Supporting Information (SI-1)

Organic Contaminants in Human Breast Milk Identified by Non-Targeted Analysis

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Materials: The solvents use for all analyses were HPLC grade acetone, GC grade hexane, pesticide grade ethyl acetate (Fisher Scientific, Pittsburgh, PA, USA); GC grade methanol and LCMS grade water (Honeywell Burdick & Jackson, Morristown, NJ, USA); and pesticide grade dichloromethane (ACROS, Waltham, MA, USA). All glassware was baked at 450 °C for 6 hours prior to use. Laboratory equipment, including solid-phase extraction (SPE) manifolds and glass pipettes were washed three times each with HPLC grade acetone and GC grade hexane prior to use.

Breast milk sample preparation: Each sample of 16 mL breast milk was centrifuged at 3,000 revolutions per minute (RPM) for 15 minutes in a 12 mL glass test tube to separate the milk into lipid and water portions. Once separate layers had formed, the sample was frozen overnight at -20 °C and the lipid portion was transferred to a separate 12 mL test tube using a spatula. This process was repeated once to obtain the entire lipid portion. The lipid portion was weighed and processed separately from the water portion. The lipid concentrations were 12.8, 22.7, and 30.1 mg lipid / mL breast milk.

The lipid fractions were dissolved in 10 mL of 1:1 acetone:hexane (all solvent ratios are volume/volume), and transferred to a new 12 mL test tube with 1 g Na_2SO_4 to remove excess water. The sample was centrifuged at 3,000 RPM for 15 min to isolate the supernatant, which was transferred to a new test tube. The sample was dissolved in 5 mL of 1:1 cyclohexane:ethylacetate and injected on to a gel permeation chromatography system (GPC, J2 Scientific, Columbia, MO, USA) to separate the lipids from the contaminants. The GPC column (2 cm i.d. and 22.5 cm length), was packed with 24 g of BioBeadsS-X3 in 1:1 ethylacetate:cyclohexane. The flowrate was 5 mL/minute and the mobile phase was 1:1 ethylacetate:cyclohexane. The fraction eluting between 12.5 and 22.5 minutes was collected for further analysis and reduced to 1 mL using N_2 gas. A solvent exchange was conducted by bringing the extract to 5 mL with hexane followed by evaporation under nitrogen to 1 mL. The extract was loaded into a Silica (SPE) cartridge, ENVIRO CLEAN® Extraction Column (UCT, Bristol, PA, USA) and eluted with 5 mL of hexane, then 5 mL of 1:1 hexane:dichloromethane, and combined as

Lipid Extract Fraction 1 (Figure S1). The final elution with 5 mL dicholoromethane was Lipid Extract Fraction 2 (Figure S1). Each fraction was mixed with 1 g Na_2SO_4 to remove excess water, concentrated under nitrogen to approximately 250 µL, and analyzed by GC×GC/TOF-MS.

The water portion of each breast milk sample was centrifuged to remove any remaining lipid, then extracted using an Oasis® SPE cartridge (Waters, Milford, MA, USA) and 4 mL of acetone, followed by 4 mL of dicholoromethane. The eluent was mixed with 1 g Na_2SO_4 to remove excess water, centrifuged at 3,000 RPM for 15 min to isolate supernatant, and transferred to a new test tube. The sample was then solvent exchanged using 4 mL dichloromethane and concentrated under nitrogen to approximately 250 µL and analyzed by GC×GC/TOF-MS.

Instrumental Conditions: Operational parameters of the GC×GC/TOF-MS are shown in Table S1.

Log KOW, water solubility, and regulatory status: The log K_{OW} for each compound was determined using the KOWWIN component of the Estimations Programs Interface for Windows (EPI Suite) software[26]. We used experimental log K_{OW} values if provided by the software; otherwise, the software estimated the log K_{OW} based on the chemical structure. Water solubility was estimated based on the chemical structure by the WATERNT component of the EPI Suite software. In the box plot of the log K_{OW} data (Figure 2), the median is represented by the bold horizontal line, and the interquartile range (IQR, or distance from the 25th to 75th percentile) by the box. The whiskers extend to the furthest data point from the box that is <1.5 × IQR and outlying points extend beyond the whiskers[27,28]. Regulatory information was obtained using SciFinder's >347,000 compound Regulated Chemicals Listing[29], a database of international lists such as high production volume chemicals, priority chemicals, and pollutant release inventories.

Table S1. GC×GC/TOF-MS conditions for non-targeted analysis of human breast milk samples.

Component	Condition
Inlet	Splitless
Injection	2 µL
Inlet temperature	300 °C
Column conditions	1st Dimension: Rtx-5ms /w Integra-Guard 30 m x 0.25 mm x 0.25 µm
	2nd Dimension: Rtx-17 1 m x 0.10 mm x 0.1 µm
Carrier Gas	Helium
Carrier gas flow	Constant flow at 1 mL/min
1st Dimension oven program	60 °C for 1 min, 6 °C/min to 300 °C and hold for 3 min, 20 °C/min to 320 °C and hold for 15 min
2nd Dimension oven program	80 °C for 1 min, 6 °C/min to 320 °C and hold for 3 min, 20 °C/min to 340 °C and hold for 15min
Modulator temperature offset relative to 1st GC oven	35 °C
Modulation period	3.5 sec
Modulation timing (hot pulse)	0.9 sec
Cool time between stages	0.85 sec
Transfer line temperature	285 °C
Ion Source	250 °C
Solvent delay	10min
Scan rate	151.51 spectra/sec
Electron Energy (volts)	-70

Figure S1. Breast milk extraction of the lipid and water fractions. DCM = dichloromethane, GPC = gel permeation chromatography, and SPE = solid phase extraction.