assessment of BAC exposure.
- BAC exposure leads to elevated levels of parent compounds and metabolites in dam and neonatal tissues.
- BACs disrupt cholesterol and lipid homeostasis both *in vitro* and *in vivo.*

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