

Supporting Information for Carignan et al., 2012

“Predictors of Tetrabromobisphenol-A and Hexabromocyclododecanes in Milk  
from Boston Mothers”

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22 pages, 13 tables and 1 figure.

## **Methods**

### ***Study Participants and Sampling:***

We recruited participants at three sites: a health center in Lowell, Massachusetts that serves an ethnically diverse, working class community; a private obstetrics office in Cambridge, Massachusetts; and a maternity center in Brookline, Massachusetts. The Cambridge and Brookline facilities serve similar populations, predominantly white and highly educated.

Questionnaires were administered to each participant in person to collect information on demographics, general health, and potential variables for flame retardant exposure such as pre-pregnancy dietary consumption, use and possession of consumer electronics, residential and occupational history, recent home renovations, home carpeting, hobbies, and typical methods of transportation. Participants were asked if they regularly chose to eat organic foods (products grown or produced without chemicals) for a particular type of food (yes/no) as well as how often. For the general food frequency questionnaire dietary serving sizes were defined by United States Department of Agriculture guidelines and visual aids were used to help participants estimate quantities consumed. Participants were asked to estimate the number of serving sizes consumed per week. The amount of dairy fat consumed per day was estimated based on questionnaire responses and industry established fat content for each product. Homes were considered carpeted if one or more rooms had wall-to-wall carpeting installed. Household electronics were categorized as follows: stereo and video electronics (i.e., TVs, CD players, DVD players and stereos), personal computers, auxiliary electronics (i.e., printer, copier, scanner and fax machines), and other small appliances.

***Determination of lipid weight in milk:***

Lipid weight was determined gravimetrically according to the European Standard EN 1528-2:1996, Part 2. Briefly, ~ 10 mL of freeze-dried human milk was weighed and transferred into 100 mL separating funnel. One mL saturated potassium oxalate was then added together with 10 mL of ethanol and 20 mL of hexane/diethyl ether (1:1, v/v). The mixture was shaken for about 15 min and the bottom layer was removed into another separating funnel before another 10 mL of hexane/diethyl ether (1:1, v/v) together with 5 mL of ethanol was added. The lower layer was removed and the upper layer combined with the upper layer from the first funnel. Ten mL of water was added to this organic phase before the lower layer was again removed. The organic phase was filtered through anhydrous sodium sulphate and then the lipid content was gravimetrically determined.

***Limits of Detection (LOD):***

Method limits of detection (LODs) were estimated based on 3:1 S:N ratios and were 9, 10, 8 and 9  $\text{pg g}^{-1}$  lipid weight (lw) for TBBP-A, HBCDs, TBCDs and PBCDs respectively.

**Table S1: Average recoveries (expressed as percent) of the <sup>13</sup>C-labelled internal standards.**

Internal standard	Average recovery (%)
<sup>13</sup> C- $\alpha$ -HBCD	78
<sup>13</sup> C- $\beta$ -HBCD	77
<sup>13</sup> C- $\gamma$ -HBCD	85
<sup>13</sup> C-TBBP-A	83

**Table S2: Recoveries (expressed as percent) of d<sub>18</sub>- $\alpha$ -HBCD used as extraction/clean-up evaluation standard.**

Sample #	Recovery (%)
1	94
2	87
3	92
4	90
5	89

**Table S3a: Concentrations of HBCD isomers in SRM2585 compared to the indicative values.**

	Average concentration (ng g <sup>-1</sup> ) $\pm$ standard deviation (n=7)	
	Measured	Indicative
$\alpha$ -HBCD	22 $\pm$ 2.3	19 $\pm$ 3.7
$\beta$ -HBCD	4.1 $\pm$ 0.9	4.3 $\pm$ 1.1
$\gamma$ -HBCD	125 $\pm$ 18	120 $\pm$ 22

**Table S3b: Summary of standard addition method results for TBBP-A.**

Mass added (ng)	Mass recovered (ng)	Recovery (%)	RSD (%) (n=5)
10.0	8.2	82.0	3.6
40.0	35.8	89.5	4.2
80.0	76.1	95.1	4.7

**Table S4.** Characteristics of recruited cohort and samples analyzed for the current study (n=43).

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Characteristic	
Age	
Mean (range)	32.6 (20-41)
Body Mass Index (BMI)	
Mean (range)	23.2 (18-39)
Race/Ethnicity	
White	38
Not White	5
Highest Education Level Achieved	
Some high school	0
High school graduate	2
Some college	2
College graduate	39
Recruitment Location	
Brookline	27
Cambridge	12
Lowell	4

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**Table S5.** Concentrations of lipids and target compounds in the analyzed human milk samples.

Sample No.	Lipid weight (g/100 ml)	Analyte concentration (pg/g lipid weight)						
		$\alpha$ -HBCD	$\beta$ -HBCD	$\gamma$ -HBCD	$\Sigma$ HBCDs	PBCDs	TBCDs	TBBP-A
1	0.79	249	37	72	359	<LOQ	<LOQ	182
2	3.09	245	46	77	368	<LOQ	<LOQ	<LOQ
3	1.12	252	40	105	398	<LOQ	<LOQ	<LOQ
4	1.86	313	38	70	421	<LOQ	<LOQ	<LOQ
5	1.50	317	39	74	429	<LOQ	<LOQ	<LOQ
6	0.82	299	53	81	433	<LOQ	<LOQ	<LOQ
7	1.00	344	32	68	443	<LOQ	<LOQ	172
8	0.82	365	37	88	491	<LOQ	<LOQ	<LOQ
9	2.55	331	53	132	516	<LOQ	58	87
10	2.05	328	59	152	539	<LOQ	<LOQ	<LOQ
11	2.30	346	51	144	542	<LOQ	<LOQ	<LOQ
12	1.45	426	57	85	568	<LOQ	<LOQ	<LOQ
13	1.33	405	67	158	630	<LOQ	62	<LOQ
14	1.32	418	65	173	657	<LOQ	<LOQ	<LOQ
15	2.65	480	63	138	681	67	96	<LOQ
16	2.27	523	47	125	695	<LOQ	<LOQ	<LOQ
17	4.26	485	61	150	696	<LOQ	<LOQ	<LOQ
18	3.77	552	63	101	716	57	89	<LOQ
19	0.57	517	82	131	730	<LOQ	<LOQ	<LOQ
20	1.83	620	79	91	791	52	122	<LOQ
21	1.36	639	51	140	830	<LOQ	<LOQ	146
22	1.69	546	104	226	875	<LOQ	84	<LOQ
23	1.22	641	58	177	876	<LOQ	53	<LOQ
24	1.33	646	72	212	930	135	278	<LOQ
25	1.82	746	68	118	932	<LOQ	84	<LOQ
26	1.88	703	41	245	989	<LOQ	<LOQ	258
27	0.92	792	83	148	1023	<LOQ	<LOQ	<LOQ
28	2.03	799	74	152	1025	94	159	<LOQ
29	0.77	793	77	172	1043	<LOQ	62	<LOQ
30	1.55	565	33	464	1062	66	80	153
31	1.43	850	67	304	1221	128	174	<LOQ
32	1.91	1250	105	293	1648	174	211	83
33	1.51	1287	99	364	1750	<LOQ	92	<LOQ
34	3.15	1290	176	573	2039	152	211	<LOQ
35	0.74	1493	114	551	2158	91	301	202
36	4.69	1666	239	260	2165	122	187	549
37	1.03	1877	165	196	2239	69	66	127
38	1.59	2332	188	306	2826	157	<LOQ	<LOQ
39	1.20	2293	372	832	3497	109	346	406
40	2.68	2374	349	924	3647	86	120	197
41	1.06	4092	282	1108	5482	317	562	345
42	0.98	4431	612	1310	6353	244	382	205
43	3.16	3279	1635	3193	8106	184	406	250

**Table S6.** Univariate logistic regression for detection of PBCDs, TBCDs, and TBBP-A in the analyzed human milk samples, Odds Ratio (95% CI).

Predictor	n	PBCDs	TBCDs	TBBP-A
$\Sigma$ HBCDs				
Low	21	Reference	Reference	Reference
High	22	12.9 (2.82 - 58.6)*	20.3 (4.18 - 98.2)*	4.25 (1.08 - 16.8)*
$\alpha$ -HBCD				
Low	21	Reference	Reference	Reference
High	22	12.9 (2.82 - 58.6)*	11.3 (2.67 - 47.4)*	4.25 (1.08 - 16.8)*
$\beta$ -HBCD				
Low	21	Reference	Reference	Reference
High	22	12.9 (2.82 - 58.6)*	20.3 (4.18 - 98.2)*	1.73 (0.49 - 6.18)
$\gamma$ -HBCD				
Low	21	Reference	Reference	Reference
High	22	12.9 (2.82 - 58.6)*	20.3 (4.18 - 98.2)*	4.25 (1.08 - 16.8)*
$\Sigma$ PBCDs				
Non-Detect	25	Reference	Reference	Reference
Detect	18	NA	43.7 (4.86 - 394)*	5.00 (1.30 - 19.3)*
$\Sigma$ TBCDs				
Non-Detect	19	Reference	Reference	Reference
Detect	24	43.7 (4.86 - 394)*	NA	3.17 (0.81 - 12.4)
$\Sigma$ TBBP-A				
Non-Detect	28	Reference	Reference	Reference
Detect	15	5.00 (1.3 - 19.3)*	3.17 (0.91 - 12.4)	NA

\*Statistically significant at the  $\alpha = 0.05$  level.

**Table S7.** Univariate logistic regression for TBBP-A detection in the analyzed human milk samples.

Predictor	n	Odds Ratio	95% CI	p-value
Dietary consumption (per serving)				
Animal products				
Meat	43	1.13	0.42 - 3.05	0.81
Beef	43	0.79	0.09 - 6.70	0.83
Pork	43	0.44	0.01 - 32.6	0.71
Poultry	43	1.72	0.36 - 8.13	0.49
Fish	43	0.91	0.17 - 4.87	0.92
Salmon	43	3.20	0.003 - >999	0.75
Tuna	43	0.49	0.008 - 32.3	0.74
Other Fish	43	3.65	0.11 - 117.3	0.46
Eggs	43	0.76	0.10 - 5.66	0.79
Dairy	43	0.71	0.42 - 1.20	0.20
Milk	43	0.70	0.25 - 1.99	0.50
Cheese	43	0.44	0.13 - 1.53	0.20
Frozen Yogurt	43	0.71	0.44 - 1.17	0.18
Egg fat	43	0.21	<0.001 - >999	0.79
Dairy fat	43	0.13	0.01 - 1.75	0.12
Milk Fat	43	0.24	<0.001 - >999	0.74
Cheese Fat	43	0.05	<0.001 - 4.66	0.20
Froz. Yogurt Fat	43	0.10	0.002 - 6.67	0.28
Makes organic choices				
Yes	26	0.64	0.18 - 2.27	0.49
No	17	Reference		



**Table S7 (Continued).** Univariate logistic regression for TBBP-A detection.

Predictor	n	Odds Ratio	95% CI	p-value
<b>Vegetarian</b>				
Yes	2	1.93	0.11 - 33.2	0.65
No	41	Reference		
<b>Lifestyle</b>				
<b>Carpeting in the home</b>				
Yes	26	1.58	0.44 - 5.64	0.49
No	17	Reference		
<b>Use of a HEPA vacuum</b>				
Yes	12	2.02	0.46 - 8.87	0.35
No	23	Reference		
<b>Transportation</b>				
<b>Regularly uses public transportation</b>				
Yes	15	0.18	0.03 - 0.94	0.04
No	28	Reference		
Hours in vehicle per week	43	0.959	0.804 - 1.142	0.6366

**Table S7 (Continued).** Univariate logistic regression for TBBP-A detection.

Predictor	n	Odds Ratio	95% CI	p-value
Electronic Items in the Home				
Counts (Odds Ratio: per item)				
Stereo and Video Electronics	43	1.00	0.73 - 1.37	0.99
Television Only	43	0.88	0.47 - 1.67	0.69
Other Stereo and Video Electronics	43	1.06	0.70 - 1.61	0.78
Computer	43	1.13	0.82 - 1.55	0.45
Auxillary electronics	43	0.87	0.47 - 1.61	0.65
Other small appliances	43	0.69	0.38 - 1.25	0.22
Usage (hours/week)				
Television Only	43	0.99	0.95 - 1.04	0.66
Other Stereo and Video Electronics	43	1.03	0.99 - 1.08	0.20
Computer	43	1.01	0.98 - 1.03	0.64
Auxillary electronics	43	0.99	0.96 - 1.02	0.64
Breastfeeding				
Day of lactation (days)	37	1.00	0.95 - 1.05	0.85
Demographics				
Maternal age (years)	43	0.96	0.84 - 1.11	0.60
Race				
White	38	0.78	0.12 - 5.27	0.80
Not White	5	Reference		

**Table S7 (Continued).** Univariate logistic regression for TBBP-A detection.

Predictor	n	Odds Ratio	95% CI	p-value
Pre-pregnancy body mass index	43	1.08	0.94 - 1.25	0.27
College Graduate				
Yes	39	0.50	0.06 - 3.96	0.51
No	4	Reference		
Cigarette Smoking				
Pre-Pregnancy Smoker				
Yes	2	<0.001	<0.001 - >999	0.97
No	41	Reference		
Ever Smoker (last 3 years)				
Yes	4	0.41	0.04 - 4.06	0.45
No	37	Reference		

**Table S8.** Univariate linear regression for average levels of ΣHBCDs in the analyzed human milk samples ( $e^\beta$ =Multiplicative Increase).

Predictor	n	$e^\beta$	95% CI	p-value
Dietary consumption (per serving)				
Animal products				
Meat	43	1.10	0.74 - 1.64	0.62
Fish	43	0.62	0.33 - 1.18	0.14
Eggs	43	1.66	0.78 - 3.55	0.18
Dairy	43	1.00	0.82 - 1.22	0.97
Dairy fat	43	1.08	0.45 - 2.60	0.86
Dietary consumption controlling for organic				
Meat	43	0.91	0.62 - 1.35	0.64
Fish	43	0.53	0.30 - 0.94	0.03
Eggs	43			
Dairy	43	1.01	0.84 - 1.21	0.93
Dairy fat	43	0.99	0.44 - 2.24	0.98
Organic controlling for dietary consumption				
Meat	43	0.49	0.30 - 0.81	0.007
Fish	43	0.47	0.30 - 0.74	0.002
Eggs	43		-	
Dairy	43	0.51	0.32 - 0.82	0.007
Dairy fat	43	0.51	0.32 - 0.82	0.007
Makes organic choices				
No	17	Reference		
Yes	26	0.51	0.32 - 0.82	0.006

**Table S8 (Continued).** Univariate linear regression for average levels of ΣHBCDs.

Predictor	n	e <sup>β</sup>	95% CI	p-value
Vegetarian				
No	41	Reference		
Yes	2	0.57	0.18 - 1.82	0.33
Lifestyle				
Carpeting in the home				
No	26	Reference		
Yes	17	1.39	0.84 - 2.29	0.19
Use of a special vacuum (i.e., HEPA)				
No	23	Reference		
Yes	12	0.94	0.55 - 1.61	0.82
Transportation				
Regularly use MBTA				
No	28	Reference		
Yes	15	0.71	0.43 - 1.18	0.18
Hours in a vehicle per week	43	0.99	0.93 - 1.06	0.83
Electronic items in the home				
Count (Odds Ratio: Per item)				
Stereo and video electronics	43	1.17	1.04 - 1.31	0.01
Televisions only	43	1.18	0.93 - 1.49	0.18
Other stereo and video electronics	43	1.22	1.05 - 1.43	0.01
Computer	43	0.99	0.87 - 1.12	0.85
Auxillary equipment	43	0.86	0.68 - 1.09	0.20
Other small appliances	43	1.09	0.87 - 1.37	0.43

**Table S8 (Continued).** Univariate linear regression for average levels of ΣHBCDs.

Predictor	n	e <sup>β</sup>	95% CI	p-value
Usage (hours/week)				
Stereo and video electronics	43	1.00	0.99 - 1.01	0.96
Televisions only	43	1.01	0.99 - 1.02	0.38
Other stereo and video electronics	43	1.00	0.98 - 1.01	0.44
Computer	43	1.00	0.99 - 1.01	0.85
Auxiliary equipment	43	1.00	0.99 - 1.01	0.86
Breastfeeding				
Day of lactation (days)	37	0.99	0.97 - 1.01	0.22
Demographics				
Maternal age (years)	43	0.97	0.92 - 1.03	0.31
Race				
Not white	5	Reference		
White	38	0.51	0.24 - 1.07	0.07
Pre-pregnancy body mass index	43	1.02	0.96 - 1.08	0.48
Education				
High school graduate	2	Reference		
Some college	2	1.39	0.28 - 6.87	0.68
College graduate	39	0.59	0.18 - 1.87	0.36
College graduate				
No	4	Reference		
Yes	39	0.50	0.22 - 1.14	0.10

**Table S8 (Continued).** Univariate linear regression for average levels of ΣHBCDs.

Predictor	n	e <sup>β</sup>	95% CI	p-value
Recruitment location				
Brookline	27	Reference		
Cambridge	12	1.10	0.63 - 1.91	0.73
Lowell	4	2.07	0.88 - 4.87	0.09
Smokes cigarettes				
Pre-pregnancy smoker				
No	41	Reference		
Yes	2	0.96	0.29 - 3.12	0.94
Ever smoker (last 3 years)				
No	37	Reference		
Yes	4	1.31	0.54 - 3.16	0.54

**Table S9.** Summary statistics for reported concentrations of TBBP-A (pg/g lw) in human milk samples.

Country	Sample Year	n	Detection Frequency	Median	Range <sup>a</sup>	Publication
USA	2004/05	43	35%	NR	<30 - 550	Current Study
UK	2009/10	34	36%	<40	<40 – 650	Abdallah et al. 2011 [1]
China	2007	1237	Pooled	NR	<60 <sup>b</sup> – 5100	Shi et al. 2009 [2]
France	2004-2006	77	44%	480	62 - 37000	Cariou et al. 2008 [3]

<sup>a</sup>Maximum value rounded

<sup>b</sup>pg/g ww

NR: Not reported



**Table S10.** Summary statistics for reported concentrations of  $\Sigma$ HBCDs (pg/g lw) in human milk samples.

Country	Sample Year	n	Detection Frequency	Median	Range <sup>d</sup>	DC	Publication
Japan	1988-2006	13-35 <sup>a</sup>	Pooled	NR	430 - 4000	$\alpha$	Kakimoto et al. 2008 [4]
France	2005	23	30%	NR	<LOD - 5000	$\alpha$	Antignac et al. 2008 [5]
Sweden	1996-2006	177	44%	250	<200 - 7800	NR	Lignell et al. 2008 [6]
Russia	2000, 2002	14, 23	57%, 13%	450, 620	200 - 1670	$\alpha$	Polder et al. 2008 [7]
USA	2002	9	NR	500 <sup>b</sup>	200 - 900 <sup>b</sup>	NA	Ryan et al. 2006 [8]
Philippines	2004	33	NR	620	130 - 3200	$\alpha$	Malarvannan et al. 2009 [9]
USA	2004/05	43	100%	790	360 - 8100	$\alpha$	Current Study
Norway	1993-2001	85	58%	NR	400 - 20000	NR	Thomsen et al. 2005 [10]
Norway	2003-2005	310	57%	860	<200 - 31000	NR	Thomsen et al. 2010 [11]
China	2007	1237	Pooled	NR	<50 - 2800 <sup>a</sup>	$\alpha$	Shi et al. 2009 [2]
Ghana	2004, 2009	67	NR	270 - 1000 <sup>e</sup>	<10 - 18000	$\alpha$	Asante et al. 2011 [12]
Belgium	2006	178	Pooled	1500 <sup>a</sup>	NA	$\alpha$	Colles et al. 2008 [13]
Canada	2002/03	8	NR	1600 <sup>b</sup>	400 - 19000 <sup>b</sup>	NA	Ryan et al. 2006 [8]
Australia	1993-2009	NR	Pooled	NR	2500-19000 <sup>a</sup>	$\alpha, \gamma$	Toms et al. 2012 [14]
Vietnam	2007	33	100%	330-2000 <sup>e</sup>	70 - 7600	$\alpha$	Tue et al. 2010 [15]
Mexico	NR	7	100%	2100 <sup>c</sup>	800 - 5400	NR	Lopez et al. 2004 [16]
UK	2009/10	34	100%	3830	1040 - 22000	$\alpha$	Abdallah and Harrad 2011 [1]
Spain	2006-2007	33	91%	27000	3000 - 188000	$\gamma$	Eljarrat et al. 2009 [17]

DC: Dominant congener

NR: Not reported

NA: Not applicable

a: Pooled

b:  $\alpha$ -HBCD only

c: Mean, as reported by Thomsen et al. 2010

d: Maximum value rounded

e: Medians presented for 4 cities

**Table S11.** Summary statistics for reported concentrations of PBCDs (pg/g lw) in human milk samples.

Country	Sample Year	n	Detection Frequency	Median	Range	Publication
USA	2004/05	39	42%	NR	<30 - 320	Current Study
UK	2009/10	34	26%	<30	<30 - 200	Abdallah and Harrad 2011 [1]

**Table S12.** Summary statistics for reported concentrations of TBCDs (pg/g lw) in human milk samples.

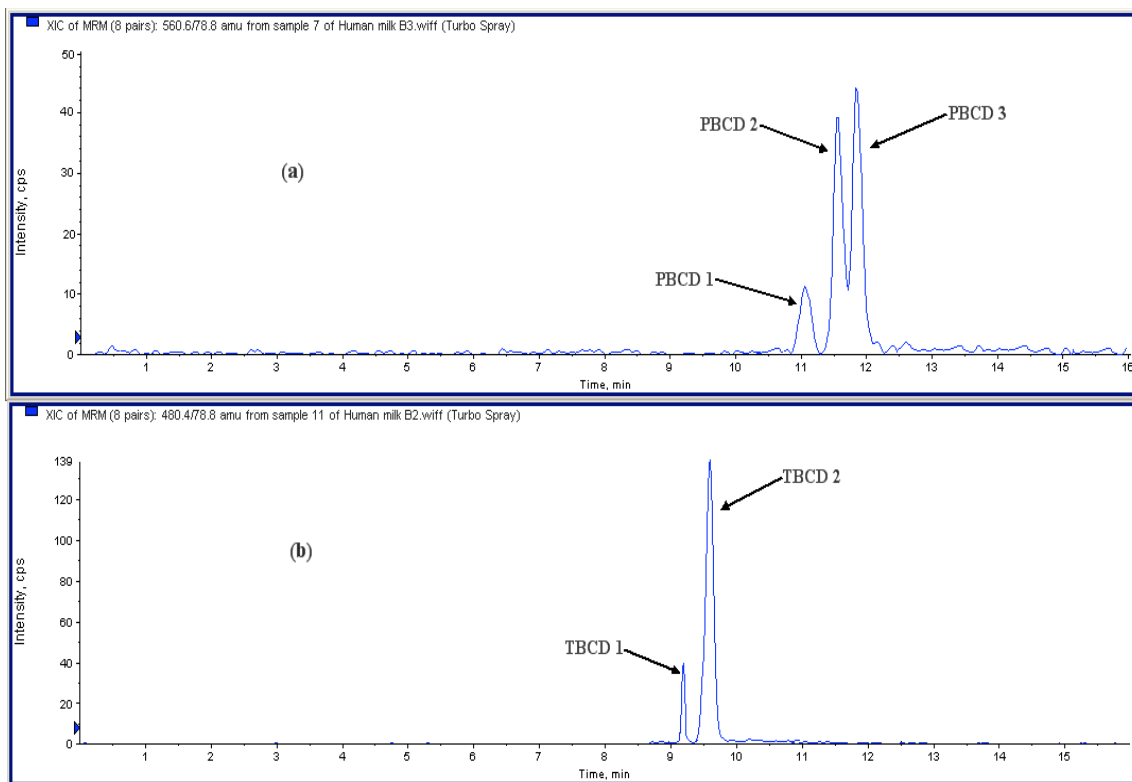
Country	Sample Year	n	Detection Frequency	Median	Range	Publication
USA	2004/05	39	56%	50	<30 - 530	Current Study
UK	2009/10	34	74%	140	<30 - 360	Abdallah and Harrad 2011 [1]

Table S13. Daily intake estimate for an infant (1 to <3 months) to BFRs from ingestion of human milk (ng/kg-day)<sup>1</sup>.

Analyte	<i>Milk Intake Rate:</i>		<i>Upper Percentile</i>	
	<i>Mean</i> (140 ml/kg-day)		<i>(190 ml/kg-day)</i>	
	GM	Max	GM	Max
TBBP-A	NR	3.1	NR	4.2
ΣHBCDs	5.7	45	7.8	62
α-HBCD	4.0	25	5.4	33
β-HBCD	0.45	9.0	0.61	12
λ-HBCD	1.1	18	1.5	24
PBCDs	NR	1.8	NR	2.4
TBCDs	0.28	3.0	0.38	4.0

<sup>1</sup>C<sub>BFR</sub> (GM or Max: ng/g-lw) \* 4.0 g/100 ml \* Milk Intake Rate (Mean or Upper Percentile: ml/kg-day)  
 NR: Not reported due to low detection frequency (<50%).

Figure S1: LC-ESI-MS/MS chromatograms of (a) PBCDs and (b) TBCDs in human milk.



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