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# **Chemical Contaminants in Raw and Pasteurized Human Milk**

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# **Abstract**

**Background:** Environmental contaminants ranging from legacy chemicals like p.p<sup>2</sup>dichlorodiphenyltrichloroethane (DDT) to emerging chemicals like phthalates are ubiquitous.

**Research aims/questions:** This research aims to examine the presence and co-occurrence of contaminants in human milk and effects of pasteurization on human milk chemical contaminants.

**Methods:** We analyzed human milk donated by 21 women to a milk bank for 23 chemicals, including the persistent organic pollutants (POPs) polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE) isomers that are known to sequester in adipose tissue, along with the current-use and nonpersistent pesticides chlorpyrifos and permethrin, phthalates, and bisphenol A (BPA). Human milk was analyzed raw and pasteurized for these chemicals using gas chromatography–tandem mass spectrometry for the POPs and high-performance liquid chromatography–tandem mass spectrometry for non-POPs.

**Results:** Within the different chemical classes, PBDE47, PCB153, ppDDE, and MEHHP (phthalate metabolite) had the highest median concentrations and were observed in all samples. We also observed chlorpyrifos and BPA in all samples and permethrin in 90% of the samples tested. Only two chemicals, chlorpyrifos and permethrin, were susceptible to substantial degradation from pasteurization, a standard method for processing donated human milk.

**Conclusion:** We detected 19 of 23 chemicals in all of our prepasteurized milk and 18 of 23 chemicals in all of our pasteurized milk. Pasteurization did not affect the presence of most of the chemicals. Future research should continue to explore human milk for potential chemical contamination and as a means to surveil exposures among women and children.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Supplementary Material

Supplementary Material may be found in the "Supplemental material" tab in the online version of this article.

# **Keywords**

human milk; milk bank

#### **Background**

Human milk is an important first food for infants. The benefits of feeding infants with human milk include protection from a variety of illnesses and infections and reduced risk of developing obesity (Office of the Surgeon General, 2011). Human milk is especially important to preterm infants for proper nutrition, immunological benefits, and growthpromoting components. In particular, it is associated with lower risk of complications that affect extremely preterm infants such as feeding intolerance, retinopathy of prematurity, and necrotizing enterocolitis (Cacho, Parker, & Neu, 2016). In fact, when preterm infants do not have their own mother's milk available to them, pasteurized donor human milk is commonly used as an alternative (Kantorowska et al., 2016; Parker et al., 2013).

Environmental contaminants ranging from legacy chemicals like DDT to emerging chemicals such as phthalates are ubiquitous. The majority of current research is dedicated to identifying chemical contaminants in traditional matrices like blood or urine, with much less focus on human milk, although it is known that chemicals in a mother's body can be transferred to her milk from recent chemical exposures or from chemicals that were stored in her fat and then remobilized during pregnancy and lactation (LaKind, Berlin, & Naiman, 2001; Landrigan, Sonawane, Mattison, McCally, & Garg, 2002). Prior human milk studies have focused on persistent organic pollutants (POPs) such as DDT, PCBs (Schlumpf et al., 2010; Weldon et al., 2011), and PBDEs that are sequestered in adipose stores in the body (Guo et al., 2016; Hoffman et al., 2016; Kalantzi et al., 2004; Marchitti, Fenton, Mendola, Kenneke, & Hines, 2017; Schecter et al., 2003; Schlumpf et al., 2010). Other classes of contaminants have been detected but are less frequently studied, including current-use pesticides (Schlumpf et al., 2010; Weldon et al., 2011), BPA (Mendonca, Hauser, Calafat, Arbuckle, & Duty, 2014), and chemicals related to personal care product exposure such as parabens (Schlumpf et al., 2010), phthalates (Fromme, Gruber et al., 2011; Hines, Calafat, Silva, Mendola, & Fenton, 2009; Kim et al., 2015; Main et al., 2006; Schlumpf et al., 2010; Zhu, Phillips, Feng, & Yang, 2006; Zimmermann, Gruber, Schlummer, Smolic, & Fromme, 2012), and triclosan (Dayan, 2007). Many concerns exist regarding potential negative effects of these types of chemicals on infant health and development. For example, phthalates may cause allergic and asthmatic symptoms, and PBDEs, permethrin and chlorpyrifos may impact neurodevelopment and behavior (Burke et al., 2017; California Office of Environmental Health Hazard Assessment, n.d.a, n.d.b; Lam et al., 2017). PCBs, DDT, and DDE are of concern for their potential effect on synaptogenesis (Pan et al., 2009). BPA is known to have estrogenic properties that may lead to multiple adverse developmental outcomes, including affecting behavior (Braun et al., 2011; Nachman, Hartle, Lees, & Groopman, 2014).

Further research is needed regarding levels of these types of contaminants in human milk. Studies that examine the co-occurrence of contaminants in human milk are also needed to

enable examination of similar etiologic mechanisms of chemical exposures and disease, a limitation of studies that typically focus on one or two classes of chemicals at a time. In addition, pasteurization is a component of donor milk processing, but to our knowledge, its impact on levels of chemical contaminants in human milk has not been evaluated.

The aim of the current study was to examine levels of a wide range of chemicals, 23 in total, from 21 human milk samples donated to a milk bank. We focused our investigation on chemicals known or suspected to have adverse health effects such as neurotoxicity or endocrine disruption: PBDEs, PCBs, organochlorines, organophosphates, pyrethroid insecticides, phthalates, and an environmental phenol, BPA. These chemicals include fatsoluble, persistent chemicals as well as water-soluble, nonpersistent chemicals. We compared levels before and after pasteurization as well as correlations among different classes of chemicals.

# **Materials and Methods**

#### **Design**

A cross-sectional design was selected, as the study aim was to examine the current chemical body burden of our sample population. The study protocol was reviewed by the Stanford University Institutional Review Board (eProtocol 34576) and determined to be exempt.

#### **Setting**

Human milk samples were obtained from women who voluntarily donated their milk in 2015 to the Mothers' Milk Bank in San José, California, which collects donations from mothers throughout California. The collection process (i.e., manual expression or breast pump) was not specified by each donor and therefore likely included samples collected from both processes.

#### **Sample**

The study population includes women who donated milk to the milk bank. Donors, who are unpaid, are recruited through social media, lactation consultants, newspaper advertisements, milk drives, and advertisements in doctors' offices and hospitals. Women donating to the milk bank are screened by questionnaire, information from their medical care provider, and with serology tests for HIV-1 and −2, HTLV −1 and 2, Hepatitis B and C, and syphilis. Donations are not accepted from women who have been identified as being in a high-risk group for transmitting blood-borne disease by their questionnaire, medical care provider, or serology. In addition, donations are not accepted by women who are taking most medications, including herbal medications, or are current smokers (Human Milk Banking Association of North America, 2015). Demographic information is provided by each woman who donates. Milk is donated to the milk bank frozen in BPA-free milk storage bags and is stored at −20 °C.

For this study, we selected 21 milk samples at random, each from a different unidentified mother. Milk bank employees drew two 5-ml aliquots from each sample. One aliquot from each pair of samples was pasteurized; after pasteurization, all samples were shipped on dry

ice to the environmental health laboratory at Emory University's Rollins School of Public Health for analysis. The pasteurization process utilized was Holder pasteurization, which is a standard method for processing donated human milk and involves heating to 62.5 °C for 30 minutes (Human Milk Banking Association of North America, 2015).

#### **Measurement**

Samples were prepared and analyzed using slight modifications of our previously published methods for PCBs and organochlorine pesticides (Marder et al., 2016; Weldon et al., 2011), PBDEs (Jacobson et al., 2016), current-use pesticides (Chen et al., 2014; Weldon et al., 2011), phthalates (Calafat, Slakman, Silva, Herbert, & Needham, 2004; Kato et al., 2003), and BPA (Joskow et al., 2006) (Table 1). Isotope dilution calibration was used to quantify all human milk contaminants. Isotope dilution calibration enables the highest accuracy and sensitivity; the isotopically labelled chemicals behave chemically identically to the target chemicals but can be discriminated based upon their mass, offering automatic recovery correction. Briefly, 500 μL to 1 mL of human milk was spiked with <sup>13</sup>C-enriched or <sup>2</sup>Henriched isotopic analogues of the target chemicals and then mixed well. Milk was denatured with formic acid (POPs) or saturated  $Na<sub>2</sub>SO<sub>4</sub>$  (for non-POPs, NPOPs). The milk was extracted with a mixed-phase solid phase extraction column to isolate the target chemicals and then eluted with solvent. The eluate was further cleaned to remove residual biogenic material by passing through a normal-phase solid phase extraction column (e.g., activated silica, florisil, or primary-secondary amine) and then collected. The eluate was concentrated to dryness and reconstituted in solvent appropriate for gas chromatography– tandem mass spectrometry (for POPs) or high-performance liquid chromatography–tandem mass spectrometry analysis (for NPOPs). The amount of lipid per gram of milk was determined gravimetrically from a separate aliquot. Quality control procedures included the use of blanks, duplicate samples, and spiked quality control materials representing about 15% of all samples tested. The accuracy of the quantified spiked value ranged from 93% to 102%, with relative standard deviations across analysis days of 7% to 13%.

#### **Data Collection**

Milk samples and demographic data were collected from study participants in 2015; women provided written informed consent to the milk bank to use their data and samples. Milk samples were selected and analyzed for chemical content as described above.

#### **Data Analysis**

For human milk samples below the limit of detection (LOD), we imputed the chemical concentration values as LOD/ 2 (Hornung & Reed, 1990; Weldon et al., 2011). Lipidadjusted concentrations were calculated for PBDEs and PCBs by dividing reported concentrations by the lipid concentration measured in the same sample.

The majority of chemical concentrations in the human milk were not normally distributed, exceptions being PCB118, PCB180, p,p'-DDE, o,p'-DDE, chlorpyrifos, and permethrin. We compared concentrations of each chemical before and after pasteurization using the Wilcoxon-Mann-Whitney test, appropriate for nonnormally distributed chemicals. We also examined correlation among chemicals as well as with the baby's age at the time of milk

collection using Spearman rank-sum tests. All statistical analyses were performed using Stata14 (StataCorp, College Station, TX).

# **Results**

#### **Demographics**

Sixty-two percent of the mothers were white, 81% were between the ages of 25 and 35 years of age, 43% had one child, and about half were from the southern and half from the northern part of California (Table 2). More than half of the samples were collected by mothers when their babies were less than 3 months of age (57%).

#### **Chemical Concentrations**

In our analysis of human milk for 23 chemicals, 19 of 23 chemicals were detected in 100% of the prepasteurization samples, and 18 of 23 chemicals were detected in 100% of the postpasteurization samples. The chemicals not detected in all samples were PBDE100, PBDE153, PBDE154, and permethrin in samples prepasteurization and chlorpyrifos, in addition, after pasteurization. The lowest detection frequencies were for PBDE100 before and after pasteurization (52%) and chlorpyrifos after pasteurization (52%) (Table 3). PBDEs were detected at a range of concentrations, with the highest median concentration being for PBDE47 (22.6 ng/g lipid). PCB concentrations were highest for PCB153 (median 10.6 ng/g lipid). Among the organochlorines DDE and DDT, the highest median concentration was for ppDDE  $(1,262 \text{ pg/g})$ . For phthalates, the highest median concentration was for MEHHP (124.4 ng/g). The environmental phenol BPA had a median concentration of 6.5 ng/g.

The chemicals were largely stable through pasteurization. On an individual basis, most chemicals decreased from pre- to postpasteurization; some increased, but these increases tended to be modest (<20% change) and to occur among values that were closer to the LOD (see Supplemental Tables A and B). The only chemicals with statistically significant reduction after pasteurization were chlorpyrifos and permethrin; chlorpyrifos' median concentration was reduced by 82% after pasteurization and 62% for permethrin (Table 4). The percent of samples with detectable levels went from 100% to 52% for chlorpyrifos and from 90% to 67% for permethrin. Additional details of chemical concentrations found before and after pasteurization, including the percentage of samples above the LODs and chemical concentration means; standard deviations; 10th, 25th, 50th, 75th, and 90th percentiles; and ranges can be found in the Supplemental Tables A and B.

#### **Correlations**

We ran Spearman rank correlation tests among chemical concentrations in the raw human milk samples (Table 5). Very strong positive correlations (correlation coefficient  $> 0.8$ ) were observed among PCBs (PCB 118 with PCBs 138 and 153, PCB 138 with PCB153), among different isomers of DDE (opDDE and ppDDE), and between the phthalate metabolites MEHHP and MEOHP. Strong correlations (correlation coefficient 0.6–0.79) were found between MEHP and both MEOHP and MEHPP. There were numerous moderate correlations (correlation coefficient 0.4–0.59), including negative moderate correlation of phthalate metabolites with PCBs and PBDEs.

Our study observed the trend that most of the correlations between the baby's age at the time the mother's milk was collected and chemical concentrations were relatively small  $(r < 0.4)$ and negative, with some exceptions. Correlations with PCBs tended to be small but positive. Larger correlations were observed for PBDE 153 (0.42), DDTs (−0.41 for ppDDT and −0.42 for opDDT), and chlorpyrifos (−0.42).

# **Discussion**

Our research showed that a range of chemicals with known adverse health effects were detectable in most of our studied human milk samples. Within the different classes of chemicals studied, PBDE47, PCB153, ppDDE, and MEHHP (a phthalate metabolite) had the highest median concentrations and were observed in all samples. We also observed the insecticide chlorpyrifos and the plasticizer BPA in all samples and the insecticide permethrin in 90% of the samples. Only two chemicals were susceptible to substantial degradation from pasteurization—chlorpyrifos and permethrin, both of which are nonpersistent insecticides.

When comparing our results to other human milk studies conducted in California, Guo et al., a study of 82 women sampled from 2003–2005 and a comparison population of 66 women sampled from 2009–2012, also found the highest PBDE concentrations for the congener PBDE47 (Guo et al., 2016). That study's median PBDE47 concentration was similar to ours  $(22.6 \text{ ng/g lipid for our study}, 16.7 \text{ ng/g lipid for Guo et al., } 2016)$ . The remaining PBDEs that both our studies analyzed were also in a comparable range. In comparison to other human milk research, including international work, our finding of PBDE47 having the highest concentration of the PBDE family is typical (Fromme, Raab et al., 2011). For PCBs, our median concentrations were nearly identical to Guo et al., except for PCB 153 (1.6 ng/g) lipid in our study and 4.9 ng/g lipid in Guo et al.). In comparing our data to Weldon et al. (2011), a study that examined human milk from 34 women living in urban and agricultural areas of California, our concentrations of PCB 118, 138, and 153 were similar to their rural participants. Our DDE and DDT concentrations were in between the levels observed for their urban and rural subjects. We had similar concentrations for chlorpyrifos, but our permethrin concentrations were much lower (28.2 pg/g milk for our study,  $>100$  pg/g for Weldon et al.). As it is known that chemical exposure and consequently human milk concentrations of chemicals vary depending on many factors (e.g., geography, occupation, personal habits, and personal characteristics), it would be helpful to have exposure information on a broader range of study subjects than currently exists within these small studies, including ours.

A strength of our study was that we measured multiple chemicals for each human milk sample at the same time. This method allowed us to examine correlations within and between the chemical groups. We found strong correlations among PCBs, PBDEs, DDE, and DDT and also some phthalates. We also observed negative moderate correlations of phthalate metabolites with PCBs and PBDEs. These inverse correlations need further research, as there is no established evidence to explain them. One speculative explanation for the co-presence of the POPs is that due to the natural variation of human milk in its fat to liquid proportions, for example between foremilk and hind milk or early milk and mature milk, the concentrations and relative proportions of the chemicals in the human milk may

also vary. The persistent chemicals are lipophilic and will concentrate in the fatty portions of human milk. The nonpersistent chemicals are more hydrophilic and will, to some extent, concentrate in the liquid portion. Applying these principles to our human milk samples, the more fatty content of milk, the less liquid content, resulting in a possible increase in the concentration of POPs such as PCBs, PBDEs, DDT, and DDE and a decrease in NPOPS such as water-soluble phthalates. An alternative explanation is that the sources of exposure are different for the chemicals that are inversely correlated; however, this explanation could not be verified with our data.

Maternal dietary intake or metabolism of fats may also contribute to contaminant levels in human milk. Dietary consumption of animal products such as dairy and red meat are the primary pathway of exposure to POPs, since POPs are lipophilic chemicals that are stored in fatty tissues including livestock and humans. Although people are mainly exposed to PBDEs through dust, PBDEs would still be found in the fatty portion of the human milk, as they are lipophilic like the other POPs we sampled for and are stored in fatty tissues in the body.

Most of the correlations of baby's age with chemical concentrations were negative and relatively small. Concentrations of POPs are known to be variable during different stages of lactation (Jakobsson, Fång, Athanasiadou, Rignell-Hydbom, & Bergman, 2012), potentially due to the fluctuations of the percent content of water, fat, proteins, and carbohydrates in the human milk (Lawrence & Lawrence, 2010). All of the milk analyzed in this study is classified as "mature milk," which is mothers' milk after the baby is 2 weeks old (Lawrence & Lawrence, 2010). Our earliest sample was collected when the baby was 26 days old. All other milk was collected after the babies were 30 days old, a time when the protein concentration and composition has stabilized (Lönnerdal, 2003). Our finding regarding DDT supports the theory that POPs in human milk decrease over time as the store of these chemicals in the mother's adipose cells is depleted. However, we also acknowledge that the literature shows a much more complex picture, with some research supporting the theory that POPs decrease with length of lactation and others showing that it can increase or stabilize (LaKind et al., 2009).

Our study was unique in that it investigated multiple chemical concentrations in human milk before and after pasteurization. Most chemicals were stable, the exceptions being two nonpersistent insecticides chlorpyrifos and permethrin. This is not surprising, given that these chemicals are structurally classified as an ester and ether, respectively, which are both acid- and heat-labile. An unexpected finding was that phthalates were not significantly degraded during the pasteurization process. However, we are not advocating pasteurization of nondonor milk, as it may also degrade heatsensitive proteins and nonmineral micronutrients.

In some samples, we found an increase in the phthalate concentration after pasteurization. We speculate that the phthalate concentrations detected were from contamination during the collection, storage, and/or analytical processes since this possibility has been documented previously (Calafat et al., 2004; Kato et al., 2003). Phthalates are the diesters of phthalic acid. Contamination of samples during collection will result in the presence of phthalates in their diester form. When people are exposed to phthalates, the phthalates are metabolized

into monoesters. To distinguish phthalate contamination from phthalate exposures, samples are analyzed for monoesters (Calafat et al., 2004; Hines et al., 2009). Monoesters detected in the raw and pasteurized human milk samples could be present due to enzymatic action after sample collection that hydrolyzes diesters into monoesters (Calafat et al., 2004). In addition, it is possible that heat from pasteurization could have broken down monoesters into phthalic acid and concomitantly diesters into monoesters. This is a potential reason why we did not detect phthalate metabolite degradation or, in some cases, detected higher phthalate metabolite concentration in the samples postpasteurization.

#### **Limitations**

The small number of samples limits our ability to generalize our findings or examine more covariates. In addition, women who self-select to donate to mother's milk banks are likely to be different from the general population of women who give birth. In particular, the study participants were less likely to be Hispanic than all California women giving birth (14% vs. close to 50%) (California Health Care Foundation, 2016), and none were less than 25 years old. We were unable to compare the levels of chemicals in human milk to levels in formula, although this is an important area of inquiry for future studies. Another area we did not explore but is worth future research is to compare the chemical concentrations found in a larger sample of human milk to health standards for chemical exposure.

# **Conclusion**

Our investigation observed the presence of a range of chemicals in human milk, demonstrating the need to study human milk further to better characterize maternal exposures and potential infant exposures. Our study demonstrated the feasibility and usefulness of simultaneously analyzing a variety of chemicals in a small aliquot of human milk, which, if applied to a larger sample size, could be a powerful tool to identify exposure patterns. There is long-standing discussion about the need for and utility of widespread human milk surveillance programs to enable population-level monitoring of exposures, modeling of potential infant exposures, and evaluation of the effectiveness of banning chemicals as evidenced by a reduction in chemical concentrations of a chemical over time (LaKind et al., 2001; Landrigan et al., 2002). Future research should continue to explore human milk as well as cow's milk and infant formula for potential chemical contamination and as a means to surveil exposures among women and children.

# **Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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#### **Key Messages**

- **•** Studies are needed that examine the co-occurrence of contaminants in human milk and the effects of pasteurization on human milk chemical contaminants.
- **•** Our investigation analyzed human milk before and after pasteurization for the presence of a range of chemicals including the persistent organic pollutants polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and dichlorodiphenyltrichloroethane (DDT); current-use nonpersistent pesticides chlorpyrifos and permethrin; phthalates; and bisphenol A (BPA).
- **•** We detected 19 of 23 chemicals in 100% of prepasteurized human milk samples, and 18 of 23 were detected in 100% of postpasteurization samples, illustrating that pasteurization did not affect most of the chemicals.
- **•** Future research should continue to explore human milk as well as cow's milk and infant formula for potential chemical contamination and as a means to surveil exposures among women and children.



# **Table 1.**

Target Chemicals Measured in Human Milk Samples From Milk Bank Donors, Their Sources, and Limits of Detection ( Target Chemicals Measured in Human Milk Samples From Milk Bank Donors, Their Sources, and Limits of Detection (N = 21).



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BFR = Brominated flame retardant; PBDE = polybrominated diphenyl ether; PCB= polychlorinated biphenyl.

# **Table 2.**

Characteristics of Mothers Donating Human Milk and Their Babies ( $N = 21$ ).



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Concentrations of Chemical Contaminants in Human Milk Samples  $\widetilde{\phantom{a}}$  $N = 21$ ).



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Human milk samples are nonpasteurized.

 $b_{\mbox{The range presented are the minimum and maximum values detected.}}$ The range presented are the minimum and maximum values detected.

Percent with concentration greater than or equal to the limit of detection, before pasteurization. Percent with concentration greater than or equal to the limit of detection, before pasteurization.

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Concentrations of Chlorpyrifos and Permethrin in Human Milk Before and After Pasteurization ( Concentrations of Chlorpyrifos and Permethrin in Human Milk Before and After Pasteurization  $(N = 21)$ .



 ${}^{4}$  Percent with concentration greater than or equal to the LOD, before pasteurization. Percent with concentration greater than or equal to the LOD, before pasteurization.

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Correlation Coefficient Correlation Category Correlation Coefficient Shading

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**Table 5.**

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Correlation coefficients are for chemical concentrations before pasteurization.

 $p < 0.05$ .

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