Supplemental Material for

Multi-omics investigation reveals benzalkonium chloride disinfectants alter sterol and lipid homeostasis in the mouse neonatal brain

Author names and affiliations

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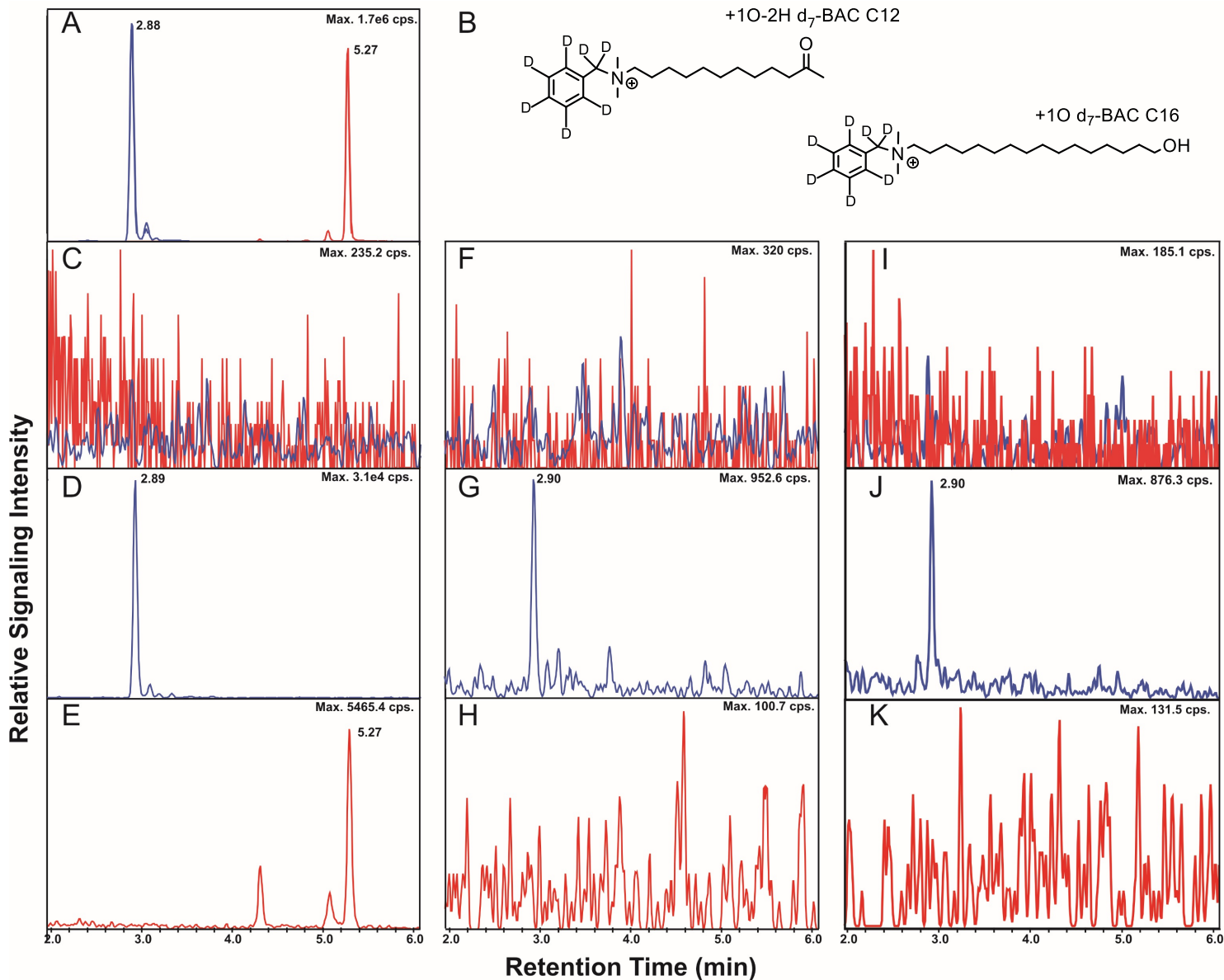
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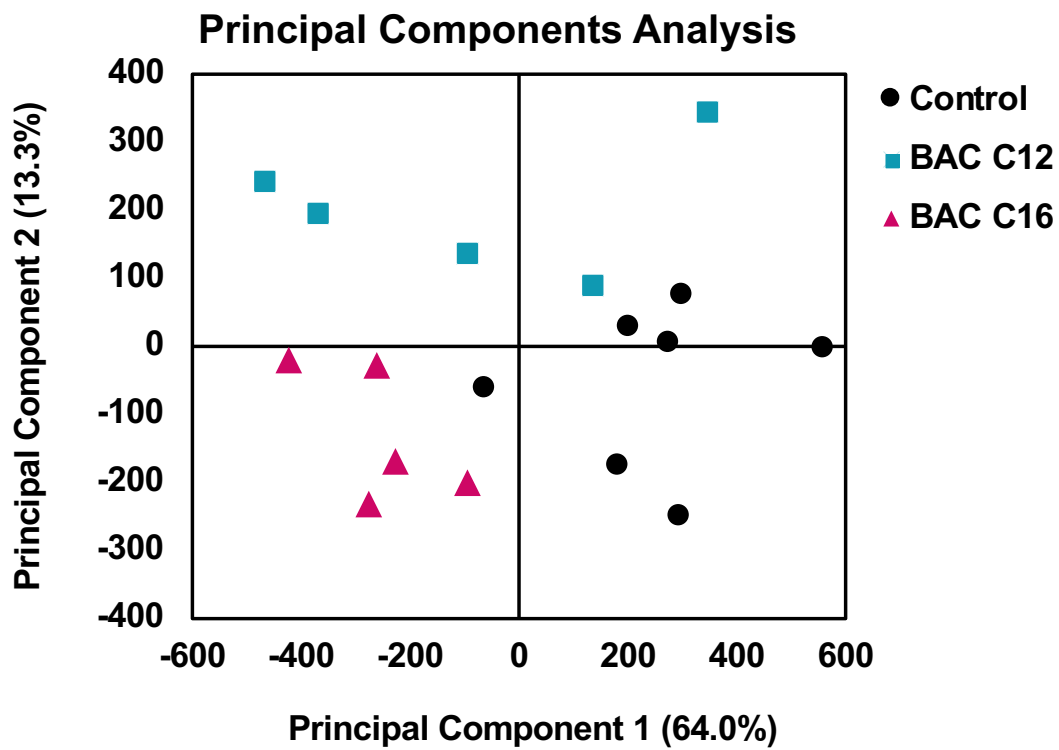
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Supplemental Table 1. Genes selected for qPCR validation and purchased from ThermoFisher Scientific.

Gene	TaqMan™ Assay ID
ACTB	Mm00607939_s1
CYP51	Mm00490968_m1
DHCR24	Mm00519071_m1
HMGCR	Mm01282499_m1
HMGCS2	Mm00550050_m1
INSIG1	Mm00463389_m1
SCAP	Mm01250176_m1
SQLE	Mm00436772_m1
SREBF1	Mm00550338_m1
SREBF2	Mm01306292_m1

Supplemental Figure 1. (A) Chromatogram of the major BAC metabolites +1O-2H d_7-BAC C12 (blue) and +1 O d_7-BAC C16 (red) and (B) corresponding structures. Metabolites in dam blood: (C) control dam blood, no metabolites detected; (D) d_7-BAC C12 -exposed dam blood; and (E) d_7-BAC C16 -exposed dam blood. Metabolites in neonatal brain: (F) control neonatal brain, no metabolites detected; (G) d_7-BAC C12 -exposed neonatal brain; and (H) d_7-BAC C16 -exposed neonatal brain, no metabolite detected. Metabolites in neonatal liver: (I) control neonatal liver, no metabolites detected; (J) d_7-BAC C12 -exposed neonatal liver, and (K) d_7-BAC C16 -exposed neonatal liver, no metabolite detected.

Principal Components Analysis

Supplemental Figure 2. In the PCA of the lipids from different treatment groups, BAC-exposed groups separate from controls primarily along PC-1, although some overlap is observed. BAC C12 separates from BAC C16 along PC-2. n=5-7 biological replicates per condition.

Supplemental Table 2. Altered lipids in neonatal brains exposed *in utero* to BAC C12 or BAC C16.

Annotation	m/z	Retention Time (min)	Mass Accuracy (ppm)	Abundance in Control	Fold Change[a,b]	
					BAC C12	BAC C16
DG 34:6 Na	639.498	0.5	11.0	453 ± 59	0.8 *	0.9
DG 36:6	595.473	0.4	0.7	481 ± 33	0.8	0.9
DG 36:2	659.501	0.4	8.0	882 ± 73	0.8	0.9
DG 32:1	549.488	0.4	3.2	1741 ± 343	0.6 **	0.8
DG 34:2	575.504	0.4	6.0	1928 ± 204	0.7 **	0.8
DG 36:1	605.551	0.4	10.7	2268 ± 197	0.7 *	0.8
DG 34:1	577.520	0.5	2.5	7228 ± 1083	0.6 ***	0.8
TG 50:5	842.723	0.5	18.3	354 ± 29	0.9	0.8
TG 48:2	820.739	0.5	6.8	737 ± 105	0.5 *	0.5
TG 54:2	904.833	0.5	11.5	1094 ± 149	0.5 *	0.6
TG 48:1	822.755	0.5	6.1	2301 ± 305	0.4 *	0.4
TG 50:2	848.770	0.5	4.4	2846 ± 497	0.5 *	0.6
TG 50:1	850.786	0.5	5.8	4284 ± 600	0.4 **	0.5
TG 52:2	876.802	0.5	5.0	6303 ± 893	0.5 *	0.6
Cer (d18:1/26:1)	658.650	0.5	10.1	174 ± 27	1.2	0.8
Cer (d18:1/20:0)	558.562	0.5	11.9	295 ± 35	1.0	0.8 *
Cer (d18:0/24:0)	634.650	0.5	19.1	501 ± 84	1.0	0.7 *
Cer (d18:1/16:1)	536.504	0.5	1.2	3880 ± 301	0.9	0.8 **
Cer (d18:1/18:0)	566.551	0.5	0.5	4272 ± 507	1.0	0.8 *
Cer (d18:1/16:0)	502.499	0.5	3.7	4487 ± 779	1.1	0.8
Cer (d18:1/22:0)	604.603	0.5	3.3	5649 ± 907	1.1	0.7 *
Cer (d18:0/16:0)	520.510	0.5	2.9	53136 ± 7419	1.0	0.8 *
Cer (d18:0/18:0)	548.541	0.5	5.1	113197 ± 14647	1.0	0.8 *
HexCer (d18:0/22:0)	750.662	0.7	15.9	222 ± 28	0.9	0.8 **
HexCer (d18:1/20:1)	770.614	0.7	6.4	312 ± 35	0.8	0.7 **
HexCer (d18:1/22:0)	766.656	0.7	16.5	313 ± 66	0.9	0.6 *
HexCer (d18:1/20:0)	738.625	0.7	13.6	400 ± 60	0.8	0.6 **
HexCer (d18:1/16:0)	700.572	0.7	13.8	1024 ± 122	0.9	0.7 *
HexCer (d18:1/20:0)	756.635	0.7	17.0	1165 ± 131	0.9	0.8 **
HexCer (d18:1/18:0)	728.604	0.7	9.5	1537 ± 231	0.9	0.7 *
HexCer (d18:1/16:0)	682.562	0.7	2.9	3639 ± 578	0.9	0.6 **
HexCer (d18:1/18:0)	750.585	0.7	4.5	5534 ± 863	0.9	0.7 **
HexCer (d18:1/18:0)	710.594	0.7	2.6	8699 ± 1622	0.9	0.6 *
PI O-40:2	927.630	3.6	17.6	84 ± 16	0.7 *	0.9
PI 40:9	887.507	3.9	17.6	217 ± 44	0.7	0.6 **
PE p34:2	722.510	5.1	7.6	3560 ± 360	0.8	0.9
SM(d18:1/18:1)	729.591	7.1	6.9	1216 ± 234	0.8	1.2

[a]Fold-change relative to control.
[b]Student's *t*-test against control with Bonferroni correction for multiple comparisons; * $P <0.025$, ** $P <0.0025$, ** $P <0.00025$

Supplemental Table 3. Top 5 significantly altered canonical pathways of DEGs in BAC C12 and BAC C16 exposed neonatal brains.

Comparison Groups	IPA Canonical Pathway	- log (P-value)	Molecules
BAC C12 vs Control	Superpathway of Cholesterol Biosynthesis	7.03	HMGCS2, SQLE, DHCR24, IDI1, HMGCR, HSD17B7, CYP51A1, MSMO1
	LXR/RXR Activation	6.49	GC, ITIH4, MMP9, HMGCR, LDLR, NGFR, SCD, APOA2, SERPINA1, APOA1, CYP51A1, HPX, TTR, ALB
	TR/RXR Activation	5.10	FGFR1, LDLR, HP, COL6A3, CAMK4, TRH, SLC2A1, ENO1, NCOA2, SLC16A3, KLF9
	Glutamate Receptor Signaling	4.60	HOMER1, GNB3, GRIA2, CAMK4,SLC1A2, GRIN2C, GRIN2B, GRM3
	Acute Phase Response Signaling	3.95	ITIH4, JAK2, SERPINA3, FN1, HP, NGFR, APOA2, SERPINA1, APOA1, HPX, RBP3, TTR, ALB
BAC C16 vs Control	EIF2 Signaling	9.37	RPL21, RPS24, RPL37A, RPL26, RPS13, RPL9, ATF4, EIF3E, RPL22L1, RPS27A, RPL36A, RPS21, RPL10
	Superpathway of Cholesterol Biosynthesis	6.37	HMGCS2, SQLE, DHCR24, HSD17B7, MSMO1
	LXR/RXR Activation	5.30	LDLR, MMP9, APOA2, APOA1, AHSG, TTR, ALB
	Glutamate Receptor Signaling	3.55	SLC17A7, GRIA2, GRIN2B, GRM3
	Acute Phase Response Signaling	3.35	APOA2, APOA1, SERPINA3, AHSG, TTR, ALB

Supplemental Table 4. Transcriptional regulators and endogenous factors predicted as upstream regulators with the Ingenuity Pathway Analysis in BAC C12 exposed neonatal brains.

Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-score	p-value of overlap
ATP7B	transporter	Activated	2.50	1.10E-12
SCAP	other	Activated	2.86	2.02E-12
HTT	transcription regulator	Inhibited	-2.16	1.67E-09
beta-estradiol	endogenous chemical	Inhibited	-2.30	3.17E-09
TGFB1	growth factor	Inhibited	-2.57	5.52E-08
SREBF2	transcription regulator		1.66	6.80E-08
SREBF1	transcription regulator		1.05	1.91E-07
T3-TR-RXR	complex		-1.19	2.02E-07
FGF2	growth factor	Inhibited	-2.22	2.29E-07
SP1	transcription regulator		-1.69	3.00E-07
EGF	growth factor	Inhibited	-2.50	3.55E-07
INSIG1	other	Inhibited	-2.29	3.60E-07
D-glucose	endogenous chemical		-0.86	8.55E-07
MED13	transcription regulator		-0.45	1.37E-06
HIF1A	transcription regulator		-1.97	1.45E-06
BDNF	growth factor		1.42	1.88E-06
ERBB2	kinase		0.01	4.62E-06
PPARG	ligand-dependent nuclear receptor		1.84	6.30E-06
APP	other		-0.78	6.38E-06
oleic acid	endogenous chemical		-0.61	7.76E-06
Vegf	group		-0.20	8.19E-06

Supplemental Table 5. Transcriptional regulators and endogenous factors predicted as upstream regulators with the Ingenuity Pathway Analysis in BAC C16 exposed neonatal brains.

Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-score	p-value of overlap
RICTOR	other	Activated	3.61	3.94E-09
L-triiodothyronine	endogenous chemical		-0.62	2.19E-07
SCAP	other	Activated	2.23	4.37E-07
dihydrotestosterone	endogenous chemical		0.97	5.81E-07
MYCN	transcription regulator	Inhibited	-2.35	6.55E-07
ATP7B	transporter	Activated	2.24	2.72E-06
SREBF1	transcription regulator		1.21	1.44E-05
IGF1R	transmembrane receptor		-1.39	2.27E-05
beta-estradiol	endogenous chemical		-1.01	2.56E-05
INSIG1	other		-1.67	2.60E-05
SREBF2	transcription regulator		1.29	6.50E-05
FOS	transcription regulator		-0.13	6.69E-05
SOX4	Transcription regulator		-1.20	9.62E-05
ABCA1	transporter		-0.64	1.36E-04
ERBB4	kinase		1.46	1.41E-04
FGF2	growth factor		-1.45	2.84E-04
fatty acid	endogenous chemical		-0.56	3.85E-04
BDNF	growth factor		1.25	4.43E-04
estrogen receptor	group		1.22	4.45E-04
GH1	growth factor		1.22	5.14E-04
Vegf	group		0.42	6.54E-04

Supplemental Table 6. Differentially expressed genes involved in sterol and lipid homeostasis in neonatal brains exposed *in utero* to BAC C12; adjusted *P* <0.05. Genes identified as related to sterol and lipid metabolism using DAVID functional annotation clustering.

Gene ID	Description	Log2 (FC)	Adjusted *P* value
AACS	acetoacetyl-CoA synthetase	0.42	9.81E-03
ACP6	acid phosphatase 6, lysophosphatidic	0.81	1.81E-02
ACSL4	acyl-CoA synthetase long-chain family member 4	0.51	4.44E-03
ADH1	alcohol dehydrogenase 1 (class I)	-1.31	2.97E-02
APOA1	apolipoprotein A-I	-1.89	4.44E-03
APOA2	apolipoprotein A-II	-2.14	4.44E-03
APOC1	apolipoprotein C-I	-1.74	1.81E-02
ART3	ADP-ribosyltransferase 3	-1.53	4.44E-03
CYP51	cytochrome P450, family 51	0.58	3.23E-02
DHCR24	24-dehydrocholesterol reductase	0.54	4.44E-03
ELOVL6	ELOVL family member 6, elongation of long chain fatty acids (yeast)	0.58	4.44E-03
ENO1	enolase 1, alpha non-neuron	-0.36	1.81E-02
ETNK1	ethanolamine kinase 1	0.31	4.11E-02
HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	0.57	4.44E-03
HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	0.69	4.44E-03
HSD17B7	hydroxysteroid (17-beta) dehydrogenase 7	1.07	4.44E-03
IDI1	isopentenyl-diphosphate delta isomerase	0.91	4.44E-03
INSIG1	insulin induced gene 1	0.41	9.81E-03
KDM8	lysine (K)-specific demethylase 8	-0.77	4.44E-03
LDHA	lactate dehydrogenase A	-0.40	4.44E-03
LDLR	low density lipoprotein receptor	0.87	4.44E-03
LOXL1	lysyl oxidase-like 1	-0.43	4.53E-02
MSMO1	methylsterol monooxygenase 1	0.66	4.44E-03
MTHFD2	methylenetetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	-0.66	4.44E-03
ODC1	ornithine decarboxylase, structural 1	-0.81	4.44E-03
PCSK9	proprotein convertase subtilisin/kexin type 9	-0.81	4.44E-03
PLCXD3	phosphatidylinositol-specific phospholipase C, X domain containing 3	0.55	4.44E-03
PNPLA2	patatin-like phospholipase domain containing 2	-0.52	2.56E-02
PPARA	peroxisome proliferator activated receptor alpha	-1.46	4.33E-02
PRKAA2	protein kinase, AMP-activated, alpha 2 catalytic subunit	0.40	7.33E-03
PRKAG2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit	0.47	2.36E-02
SCD1	stearoyl-Coenzyme A desaturase 1	-0.36	1.98E-02
SESN2	sestrin 2	-0.61	4.44E-03
SLC16A1	solute carrier family 16 (monocarboxylic acid transporters), member 1	0.48	4.44E-03
SQLE	squalene epoxidase	0.62	4.44E-03
TPI1	triosephosphate isomerase 1	-0.45	4.44E-03
UQCRQ	ubiquinol-cytochrome c reductase, complex III subunit VII	-0.53	4.86E-02

Supplemental Table 7. Differentially expressed genes involved in sterol and lipid homeostasis in neonate brains exposed *in utero* to BAC C16; adjusted *P* <0.05. Genes identified as related to sterol and lipid metabolism using DAVID functional annotation clustering.

Gene ID	Description	Log2 (FC)	Adjusted *P* value
AACS	acetoacetyl-CoA synthetase	0.35	3.84E-02
ADH1	alcohol dehydrogenase 1 (class I)	-1.40	2.92E-02
APOA1	apolipoprotein A-I	-2.10	9.98E-03
APOA2	apolipoprotein A-II	-2.75	9.98E-03
APOC1	apolipoprotein C-I	-1.54	3.35E-02
ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	-0.35	2.38E-02
ATP5L	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G	-0.40	9.98E-03
CFAP61	cilia and flagella associated protein 61	1.95	2.38E-02
DHCR24	24-dehydrocholesterol reductase	0.49	9.98E-03
HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	0.61	9.98E-03
HSD17B7	hydroxysteroid (17-beta) dehydrogenase 7	0.71	9.98E-03
LDLR	low density lipoprotein receptor	0.73	9.98E-03
MSMO1	methylsterol monoxygenase 1	0.48	9.98E-03
MTHFD2	methylenetetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	-0.53	9.98E-03
NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4	-0.32	2.92E-02
ODC1	ornithine decarboxylase, structural 1	-0.69	9.98E-03
PGP	phosphoglycolate phosphatase	0.42	2.38E-02
POLR1D	polymerase (RNA) I polypeptide D	-0.40	9.98E-03
SQLE	squalene epoxidase	0.45	9.98E-03