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Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching–high performance liquid chromatography–isotope dilution tandem mass spectrometry

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Abstract

Breast milk is one possible route of exposure to environmental chemicals, including phenols and chlorinated organic chemicals for breast-fed infants. We developed a highly sensitive method of analyzing breast milk for triclocarban (3,4,4′-trichlorocarbanilide) and eight phenolic compounds: bisphenol A (BPA), 4-*tert*-octylphenol (4-tOP), *ortho*-phenylphenol (OPP), 2,4-dichlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and 2-hydroxy-4-metoxybenzophenone (BP-3). The method includes adding a solution containing a stable isotope of each chemical, enzymatic hydrolysis of the conjugated chemicals in the milk, and on-line solid-phase extraction coupled with high performance liquid chromatography–tandem mass spectrometry. It can also be used to measure the free (unconjugated) species by omitting the enzymatic deconjugation step. The method, validated using pooled breast milk samples, has inter-day coefficient of variations ranging from 4.8 to 18.9% for most analytes, and spiked recoveries generally about 100%. Detection limits for most analytes are below 1 ng/mL in 100 μL of breast milk. We tested the usefulness of the method by measuring concentrations of these nine compounds in 20 breast milk samples. BPA, OPP, and BP-3 were detected in more than 60% of the samples tested. The free species of these compounds appear to be most prevalent in milk.

1. Introduction

Humans are exposed to many environmental chemicals, including phenols and chlorinated organic chemicals, through industrial pollution, pesticide use, food consumption, and use of personal care and consumer products. Bisphenol A (BPA) is used to manufacture polycarbonate plastic and epoxy resins, which are used in baby bottles, as protective coatings on food containers, and for composites and sealants in dentistry. Alkylphenols (APs), such as 4-*tert*-octylphenol (4-tOP), are precursors in the manufacture of nonionic surfactants used in detergents and some pesticide formulations. Some phenols, such as 2-hydroxy-4-methoxybenzophenone (benzophenone-3, BP-3), are used as sunscreen agents in personal care products. Chlorophenols have been used in the wood preservation industry, as intermediates in producing pesticides, and as disinfectants or fungicides for industrial and indoor home use. Chlorophenols can also be produced as byproducts during the manufacture of other chlorinated aromatic compounds. Triclocarban (TCC, 3,4,4′-trichlorocarbanilide) is added to detergents, cosmetics, and other personal care products to prevent microbial growth.

Several of these compounds are toxic in animals. However, for the most part, toxic effects of these compounds in humans are largely unknown. BPA and APs display weak estrogenic activity in vitro and in vivo [1–3]. Some chlorinated phenols are carcinogenic in experimental animals [4]. BP-3 exerts a uterotrophic effect in vivo, stimulates proliferation of MCF-7 breast cancer cells, and increases the secretion of tumor marker pS2 in vitro [5]. TCC interferes with mammalian reproduction and can cause methemoglobinemia in humans [6].

The extensive use of phenols and chlorinated organic chemicals results in widespread human exposure in the general US population [7–9]. Interest in monitoring breast milk for environmental contaminants is increasing because breast milk is the main route of exposure to environmental chemicals for breastfed infants. Methods that have been reported use

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several analytical techniques to measure environmental phenols in milk [10–13]. BPA was measured by high performance liquid chromatography (HPLC) with column-switching and fluorescence detection [11]; and by alkaline digestion followed by solid-phase extraction (SPE), derivatization, and detection by gas chromatography–mass spectrometry (GC–MS) [10]. Chlorophenols were measured using solid-phase microextraction coupled with GC-electron capture detection [13]. GC–MS was used to measure several sunscreen agents, including BP-3, in breast milk [12]. All these analytical methods involve intensive sample preparation and derivatization steps. In addition, some of them require relatively large amounts of sample (10 mL) to achieve the desired sensitivity.

We developed a highly sensitive analytical method using isotope dilution on-line SPE coupled with HPLC–tandem mass spectrometry (MS/MS) for measuring in breast milk both the free and conjugated species of TCC and eight environmental phenols: BPA, 4-tOP, *ortho*-phenylphenol (OPP), 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,4,6-trichlorophenol (2,4,6-TCP), and BP-3. Compared with other methods [10,13], our on-line SPE–HPLC–MS/MS method provides similar sensitivity, precision, and accuracy, without complicated and time-consuming sample preparation. Our method can be used for quick, accurate, and cost-effective analysis of large numbers of samples in epidemiologic studies to assess the prevalence of infant exposure to selected phenols and chlorinated organic chemicals via breastfeeding.

2. Experimental

2.1. Analytical standards and reagents

Methanol (MeOH) and water, purchased from Caledon (Ontario, Canada) were analytical or HPLC grade. Formic acid (98%) was purchased from EM Science (Gibbstown, NJ, USA). 2-Propanol (HPLC grade) was from J.T. Baker (Phillipsburg, NJ, USA). BPA; 4-tOP; OPP; TCC; 2,4-DCP; 2,5-DCP; 2,4,5-TCP; 2,4,6-TCP; 4-methylumbelliferyl glucuronide; 4-methylumbelliferyl sulfate; ammonium acetate (>98%); and β-glucuronidase (*Helix pomatia*, H1) were purchased from Sigma–Aldrich Laboratories Inc. (St. Louis, MO, USA). EMD Chemicals Inc. (Hawthorne, NY, USA) kindly provided BP-3. $^{13}C_{12}$-BPA; $^{13}C_6$-OPP; D_7-TCC; $^{13}C_6$-2,4-DCP; $^{13}C_6$-2,5-DCP; $^{13}C_6$-2,4,5-TCP; and $^{13}C_6$-2,4,6-TCP were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). D_4-4-tOP was purchased from Hayashi Pure Chemical Ind., Co. Ltd. (Japan).

2.2. Preparation of standards and quality control materials

Initial stock solutions were prepared by dissolving measured amounts of the analytes of interest in methanol. Nine working standard spiking solutions, containing all nine compounds, were generated by serial dilution of the initial stock solutions with MeOH. These standards covered concentration ranges of 0.1–50 ng/mL (2,4-DCP; 2,5-DCP; 2,4,5-TCP; 2,4,6-TCP; and OPP), and 0.1–100 ng/mL (BPA, 4-tOP, BP-3, and TCC). The isotope-labeled standard spiking solution was also prepared in MeOH. All standard stock solutions and spiking solution were dispensed into vials and stored at −20 °C until use. Quality control (QC) materials were prepared by pooling breast milk obtained from multiple anonymous donors. The milk pool was divided into two for QC low (QCL) and QC high (QCH) concentration pools. QCL and QCH pools were enriched with different levels of native target compounds, mixed thoroughly after preparation, and dispensed in aliquots of 1.5 mL in glass autosampler vials. All QC materials were stored at −20 °C until use.

An aliquot (10 μL) of a solution prepared by dissolving 240 μg of 4-methylumbelliferyl glucuronide and 200 μg of 4-methylumbelliferyl sulfate in 100 mL of methanol was added to all samples and used as a deconjugation standard. The 4-methylumbelliferone/$^{13}C_4$-4-methylumbelliferone peak area ratio was monitored to check the extent of the deconjugation reaction. The enzyme solution was prepared by dissolving 0.1 g of β-glucuronidase/sulfatase (*H. pomatia*, 463,000 U/g solid) in 50 mL of 1 M ammonium acetate buffer solution (pH 5.0).

2.3. Sample preparation

To measure both free and conjugated species, each unknown sample was prepared in two different ways: one sample was processed without enzyme treatment, and the other was treated with β-glucuronidase (*H. Pomatia*). Breast milk was thawed and vortex mixed before aliquoting. A 100 μL aliquot of breast milk was mixed with 50 μL of internal standard solution, 10 μL of 4-methylumbelliferyl glucuronide/4-methylumbelliferyl sulfate standard, and 50 μL of enzyme solution in a disposable 1.5 mL CLIKLOK microcentrifuge tube (Simport, Beloeil, Canada). After gentle mixing, the sample was incubated overnight at 37 °C. After incubation, 190 μL of 2-propanol was added to the deconjugated breast milk, the sample was vortex mixed, and then centrifuged at 8000 × *g* for 15 min. Of the milk supernatant, 200 μL was transferred to an autosampler vial, 300 μL of 0.1 M formic acid was added, and the 500 μL sample was vortexed before being placed on the HPLC autosampler for on-line SPE–HPLC–MS/MS analysis. To determine concentrations of the free species, we followed the procedures described above, but added 50 μL of 1 M ammonium acetate buffer instead of the enzyme solution, and skipped the incubation step.

2.4. On-line SPE–HPLC–MS/MS

The on-line SPE–HPLC–MS/MS system was built from several Agilent 1100 modules (Agilent Technologies, Wilmington, DE, USA) coupled with an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) interface, similar to our previously described on-line SPE–HPLC–MS/MS setup [14], but using a 6-port instead of a 10-port switching valve. The SPE column was a LiChrosphere RP-18 ADS (25 mm × 4 mm, 25 μm particle size, 60 Å pore size, Merck KGaA, Germany), and the HPLC columns were two

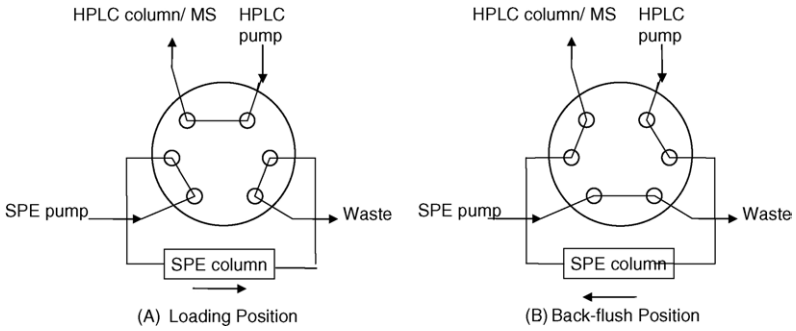

Fig. 1. On-line SPE–HPLC–MS/MS set up.

Chromolith^{TM} Performance RP-18 (100 mm × 4.6 mm; Merck KGaA, Germany). Two HPLC columns were needed to separate two pair of structural isomers, 2,4-DCP and 2,5-DCP, and 2,4,5-TCP and 2,4,6-TCP. The mass spectrometer and Agilent modules were programmed and controlled using the Analyst 1.4 software (Applied Biosystems), and the HPLC–MS/MS acquisition method was built in 'LC sync' mode.

The sample (500 μL) was loaded on the SPE column using the first binary pump with 100% H_2O at 1 mL/min for 3 min with the switching valve in the sample-loading position (Fig. 1A). After 3 min, the valve automatically switched to its alternate position (Fig. 1B) allowing the analytes to be transferred from the SPE column onto the HPLC analytical column in backflush mode using 50% MeOH (0.75 mL/min) provided by the second binary pump. After 5 min, the valve switched back to the loading position, and the first binary pump was used to regenerate the SPE column with 100% MeOH from 5.1 to 8 min and with 100% H_2O from 8.1 to 24 min at a flow rate of 1.0 mL/min. At the same time, the second binary pump was used to elute the analytes from the HPLC column using the following gradient program (mobile phase A: water, and mobile phase B: MeOH) for 24 min at a flow rate of 0.75 mL/min: 50% B for 5 min, increased to 65% B from 5 to 13 min, increased to 100% B from 13 to 20 min, kept at 100% B from 20 to 22 min, then decreased to 50% B at 22.1 min, and kept at 50% B for 2 min to equilibrate the HPLC column. This relatively slow gradient program was needed to separate 2,5-DCP from its structural isomer 2,4-DCP (Fig. 2), a metabolite of several xenobiotics, including the herbicide 2,4-dichlorophenoxyacetic acid.

2.5. Mass spectrometry

The API 4000 mass spectrometer was used in negative ion APCI mode. The APCI settings were curtain gas (N_2) flow: 20 arbitrary units (au), collision gas flow: 9 au, nebulizer gas (air) flow: 50 au, nebulizer gas temperature: 500 °C, and corona needle voltage: −3 V. Ionization parameters and collision cell parameters were optimized separately for each analyte. The

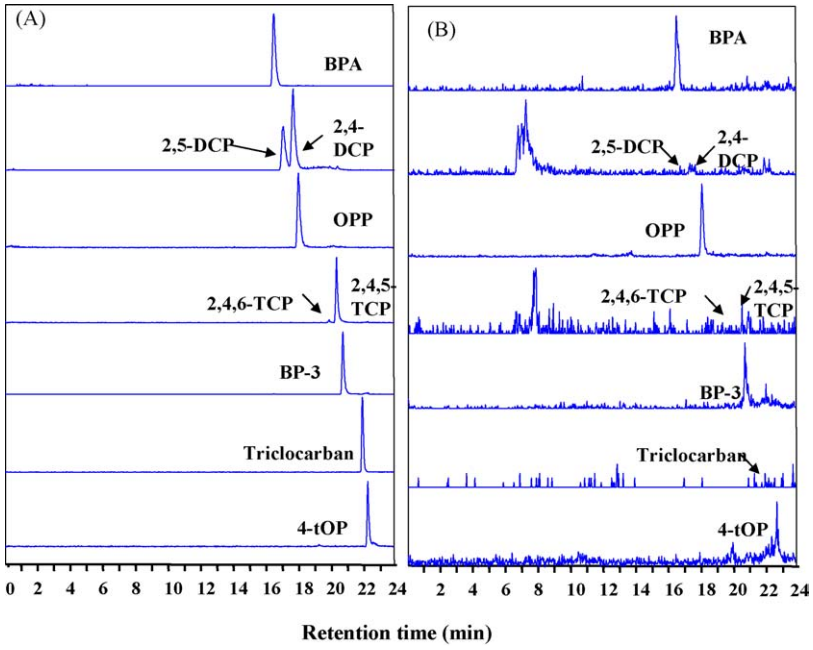

Fig. 2. HPLC–APCI–MS/MS chromatogram of (A) a standard mixture (concentrations are 5 ng/mL for 2,4-DCP, 2,5-DCP, 2,4,5-TCP, 2,4,6-TCP, and OPP, and 10 ng/mL for BPA, 4-tOP, BP-3, and triclocarban) and (B) a nonspiked human breast milk extract. Concentrations (in ng/mL) were as follows: BPA, 1.0; OPP, 2.9; all other analytes were not detected.

Table 1
Analyte retention time, and precursor ion → product ion transitions monitored for quantitation (and confirmation) of native compounds and corresponding isotope-labeled internal standards

Analyte	RT (min)	Precursor ion → Product ion (*m/z*)	
		Native analyte	Internal standard
Bisphenol A	16.6	227 → 133 (227 → 212)	239 → 139
4-*t*-Octyl phenol	22.4	205 → 133	209 → 137
Benzophenone-3	20.9	227 → 183 (227 → 211)	–
o-Phenylphenol	18.1	169 → 115 (169 → 141)	175 → 121
2,4-Dichlorophenol	17.7	161 → 125 (163 → 125)	167 → 131
2,5-Dichlorophenol	17.1	161 → 125 (163 → 125)	167 → 131
2,4,5-Trichlorophenol	20.5	195 → 159 (197 → 161)	201 → 165
2,4,6-Trichlorophenol	20.0	195 → 159 (197 → 161)	201 → 165
Triclocarban	22.0	313 → 160 (313 → 126)	320 → 163

RT = retention time. $^{13}C_{12}$-bisphenol A was used as the internal standard for benzophenone-3.

negative fragment ions used for quantification and the retention time for the analytes are listed in Table 1.

3. Results and discussion

3.1. Breast milk sample cleanup

Breast milk is a complex matrix that contains lipids, proteins, carbohydrates, minerals, and vitamins [15]. To maximize the lifetime of the SPE and HPLC analytical columns, we centrifuged the milk sample and injected the supernatant on the on-line SPE–HPLC–MS/MS system. Adding 2-propanol to the breast milk before centrifuging resulted in a precipitate and a clear supernatant with no lipid layer. By contrast, if the milk was centrifuged without adding any organic solvent, we obtained a precipitate at the bottom of the centrifuge tube and a supernatant consisting of a clear liquid with a lipid layer (thin film) on top. Transferring the supernatant to the autosampler vial for analysis easily mixed some of the lipid layer with the clear part of the supernatant and suppressed ionization of some target compounds. Therefore, for maximum sensitivity, we chose to add 2-propanol to the milk.

We also found that adding formic acid to the supernatant facilitated retention of the more acidic chlorophenols, especially trichlorophenols, to the SPE sorbent. We speculate that interactions facilitating retention of the acidic compound by SPE sorbent result from competition between formic acid, the acidic chlorophenols, and other milk components for interaction sites on the sorbent [16].

3.2. Method performance and quality control

Breast milk spiked with standard and isotope-labeled standard solutions was analyzed repeatedly to determine the limit of detection (LOD), accuracy, and precision of the method. The LOD and limit of quantification were calculated as $3S_0$ and $10S_0$, where S_0 is the standard deviation as the concentration approaches zero [17]. S_0 was determined from five repeated measurements of low-level standards. The calculated method LODs, except for 4-tOP (2.6 ng/mL), were 0.1–1.2 ng/mL (Table 2). These values reflect the good sensitivity of the method, especially considering the relatively low sample volume (100 μL) used.

Method accuracy was assessed by five replicate analyses of milk spiked at four different concentrations and expressed as the percentage of expected levels. The intraday variability, reflected in the method accuracy, ranged from 85 to 127% (Table 2); for most analytes, it was very good at all spike levels (90–110%). We determined method precision by calculating the relative standard deviation (R.S.D.) of 50 repeated measurements of QCL and QCH materials over a period of 2 weeks (Table 3). These R.S.D.s, which reflect the intraday and inter-day variability of the method, ranged from 4.8 to 25.6%. The precision was good for most analytes (4.8–18.9%), including BP-3 for which a labeled internal standard was not available.

Table 2
Solid-phase extraction recoveries, spiked standard concentration recoveries, and limits of detection

Analyte	SPE recovery	(Standard concentration, ng/mL) spiked recovery (%)				LOD (ng/mL)
Bisphenol A	93.7	(1) 106	(10) 102	(50) 97	(100) 104	0.28
4-*t*-Octyl phenol	56.0	(10) 127	(25) 98	(50) 99	(100) 90	2.55
Benzophenone-3	94.7	(5) 96	(10) 104	(50) 95	(100) 112	0.51
o-Phenylphenol	93.5	(1) 126	(5) 99	(10) 98	(50) 103	0.33
Triclocarban	20.0	(5) 113	(10) 98	(50) 102	(100) 103	0.91
2,4-Dichlorophenol	95.3	(1) 109	(5) 99	(10) 96	(50) 104	0.16
2,5-Dichlorophenol	100	(1) 85	(5) 103	(10) 109	(50) 105	0.42
2,4,5-Trichlorophenol	88.2	(1) 94	(5) 98	(10) 93	(50) 106	0.10
2,4,6-Trichlorophenol	86.1	(2.5) 89	(10) 103	(25) 99	(50) 99	1.22

SPE = solid-phase extraction; LOD = limit of detection.

Table 3
Precision of concentration measurements in spiked quality control samples

Analyte	QC Low		QC High	
	Mean	R.S.D.	Mean	R.S.D.
Bisphenol A	4.8	11.4	24.8	8.2
4-*t*-Octyl phenol	8.5	18.9	29.3	13.7
Benzophenone-3	3.6	18.0	17.3	12.7
o-Phenylphenol	4.6	5.8	18.6	5.9
2,4-Dichlorophenol	4.8	7.6	19.1	5.5
2,5-Dichlorophenol	4.5	5.7	18.4	4.8
2,4,5-Trichlorophenol	4.5	5.4	18.6	5.7
2,4,6-Trichlorophenol	5.4	25.6	19.2	9.9
Triclocarban	4.6	17.9	22.9	11.0

QC = quality control; R.S.D. = relative standard deviation.

Calibration curves were obtained from the standards spiked in water and in milk. The slopes in milk (water) were: 0.0305 (0.0282) for BPA; 0.072 (0.0736) for 4-tOP; 0.0141 (0.0132) for BP-3; 0.0165 (0.0169) for OPP; 0.018 (0.0188) for 2,4-DCP; 0.0172 (0.0182) for 2,5-DCP; 0.0275 (0.0288) for 2,4,5-TCP; 0.0436 (0.0403) for 2,4,6-TCP; and 0.0266 (0.026) for TCC. Because slopes of the calibration curves from both matrices were very similar, only the calibration curve obtained from water was used for quantification. The peak area ratio of each analyte to internal standard (i.e. response factor, [RF]) was used for quantification. Stable isotope-labeled internal standards were available for all of the analytes, except for BP-3. $^{13}C_{12}$-BPA was used as the internal standard for BP-3. Nine standard analyte concentrations ranging from 0.1 to 50 ng/mL (2,4-DCP; 2,5-DCP; 2,4,5-TCP; 2,4,6-TCP; and OPP), and 0.1–100 ng/mL (BPA, 4-tOP, BP-3, and TCC) spiked into water were used to construct daily calibration curves weighted by the reciprocal of the standard amount ($1/x$) of RF versus standard amount. Since standards and unknowns went through the same extraction procedure, reagent contributions were automatically corrected by the calibration curve intercept. Calibration curves in water showed adequate linearity (i.e. correlation coefficients greater than 0.99). Inter-day variation of calibration curve slopes, measured as the R.S.D. (%), was 2.4–9.8%.

To calculate SPE recoveries of compounds from milk, we performed the following experiment: First, 100 μL of blank milk spiked with a known amount of native standards was injected on the SPE column, and 50 μL of internal standard solution was injected into the HPLC gradient flow (using a second Agilent 1100 autosampler) when the compounds were backflushed from the SPE column and before starting the HPLC separation. Although native compounds and isotope-labeled standards were injected separately, they all eluted from the HPLC column and were detected by MS/MS at the same time. A response factor (RF_a) for each analyte was calculated from this experiment as the ratio of areas of native compound and its corresponding labeled analog. Second, blank milk, spiked with the same amount of native and internal standards, was injected on the SPE column, and 50 μL of MeOH was injected into the HPLC flow. RF_b was calculated as before. The two experiments differed in that for the first (RF_a), internal standards did not go through the SPE cleanup, but for the second (RF_b), they did. SPE recovery was calculated from RF_a/RF_b because the internal standard amount used for both experiments was the same and matrix effects were equivalent.

Very good SPE recoveries (86–100%) (Table 2) were obtained for most analytes except 4-tOP (56%) and TCC (20%). 4-tOP and TCC eluted last from the HPLC column. Because the SPE and HPLC columns contain similar RP-18 sorbents, these results suggest that 4-tOP and TCC are retained strongly on both the SPE and HPLC columns. Backflushing the SPE column with 50% MeOH for 2 min may not be enough to transfer 4-tOP and TCC completely onto the HPLC column. We tried extending the backflush time from 2 to 5 min, but SPE recovery improved only slightly. This suggests that either a backflush solvent with higher organic content or a weaker SPE sorbent must be used to completely elute 4-tOP and TCC from the SPE column. However, using a SPE backflsuh solvent with higher organic content compromised separation of the two pair of di- and tri-chlorophenol isomers. When we used another type of SPE column, Oasis HLB, SPE recoveries of 4-tOP, and TCC were still low. Nevertheless, although SPE recoveries for these two compounds are low, sensitivity ($LOD_{4\text{-tOP}}$ = 2.6 ng/mL, LOD_{TCC} = 1.2 ng/mL) and accuracy (spiked recoveries between 90 and 127% at four spiking levels) are still quite good (Table 2).

3.3. Environmental levels of chlorinated organic chemicals and phenols in breast milk

We applied our method to determine free and total (free plus conjugated) concentrations of TCC and eight environmental phenols in 20 breast milk samples collected from a group of anonymous lactating women who had no known occupational exposure to these compounds. BPA, OPP, and BP-3 were frequently detected in the milk samples (Table 4; Fig. 2). Median concentrations of free BPA (0.4 ng/mL) and total BPA (1.1 ng/mL) were comparable to median levels (0.6 ng/mL) of BPA found in a study of 23 lactating women [11]. Furthermore,

Table 4
Frequency of detection, mean and median concentrations of free, and total (free plus conjugated) species, and range of concentrations in breast milk of selected environmental phenols

Compound	Frequency of detection (%)	Mean (ng/mL)	Median (ng/mL)	Range (ng/mL)
BPA free	60	1.3	0.4	<LOD–6.3
BPA total	90	1.9	1.1	<LOD–7.3
4-tOP free	10	<LOD	<LOD	<LOD–5.0
4-tOP total	25	2.7	<LOD	<LOD–7.6
BP-3 free	15	<LOD	<LOD	<LOD–1.5
BP-3 total	60	0.9	0.7	<LOD–3.2
OPP free	70	1.6	1.7	<LOD–4.3
OPP total	85	1.8	2.2	<LOD–3.9

N = 20. Bisphenol A (BPA), 4-*tert*-octylphenol (4-tOP), benzophenone (BP-3), and orthophenyl phenol (OPP). The limits of detection (LODs) were 0.3 ng/mL (BPA), 2.6 ng/mL (4-tOP), 0.5 ng/mL (BP-3), and 0.3 ng/mL (OPP). Concentrations <LOD were imputed a value of LOD divided by the square root of 2 for the statistical calculations. Triclocarban and the other phenols were detected in less than 5% of the samples.

median concentration of BPA in milk in our population of 20 women was similar to median urinary concentrations of BPA (1.28 ng/mL) in a group of 394 adult U.S. residents [7]. Relatively high frequency of detection of BPA (free, 60%; total, 90%) in our study population suggests a high potential for exposure of breastfed infants to BPA via breast milk. We also detected OPP frequently in milk (free, 70%; total, 85%) at median concentrations around 2 ng/mL (Table 4), slightly higher than median urinary concentrations (0.49 ng/mL) in the general adult U.S. population [8]. To our knowledge, this is the first study to report concentrations of OPP in breast milk. 4-tOP and BP-3 were also detected in some breast milk samples (Table 4). In a previous study, BP-3 was detected in four of six samples collected at concentrations ranging from 16 to 417 ng/g (fat basis) from women in Germany [12]. Other compounds were detected infrequently (2,4-DCP, 5%; 2,4,5-TCP, 5%; 2,4,6-TCP, 5%) or not at all (2,5-DCP and TCC).

In the present study, levels of free species were very similar to levels of total (free + conjugated) species in breast milk. The presence of mostly unconjugated compounds in breast milk might be attributed to the more lipophilic character of free species compared with corresponding conjugates that might have favored their transport into breast milk during synthesis of the milk [18]. Conjugation (e.g. glucuronidation, sulfation) may reduce potential toxicity of compounds if only the free species is bioactive. In this case, although the free species might be available to induce health effects, their conjugated forms could be rendered essentially inactive. Because of potential health effects for nursing children, additional studies are needed to determine the degree of conjugation in milk of environmental chemicals, including phenols and chlorinated organic compounds.

In summary, we developed a sensitive, selective, and precise automated on-line SPE–HPLC–MS/MS method to measure simultaneously nine environmental chemicals, including TCC and eight phenols in breast milk. This method is rugged, labor- and cost-effective, and allows analysis of large numbers of samples for epidemiologic studies to assess the prevalence of human exposure to chlorinated organic chemicals and selected environmental phenols.

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