Supporting information

Suspect screening of bisphenol A (BPA) structural analogues and functional alternatives in human milk from Canada and South Africa

Zhi Hao Chi[1], Lan Liu[1], Jingyun Zheng[1], Lei Tian[1], Jonathan Chevrier[2], Riana Bornman[3], Muvhulawa Obida[3], Cindy Gates Goodyer[4], Barbara F. Hales[5,6] and Stéphane Bayen [1,*]

[1] Department of Food Science and Agricultural Chemistry, McGill University, Montreal, QC, Canada.

[2] Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, QC, Canada.

[3] University of Pretoria, Pretoria, South Africa.

[4] McGill University Health Centre, Montreal, QC, Canada.

[5] Department of Pharmacology and Therapeutics, McGill University, Montreal, QC, Canada. [6] Deceased: Barbara F. Hales.

*Correspondence to Stéphane Bayen. Email: stephane.bayen@mcgill.ca

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The supporting information also includes separate file *NTA_SRT_V4.xlsx* : NTA Study Reporting Tool for the present study.

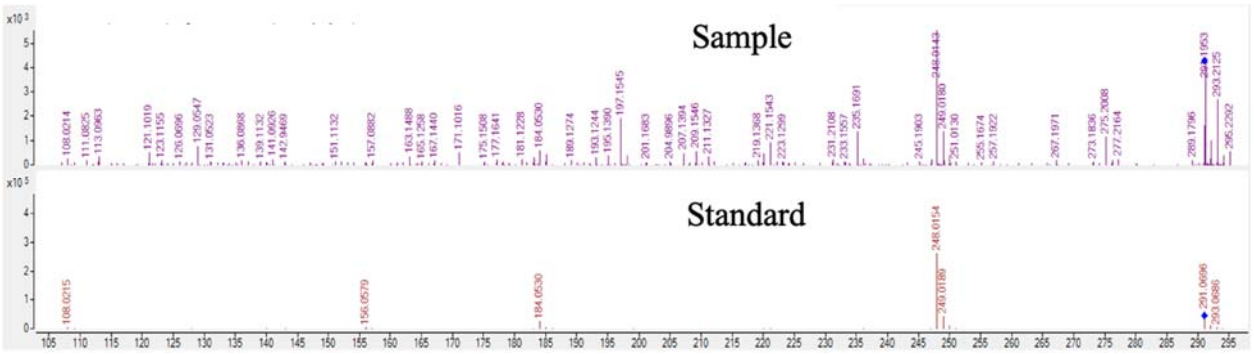


Figure S1. MS/MS spectra of D8 in an unspiked human milk sample and pure analytical standard (20 V) (Fragmented parent ion at *m/z* of 291.07)

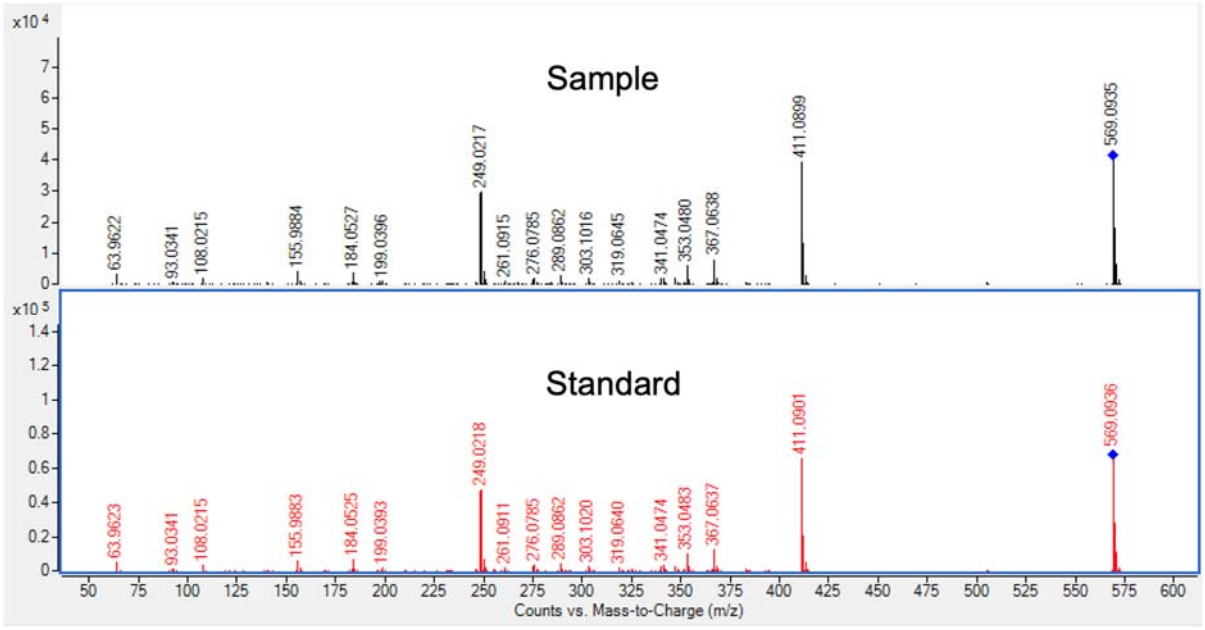


Figure S2. MS/MS spectra of D90 in unspiked human milk sample and pure analytical standard (35 V) (Fragmented parent ion at *m/z* of 569.09)

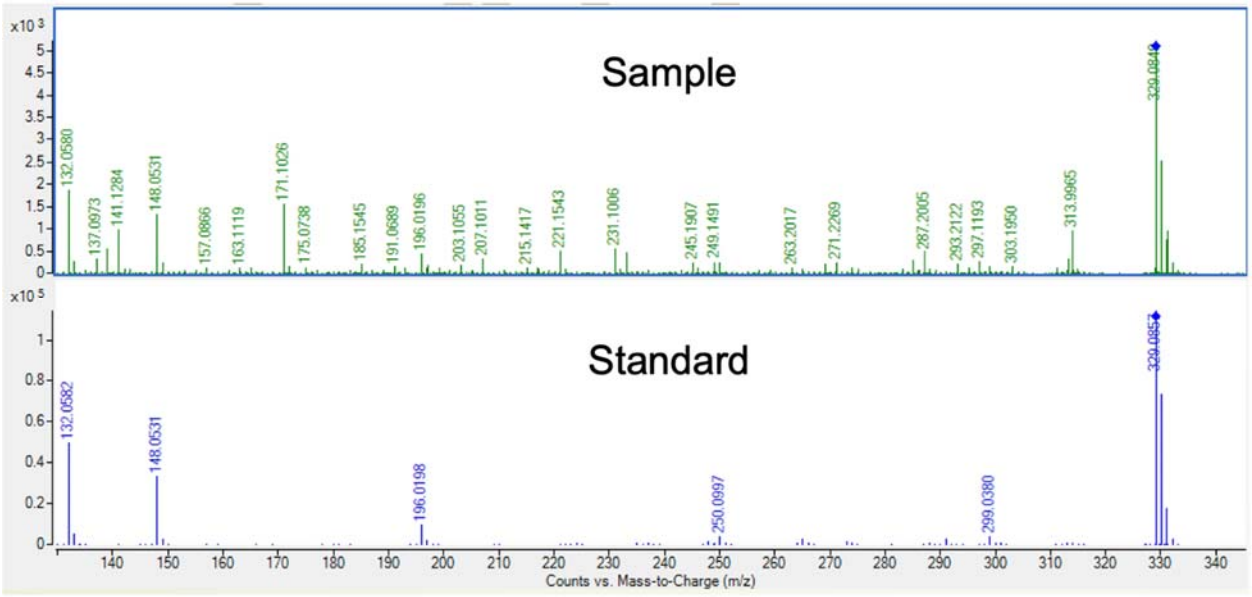


Figure S3. MS/MS spectra of TGSA in an unspiked human milk sample and pure analytical standard (20 V) (Fragmented parent ion at *m/z* of 329.09)

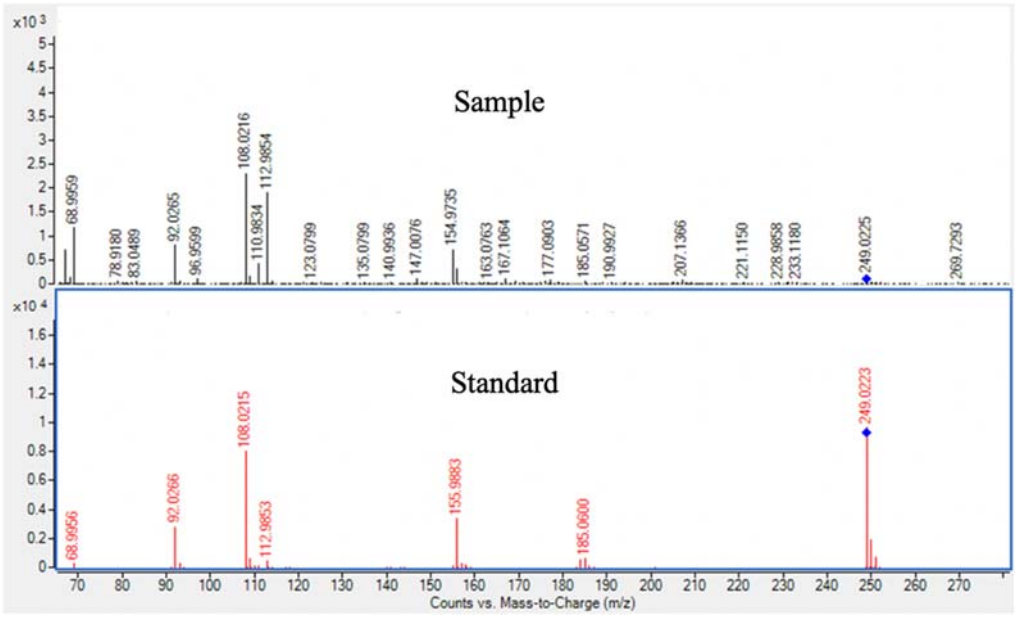


Figure S4. MS/MS spectra and chromatograph peak of 2,4-BPS in an unspiked human milk sample and pure analytical standard (20 V) (Fragmented parent ion at *m/z* of 249.02)

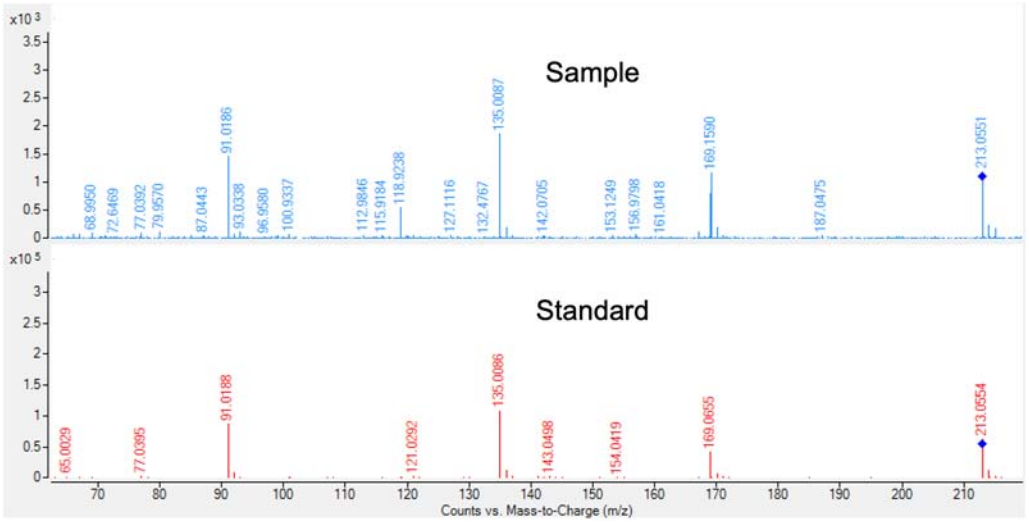


Figure S5. MS/MS spectra of benzophenone-1 in an unspiked human milk sample and pure analytical standard (20 V) (Fragmented parent ion at *m/z* of 213.06)

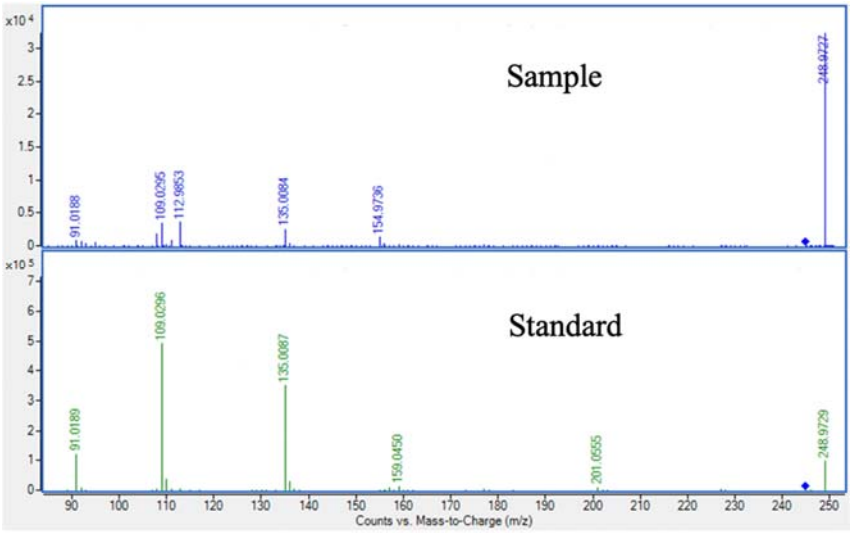


Figure S6. MS/MS spectra of benzophenone-2 in an unspiked human milk sample and pure analytical standard (20 V) (MS1 value at *m/z* 245.05 not visible)

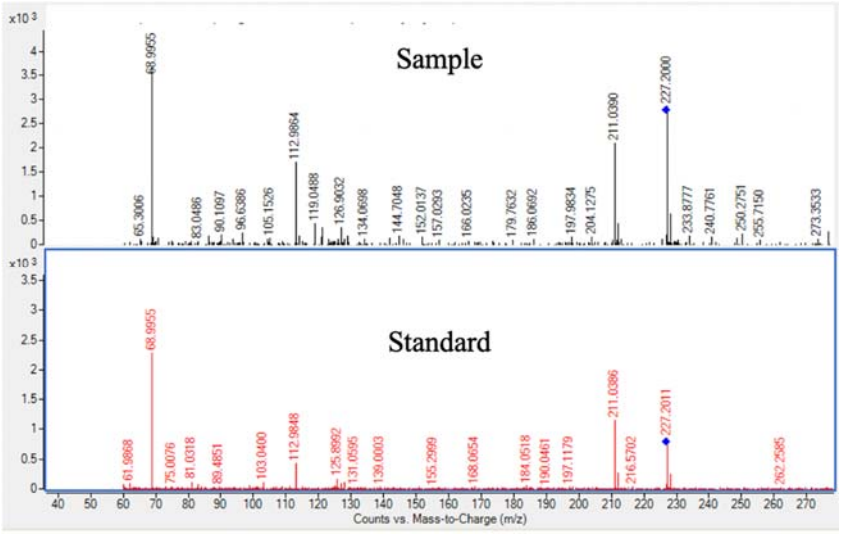


Figure S7. MS/MS spectra of benzophenone-3 in an unspiked human milk sample and pure analytical standard (20 V) (Fragmented parent ion at *m/z* of 227.07)

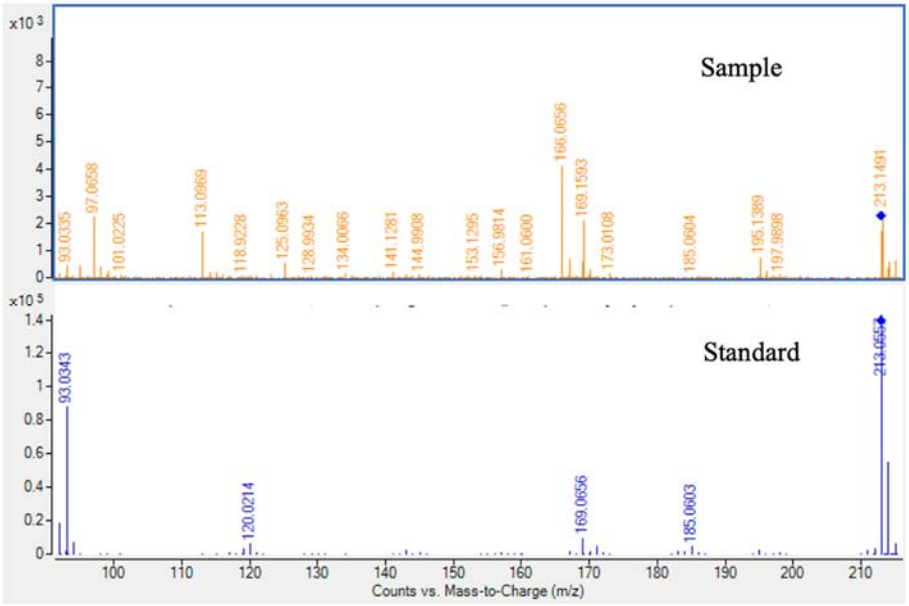


Figure S8. MS/MS spectra of 4,4'-dihydroxybenzophenone in an unspiked human milk sample and pure analytical standard (20 V) (Fragmented parent ion at *m/z* of 213.06)

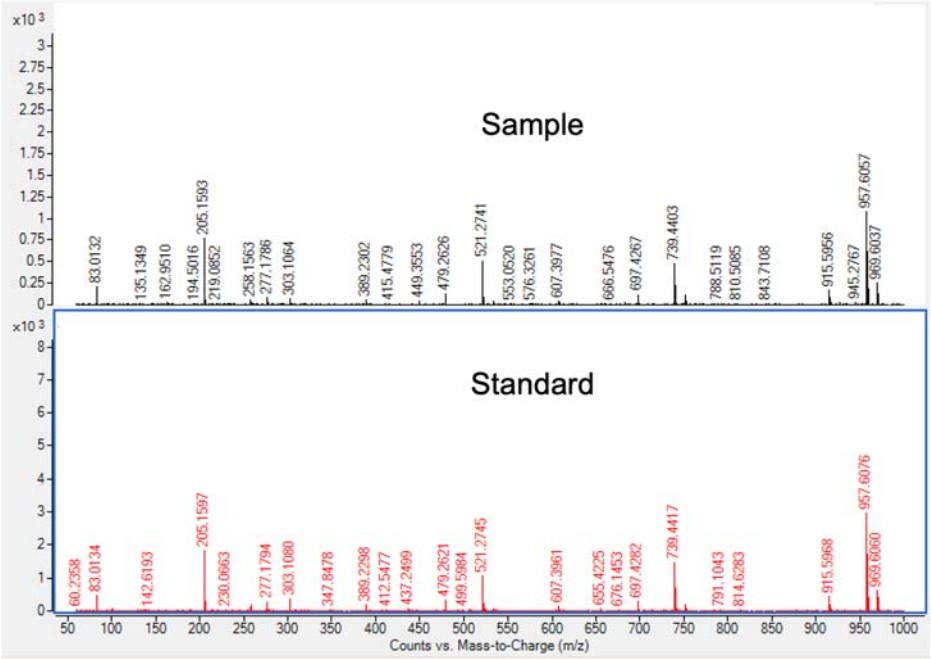


Figure S9. MS/MS spectra of Irganox 1010 in an unspiked human milk sample and pure analytical standard (40 V) (MS1 value at *m/z* 1175.78 not visible)

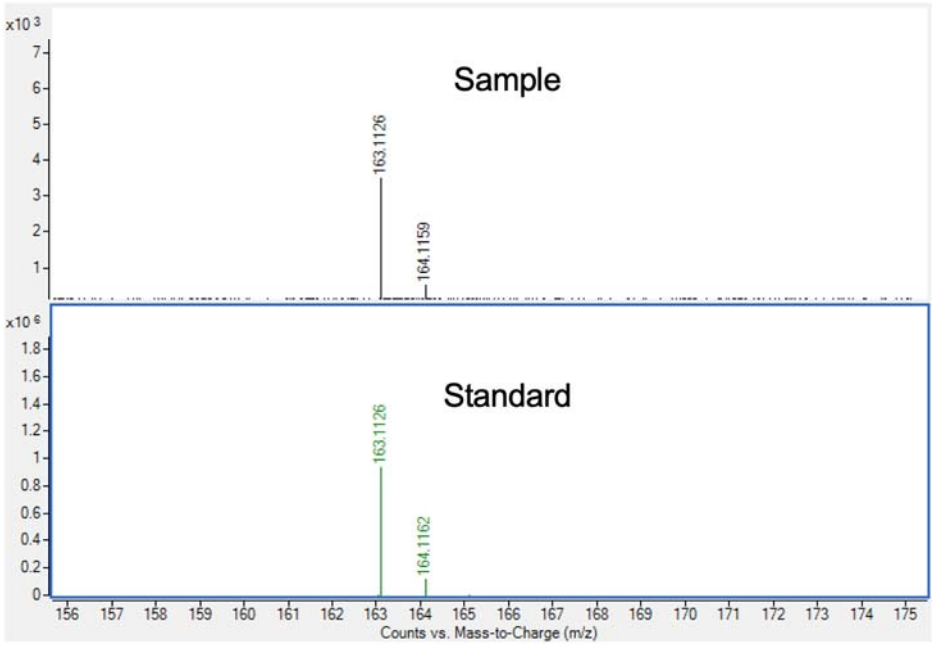


Figure S10. MS/MS spectra of Cyanox 2246 (Antioxidant 2246) in an unspiked human milk sample and pure analytical standard (40 V) (MS1 value at *m/z* 339.20 not visible)

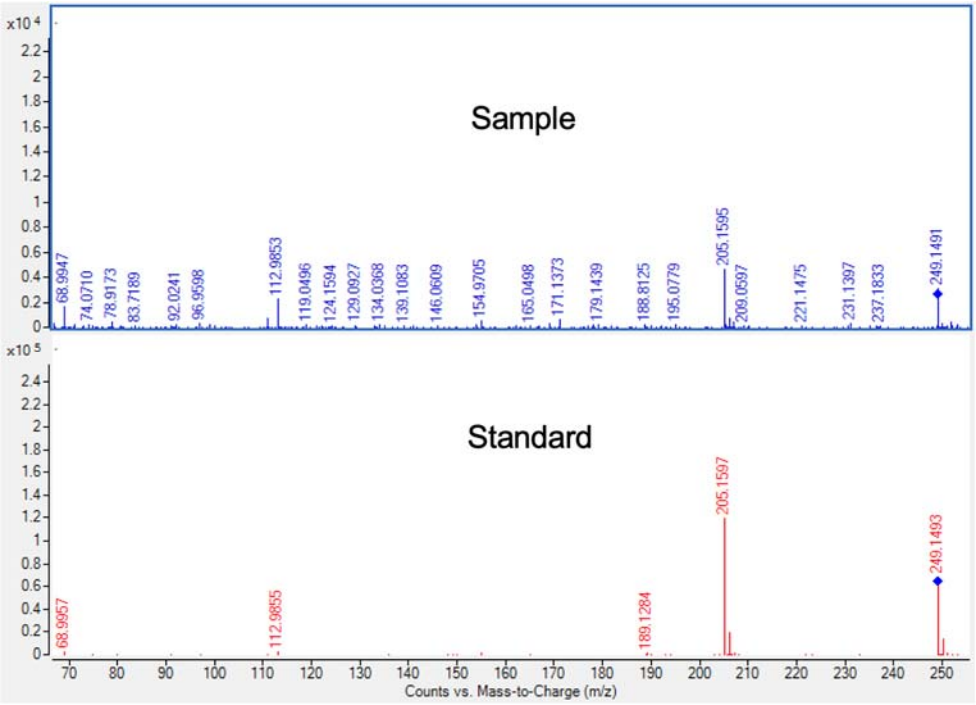


Figure S11. MS/MS spectra of BHT-COOH in an unspiked human milk sample and pure analytical standard (20 V) (Fragmented parent ion at *m/z* of 249.1)

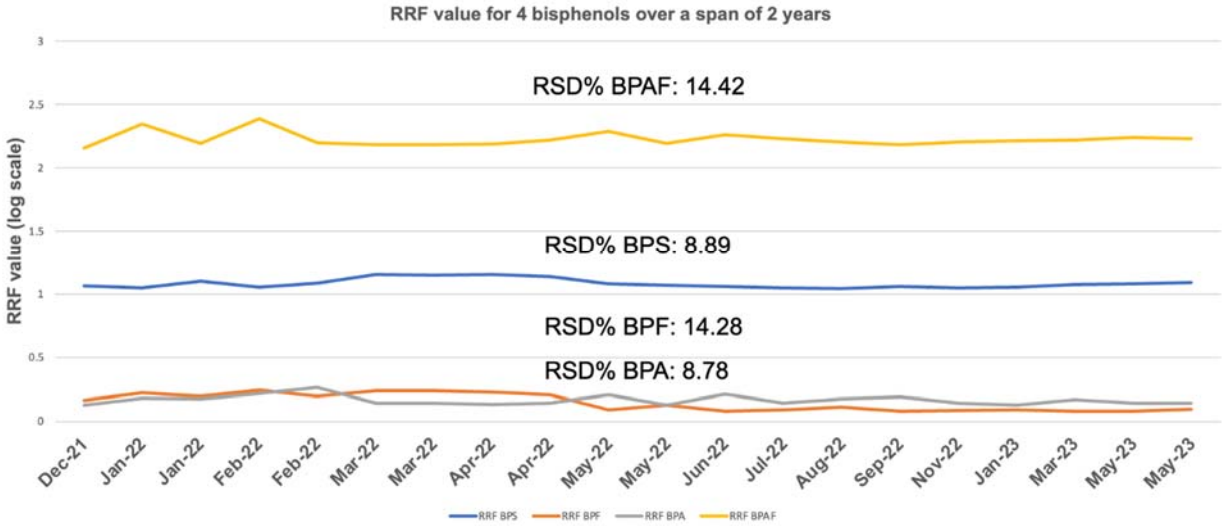
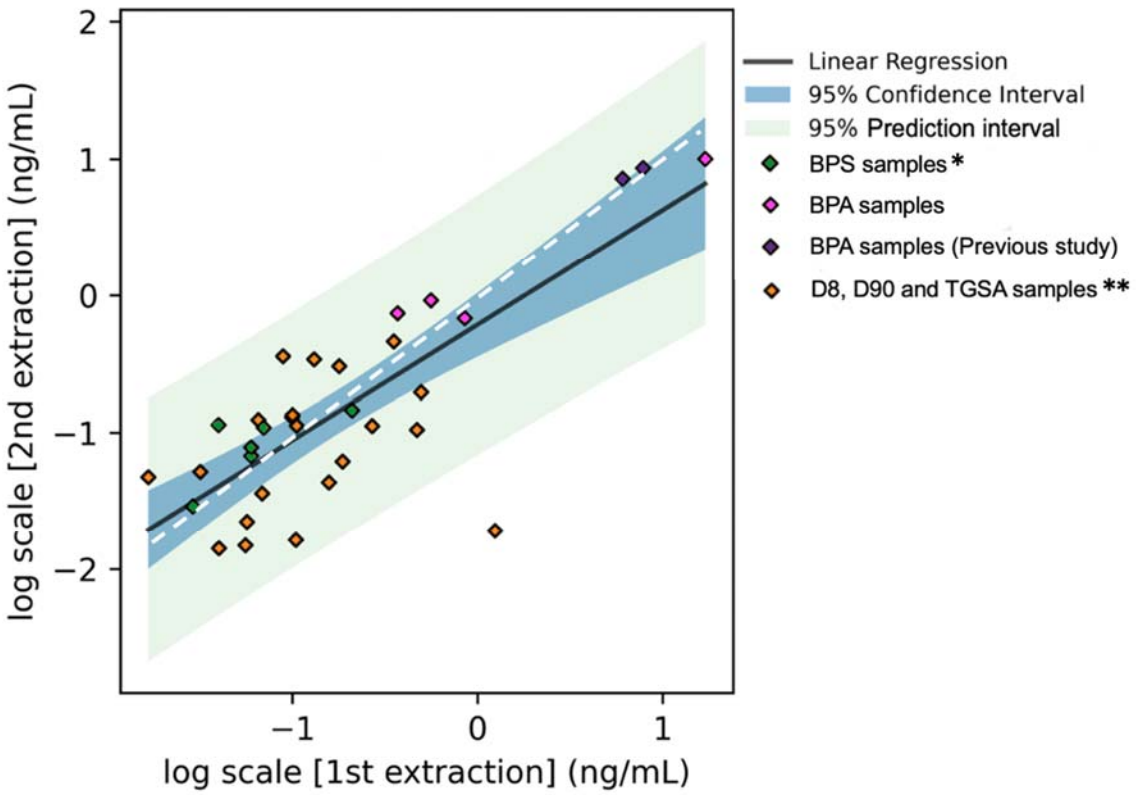


Figure S12. Relative response factors (RRFs) for BPA, BPF, BPAF and BPS versus their respective $^{13}C_{12}$ and d_4 labelled surrogates across all 20 analyzed batches and the date of their analysis (RRF for BPS was constant with relative standard deviation of 8.89 %)



***BPS and BPA for 1st and 2nd extractions were quantified using $^{13}C_{12}$-BPS and D_4-BPA, respectively**
****D8, D90 and TGSA for 1st and 2nd extractions were semi-quantified using $^{13}C_{12}$-BPS**

Figure S13. Linear regression plot of BPS, D8, D90, TGSA and BPA for 1st and 2nd extraction (The dashed white line represents the 1:1 line)

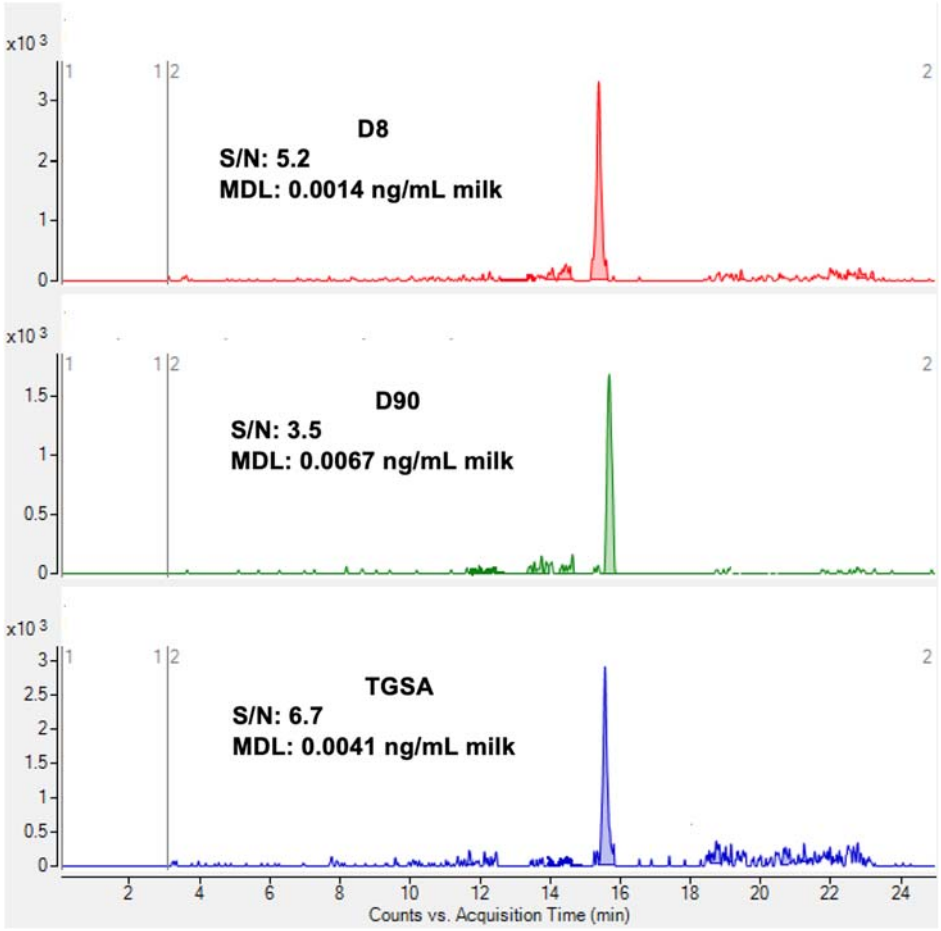


Figure S14. Examples of extracted signals for D8, D90 and TGSA in one incurred milk samples at levels close to their detection limits

Table S1. Additional compounds added to the database library (n=18)

Name	Formula	CAS RN
4,4'-Dihydroxybenzophenone	$C_{13}H_{10}O_3$	611-99-4
Benzophenone 1	$C_{13}H_{10}O_3$	131-56-6
Benzophenone 3	$C_{14}H_{12}O_3$	131-57-7
Butylated hydroxyanisole (BHA)	$C_{11}H_{16}O_2$	25013-16-5
Butylated Hydroxytoluene (BHT)	$C_{15}H_{24}O$	128-37-0
3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO)	$C_{15}H_{22}O_2$	1620-98-0
2,6-di-*tert*-butyl-4-(hydroxymethyl) phenol (BHT-OH)	$C_{15}H_{24}O_2$	88-26-6
2,6-di-*tert*-butyl-1,4-benzoquinone (BHT-Quinone)	$C_{14}H_{20}O_2$	719-22-2
3,5-Di-tert-butyl-4-hydroxybenzoic acid (BHT-COOH)	$C_{15}H_{22}O_3$	1421-49-4
2,6-di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone (BHT-quinol)	$C_{15}H_{24}O_2$	10396-80-2
Octyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionate (Irganox 1135)	$C_{25}H_{42}O_3$	13417-12-4
Octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionate (Irganox 1076)	$C_{35}H_{62}O_3$	2082-79-3
Pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate) (Irganox 1010)	$C_{73}H_{108}O_{12}$	6683-19-8
4-tert-Octylphenol	$C_{14}H_{22}O$	140-66-9
4-Nonylphenol	$C_{15}H_{24}O$	104-40-5
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	96-76-4
2,4-di-tert-amylphenol	$C_{16}H_{26}O$	120-95-6
4-sec-Butyl-2,6-di-tert-butylphenol	$C_{18}H_{30}O$	17540-75-9

Table S2: Non-targeted analysis selected parameters (MassHunter Profinder)

Parameters	Values
Match tolerance mass	10 ppm
Expansion values for chromatogram extraction (*m/z*) (+/-)	50 ppm
Isotope abundance score	60%
Retention time window	±0.3 min
Peak filter (absolute height)	≥ 300 counts
Integrator method	Agile 2
Peak spectra: spectra to include how much percent of average scan	>10%
TOF spectra: exclude if above how much saturation	20%
Post processing: Find by formula peak filter (absolute height)	≥ 300 counts

Table S3: Non-targeted identification of 42 selected bisphenol-related candidates in breast milk from Montreal, Vhembe and Pretoria (Profinder score >90%)

ESI- m/z [M-H]-	*Retention time (min)*	*Region (Montreal, Vhembe or Pretoria)*	*Formula*	*Suspected identity (Confidence level 3-Schymanski scale)* **[1]**	*Further identification*
291.0691	14.40	All	$C_{15}H_{16}O_4S$	4-(4-propan-2-yloxyphenyl) sulfonylphenol (D8)	Confirmed (level 1) [1]
569.0940	14.65	All	$C_{12}H_{10}O_4S[C_{16}H_{16}O_5S]_n$ (n=1)	Phenol, 4,4'-sulfonylbis-, polymer with 1,1'-oxybis[2-chloroethane] (D90)	Confirmed (level 1)
329.0848	14.55	All	$C_{18}H_{18}O_4S$	4-(4-hydroxy-3-prop-2-enylphenyl)sulfonyl-2-prop-2-enylphenol (TGSA)	Confirmed (level 1)
249.0224	12.20	Montreal	$C_{12}H_{10}O_4S$	2,4- bisphenol S	Confirmed (level 1)
245.0450	12.11	Montreal	$C_{13}H_{10}O_5$	2,2',4,4'-Tetrahydroxybenzophenone (Benzophenone 2)	Confirmed (level 1)
227.0708	14.64	Montreal	$C_{14}H_{12}O_3$	Oxybenzone (Benzophenone 3)	Confirmed (level 1)
257.081	14.16	All	$C_{15}H_{14}O_4$	Methyl bis(4-hydroxyphenyl)acetate (MBHA)	Inconclusive (suggested identity by SIRIUS to be Davidigenin; standard was not purchased)
379.1698	13.84	All	$C_{27}H_{24}O_2$	2,2-Bis(2-hydroxy-5-biphenylyl) propane (BPPH)	Inconclusive (Poor SIRIUS score)

287.0556	**12.57**	**All**	**$C_{15}H_{12}O_6$**	**3,3'-Methylenedisalicylic acid**	**Inconclusive (Poor SIRIUS score)**
201.0552	11.49	All	$C_{12}H_{10}O_3$	p-(p-Hydroxyphenoxy)phenol	Inconclusive (Poor SIRIUS score)
109.0290	9.41	All	$C_6H_6O_2$	Hydroquinone	Incorrect identity using standard
434.1604	15.03	All	$C_{25}H_{25}NO_6$	3,3'-bis(6-hydroxy-5-methylbenzoic acid)	Inconclusive (Poor SIRIUS score)
305.1178	15.18	All	$C_{20}H_{18}O_3$	1,1,1-Tris(4-hydroxyphenyl)ethane	Inconclusive (Poor SIRIUS score)
459.3263	18.32	All	$C_{32}H_{44}O_2$	2,2'-(Octahydro-4,7-methano-1H-indenediyl)bis[6-tert-butyl-p-cresol]	Inconclusive (Poor SIRIUS score)
507.1573	15.67	All	$C_{28}H_{31}O_7P$	Thymolphthalein monophosphate ([4-[1-(4-hydroxy-2-methyl-5-propan-2-ylphenyl)-3-oxo-2-benzofuran-1-yl]-5-methyl-2-propan-2-ylphenyl] phosphate)	Inconclusive (Poor SIRIUS score)
544.3942	17.85	All	$C_{37}H_{52}O_3$	4,4',4"-(Butane-1,1,3-triyl)tris(2-tert-butyl-5-methylphenol)	Inconclusive (Poor SIRIUS score)
464.1657	14.84	Montreal	$C_{27}H_{29}O_5S$	Thymol blue (4-[3-(4-hydroxy-2-methyl-5-propan-2-ylphenyl)-1,1-dioxo-2,1λ6-benzoxathiol-3-yl]-5-methyl-2-propan-2-ylphenol)	Inconclusive (Poor SIRIUS score)

621.4308	18.75	Vhembe and Pretoria	$C_{43}H_{58}O_3$	1,1,3-Tris(5-cyclohexyl-4-hydroxy-o-tolyl)butane	Inconclusive (Poor SIRIUS score)
341.2230	16.72	Vhembe and Pretoria	$C_{21}H_{30}N_2O_2$	2,2'-methylenebis[5-(diethylamino)phenol]	Inconclusive (Poor SIRIUS score)
339.2324	17.10	Vhembe and Pretoria	$C_{23}H_{32}O_2$	2,2'-Methylenebis(4-methyl-6-tert-butylphenol) (Cyanox 2246)	Confirmed (level 1)
269.1542	15.04	Montreal	$C_{18}H_{22}O_2$	4,4'-(1,3-Dimethylbutylidene)diphenol	Inconclusive (Poor SIRIUS score)
309.1854	17.29	All	$C_{21}H_{26}O_2$	(4-[1-(4-hydroxyphenyl)-3,3,5-trimethylcyclohexyl]phenol) (Bisphenol TMC)	Inconclusive (Same retention time as the standard, and several MS/MS fragments also matching with the standard but missing important fragment of bisphenol TMC at m/z value at 215.14)
381.2794	17.22	All	$C_{26}H_{38}O_2$	(4,4'-(Butane-1,1-diyl)bis(2-(tert-butyl)-5-methylphenol) (Santowhite)	Inconclusive (Poor SIRIUS score)
423.3263	18.28	Vhembe and Pretoria	$C_{29}H_{44}O_2$	4,4'-Dihydroxy,3,3'-5,5'-tetra-t-butyldiphenylmethane (Ionox 220)	Inconclusive (Poor SIRIUS score)
365.0725	14.70	Vhembe and Pretoria	$C_{15}H_{12}F_6N_2O_2$	2-Amino-4-[2-(3-amino-4-hydroxyphenyl)-1,1,1,3,3,3-hexafluoro-2-propanyl]phenol	Inconclusive (Poor SIRIUS score)
657.3917	21.71	Vhembe and Pretoria	$C_{41}H_{50}N_6O_2$	Bisoctrizole (2-(benzotriazol-2-yl)-6-	Inconclusive (Poor SIRIUS score)

				[[3-(benzotriazol-2-yl)-2-hydroxy-5-(2,4,4-trimethylpentan-2-yl)phenyl]methyl]-4-(2,4,4-trimethylpentan-2-yl)phenol)	
219.1749	16.68	All	$C_{15}H_{24}O$	2,4-Di-tert-butyl-5-methylphenol	Inconclusive (Poor SIRIUS score)
177.1279	10.32	All	$C_{12}H_{18}O$	5-tert-butyl-2,3-dimethylphenol	Inconclusive (Poor SIRIUS score)
273.0763	11.05	Montreal	$C_{15}H_{14}O_5$	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone (Benzophenone-6)	Incorrect identity using standard
311.2011	17.10	All	$C_{21}H_{28}O_2$	4,4'-(Propane-2,2-diyl)bis(2-isopropylphenol (Bisphenol G)	Inconclusive (Poor SIRIUS score)
243.0658	12.95	All	$C_{14}H_{12}O_4$	Bis(4-hydroxyphenyl)acetic acid	Inconclusive (Poor SIRIUS score)
1175.7763	18.10	All	$C_{73}H_{108}O_{12}$	[3-[3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoyloxy]-2,2-bis[3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoyloxymethyl]propyl] 3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoate) (Irganox 1010)	Confirmed (level 1)
287.0556	14.63	All	$C_{15}H_{12}O_6$	5,5'-Methylenedisalicylic acid	Inconclusive (Poor SIRIUS score)
807.5481	18.32	All	$C_{53}H_{77}O_4P$	Phosphorous acid, 2-(1,1-dimethylethyl)-4-[1-[3-(1,1-dimethylethyl)-4-hydroxyphenyl]-1-methylethyl]phenyl bis(4-nonylphenyl) ester	Inconclusive (Poor SIRIUS score)

213.0552	11.53	Montreal	$C_{13}H_{10}O_3$	4,4'-Dihydroxybenzophenone	Confirmed (level 1)
213.0552	11.21	Montreal	$C_{13}H_{10}O_3$	2,4-Dihydroxybenzophenone (Benzophenone 1)	Confirmed (level 1)
219.1749	15.09	All	$C_{15}H_{24}O$	Butylated Hydroxytoluene (BHT)	Inconclusive (Poor SIRIUS score)
249.1490	14.37	All	$C_{15}H_{22}O_3$	3,5-di-tert-butyl-4-hydroxybenzoic acid (BHT-COOH)	Confirmed (level 1)
219.1385	14.61	All	$C_{14}H_{20}O_2$	2,6-di-tert-butyl-p-benzoquinone (BHT-quinone)	Inconclusive (Poor SIRIUS score)
233.1542	14.20	All	$C_{15}H_{22}O_2$	3,5-di-tert-butyl-4-hydroxybenzaldehyde BHT-CHO	Inconclusive (Poor SIRIUS score)
179.1072	12.32	All	$C_{11}H_{16}O_2$	Butylated hydroxyanisole (BHA)	Inconclusive (Poor SIRIUS score)
205.1593	16.31	Vhembe and Pretoria	$C_{14}H_{22}O$	4-tert-Octylphenol	Inconclusive- acceptable SIRIUS score, but retention time different than standard (presence of possible isomers)

Table S4. The relative intensities of qualifier to quantifier ions for BPS, D8, D90 and TGSA in pure solvent and human milk matrix

Compound	Matrix	Base peak [M-H]- (m/z)	Qualifier 1 (m/z)	Relative Intensity %	Relative difference %*	Qualifier 2 (m/z)	Relative intensity %	Relative difference %*	Collision energy (V)
BPS**	Solvent	249.0222	108.0215	82.9	-	155.9883	34.7	-	20
	Milk			84.3	1.65		33	-4.73	
D8	Solvent	291.0691	248.0144	535.8	-	184.0527	60.1	-	20
	Milk			515.8	-3.74		58.9	2.00	
D90	Solvent	569.094	411.0901	91.1	-	249.0217	69.0	-	35
	Milk			92.42	-1.46		67.3	2.40	
TGSA	Solvent	329.0848	132.0579	17.7	-	148.0529	11.94	-	20
	Milk			17.1	3.57		11.53	3.57	

*Relative difference was calculated as the ratio using the difference between the relative intensities obtained in milk matrix and solvent
**BPS values obtained from previous study

Table S5: Relative recovery of spiked D8, D90 and TGSA in pooled human milk (n=3 replicates)

Analyte	Relative response factor (RRF)	Linearity r^2	Matrix effects	Average intraday relative recovery % (30 ppb)	Relative standard deviation (RSD%)	Method detection limit (MDL) (ng/mL milk)	Limit of quantification (LOQ) (ng/mL milk)
BPS*	12.07**	0.99*	21%*	102*	3.7*	0.0020*	0.007*
D8	13.99	0.99	46%	100	8.9	0.0014	0.005
D90	3.40	0.99	47%	86	4.9	0.0067	0.024
TGSA	11.74	0.99	47%	103	5.2	0.0041	0.014

**BPS values obtained from previous study*
***Average RRF obtained within a span of 2 years throughout 20 batches*

Table S6. Concentrations (ng/mL) of BPS, D8, D90, TGSA and BPA for 1st and 2nd extractions (ND: not-detected)

Sample	1st extraction data												2nd extraction data											
	BPS	BPS (log-scaled)	D8	D8 (log-scaled)	D90	D90 (log-scaled)	TGSA	TGSA (log-scaled)	BPA	BPA (log-scaled)	BPA [2]	BPA (log-scaled) [2]	BPS	BPS (log-scaled)	D8	D8 (log-scaled)	D90	D90 (log-scaled)	TGSA	TGSA (log-scaled)	BPA	BPA (log-scaled)	BPA [2]	BPA (log-scaled) [2]
Sample 1	ND	ND	ND	ND	ND	ND	ND	ND	0.85	-0.071	ND	ND	0.043	-1.37	ND	ND	ND	ND	0.13	-0.89	0.68	-0.17	0.10	-1.00
Sample 2	ND	ND	ND	ND	ND	ND	ND	ND	0.37	-0.43	6.07	0.78	0.068	-1.17	ND	ND	ND	ND	0.16	-0.80	0.74	-0.13	7.21	0.86
Sample 3	ND	ND	ND	ND	ND	ND	ND	ND	16.98	1.23	7.83	0.89	0.038	-1.42	ND	ND	ND	ND	0.095	-1.02	10.06	1.00	8.64	0.94
Sample 4	0.025	-1.60	ND	ND	ND	ND	0.03	-1.50	0.56	-0.25	-		ND	ND	ND	ND	ND	ND	0.052	-1.28	0.92	-0.036	-	
Sample 5	0.060	-1.22	ND	ND	ND	ND	0.27	-0.57	0.06	-1.22	-		0.07	-1.17	ND	ND	ND	ND	0.11	-0.95	ND	ND	-	
Sample 6	ND	ND	ND	ND	ND	ND	0.49	-0.31	2.75	0.44	-		0.06	-1.22	ND	ND	ND	ND	0.20	-0.71	ND	ND	-	
Sample 7	0.06	-1.22	ND	ND	ND	ND	0.35	-0.45	ND	ND	-		0.08	-1.11	0.023	-1.64	ND	ND	0.46	-0.34	ND	ND	-	
Sample 8	0.03	-1.54	0.040	-1.40	ND	ND	0.47	-0.33	ND	ND	-		0.03	-1.53	0.014	-1.85	0.34	-0.47	0.11	-0.96	ND	ND	-	
Sample 9	0.04	-1.40	0.056	-1.25	0.18	-0.75	0.02	-1.78	ND	ND	-		0.11	-0.94	0.015	-1.83	0.30	-0.52	0.048	-1.32	ND	ND	-	
Sample 10	0.07	-1.15	0.056	-1.25	0.10	-1.00	0.19	-0.73	ND	ND	-		0.11	-0.96	0.022	-1.65	0.13	-0.89	0.062	-1.21	ND	ND	-	
Sample 11	0.21	-0.68	0.066	-1.18	0.13	-0.88	0.16	-0.80	ND	ND	-		0.14	-0.84	0.12	-0.91	0.34	-0.47	0.044	-1.36	ND	ND	-	
Sample 12	ND	ND	0.089	-1.05	0.07	-1.16	0.1	-1.00	ND	ND	-		ND	ND	0.36	-0.44	0.04	-1.44	0.14	-0.87	ND	ND	-	
Sample 13	ND	ND	0.11	-0.98	0.10	-0.98	ND	ND	ND	ND	-		ND	ND	0.016	-1.79	0.11	-0.95	ND	ND	ND	ND	-	
Sample 14	ND	ND	1.24	0.094	ND	ND	ND	ND	ND	ND	-		ND	ND	0.019	-1.72	0.26	-0.58	ND	ND	ND	ND	-	

To improve robustness, the concentrations for each compound for both 1st and 2nd extractions were log scaled, excluding non-detects. Samples that had non-detectable levels in either the 1st or 2nd extraction were excluded for the assessment of any difference between the 2 extractions.

Table S7. Paired sample t-test for BPS, D8, D90, TGSA and BPA between 1st and 2nd extractions, excluding non-detects in either extraction

Analyte	t-value	df	Significance (p-value)
BPS	1.31	5	0.25
D8	-1.54	6	0.18
D90	0.89	4	0.43
TGSA	-1.3	8	0.23
BPA	0.39	3	0.73
BPA [2]	4.33	1	0.14

Table S8: Mean mass measurement errors (ppm) for D8, D90 and TGSA in pure solvent and sample matrices

Compound	*m/z*	Mass measurement error (ppm) of standards in pure solvent (n=3)	Mass measurement error (ppm) in human milk matrix (n=3)
D8	291.0691	0.34 ±0.27	1.00±0.34
D90	569.0940	0.11±0.52	0.18±0.77
TGSA	329.0849	0.45±0.18	0.90±0.54

References

1. Schymanski, E.L., et al., *Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis.* Analytical and Bioanalytical Chemistry, 2015. **407**(21): p. 6237-6255.
2. Chi, Z.H., et al., *Biomonitoring of bisphenol A (BPA) and bisphenol analogues in human milk from South Africa and Canada using a modified QuEChERS extraction method.* Environ Pollut, 2024: p. 123730.