


## Research Paper

# Persistent Organic Pollutants in Human Milk: Exposure Levels and Determinants among Lactating Mothers in Lebanon

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## ABSTRACT

Exposure of newborns to persistent organic pollutants (POPs) is a public health concern. The objective of this study was to assess the prevalence of POPs in human milk collected from lactating mothers in Lebanon and to investigate the sociodemographic, nutritional, and other lifestyle determinants. Fifty-four breast milk samples were collected as per World Health Organization guidelines. A survey was used to assess the anthropometric and demographic characteristics of participants. Dietary habits were evaluated based on a semiquantitative food frequency questionnaire. Organochlorine pesticides and polychlorinated biphenyls were measured in milk samples with liquid-liquid extraction and gas chromatography. Among the screened POPs, dichlorodiphenyldichloroethylene (DDE) was the only POP detected in breast milk samples and was found in only 17.9% of the samples, with a mean (SD) of 11.6 (5.0) µg/L and a range of 5.7 to 21.4 µg/L. Prepregnancy body mass index and age were positively associated with DDE contamination in breast milk. Women who consumed cereals at least two times per week had detectable DDE contamination in their breast milk. Consumption of potatoes and beans at least once per week was also associated with DDE contamination. Our study is the first to assess the presence of POPs in breast milk in Lebanon. The benefits of breastfeeding compensate for the low prevalence of DDE in the breast milk. Our findings highlight the high need to implement monitoring policies, good agricultural practices, and education programs for breastfeeding mothers.

## HIGHLIGHTS

- DDE was the only POP detected in breast milk, in 17.9% of samples.
- Age and prepregnancy body mass index were associated with DDE contamination.
- Women who consumed cereals at least twice per week had detectable DDE.
- Consumption of potatoes and beans once per week was associated with DDE contamination.
- Consumption of dairy products and fresh produce was not linked to DDE contamination.

Key words: Breast milk; Determinants; Dichlorodiphenyldichloroethylene (DDE); Lebanon; Persistent organic pollutants

Human milk is the ideal nutrition for infants. Breast milk is nutritionally complete, promotes optimal growth, and possesses immunological and other health properties that protect infants from acute and chronic diseases (23). The World Health Organization (WHO) (41) endorses the benefits of breastfeeding for both the infant and the lactating mother and since 2001 has recommended exclusive breastfeeding for 6 months after delivery and nonexclusive breastfeeding with complementary foods for  $\geq 2$  years. However, past and present exposure of the lactating mother to environmental and dietary pollutants increases the risk of contaminating her milk, with potentially adverse health consequences on the lactating infant (5, 13, 32). Such

contaminants include persistent organic pollutants (POPs), which are synthetic organic compounds used primarily in industry and pesticides. These very stable chemicals are neither readily degraded in the environment nor completely metabolized or excreted by organisms. Because of their long half-life (10 to 15 years), POPs can travel up the food chain, and their concentration can biomagnify inside the body after being consumed and stored (22). The main route of human exposure is ingestion of contaminated food, particularly foods high in fat. Women store these lipophilic chemicals mainly in adipose tissue, and these chemicals are released during milk production when the mother uses her fat reserves, transferring a portion of the POPs that have been accumulating in her body to her newborn during breastfeeding (9).

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POPs are separated into three main groups: (i) organochlorine pesticides (OCPs), including aldrin, dieldrin, endrin, and dichlorodiphenyltrichloroethane (DDT), (ii) polychlorinated biphenyls (PCBs), and (iii) unintended by-products of incomplete combustion and chemical reactions such as dioxins and furans (22). POPs can compromise human health. Exposure to POPs is associated with reproductive system dysfunctions (40) and several types of cancers, such as breast, endometrial, colon, and prostate cancers and non-Hodgkin's lymphoma (6). POPs also can be endocrine disruptors, capable of altering hormonal function (27), which might increase the risk of metabolic disorders (25, 28) such as obesity, insulin resistance (26), dyslipidemia (34), and hypertension (16, 29). Lactating infants are at the highest risk from POP toxicity because they are at the very top of the food chain. Infants also may be more susceptible to POP toxicity than older individuals because of their low body weight, immature immune systems, and decreased detoxification abilities. Infant exposure to POPs may adversely affect physiological and intellectual development (6).

The recognized detrimental health effects of POPs on humans and animals prompted the Stockholm Convention on Persistent Pollutants Organic Pollutants in 2001 (which came into force in May 2004), a prominent global treaty to protect human health and the ecosystem by eliminating or reducing the use of POPs (24). However, POPs are still present in the environment. Studies conducted around the world, including New Zealand (31), South Africa (12), Canada (35), Tunisia (17), Asia (18, 36, 44), and Europe (8, 10, 15), have revealed the presence of various types and concentrations of POPs in human milk. Maternal sociodemographic and nutritional factors affecting the presence of POPs in human milk were also identified and included maternal age, parity, body mass index (BMI), and the mother's consumption of fish, fruits, vegetables, and dairy products.

In Lebanon, OCPs and PCBs have been found in rivers, groundwater, sediments, and soil from various regions (19). Helou et al. (20) also found OCPs and PCB in maternal and cord sera from pregnant women in Lebanon. In a study of POP contamination in milk from mothers ( $n = 32$ ) in Beirut, Lebanon, residues of DDT and its derivative dichlorodiphenyldichloroethane (DDD) were found in 33 and 22% of the samples, respectively. Dichlorodiphenyldichloroethylene (DDE), another DDT residue, was present in 97% of the breast milk samples in concentrations that surpass current maximums defined by the WHO (20  $\mu\text{g/L}$ ). Significant positive associations were found between the presence of DDE in samples and maternal age and consumption of high-fat meat and tuna (11).

Although POP contamination in the environment and human milk may decrease over time (33), the present study was conducted to assess the presence of POPs in the milk of lactating mothers in Lebanon between November 2015 and December 2016 and to investigate the sociodemographic, nutritional, and other lifestyle determinants of breast milk contamination by POPs.

## MATERIALS AND METHODS

**Breast milk samples.** The Lebanese Ministry of Public Health endorsed our study, which helped recruit participants. Fifty-four breast milk samples were collected from lactating volunteers living in various Lebanese governorates between November 2015 and December 2016. The Institutional Review Board (Lebanese American University) approved the study before we reached out to the participants, who signed informed consent forms. Breast milk samples were collected according to the WHO protocol (42). Criteria for the mothers were good health, younger than 30 years of age, primiparous with a normal pregnancy, and breastfeeding only one child. Participants could not reside in areas where high POP emissions were known or suspected and must have resided in their present area for at least the last 10 years. Each participant needed to be available within 3 to 8 weeks of delivery to collect her milk. Samples (10 to 75 mL) were collected in sterile plastic containers and transported in an icebox to the laboratory freezer, where they were stored at  $-20^{\circ}\text{C}$  until analyzed.

**Participant information.** A culturally tailored questionnaire was used to assess the sociodemographic characteristics of participants, such as age, height, weight, educational level, lactation period, and occupational status. The participants' dietary habits were assessed via a semiquantitative food frequency questionnaire, which collected information on various food groups: dried fruits, pulses, cereals, eggs, meat, milk, fats and oils, nuts, oilseeds, and drinks. This questionnaire was based on the WHO guidelines, and data were recorded as grams of food per person per week (42). Certified translators translated the questionnaires into Arabic and then back-translated them into English.

**Chemicals.** A standard mixture of 16 OCPs (catalog no. 41001) in methanol was purchased from Absolute Standards (Hamden, CT) and contained aldrin (100  $\mu\text{g/mL}$ ),  $\alpha$ -benzene hexachloride (BHC; 100  $\mu\text{g/mL}$ ),  $\beta$ -BHC (100  $\mu\text{g/mL}$ ),  $\delta$ -BHC (100  $\mu\text{g/mL}$ ),  $\gamma$ -BHC (lindane; 100  $\mu\text{g/mL}$ ), 4,4'-DDD (600  $\mu\text{g/mL}$ ), 4,4'-DDE (200  $\mu\text{g/mL}$ ), 4,4'-DDT (600  $\mu\text{g/mL}$ ), dieldrin (200  $\mu\text{g/mL}$ ), endosulfan I (200  $\mu\text{g/mL}$ ), endosulfan II (200  $\mu\text{g/mL}$ ), endosulfan sulfate (600  $\mu\text{g/mL}$ ), endrin (200  $\mu\text{g/mL}$ ), endrin aldehyde (600  $\mu\text{g/mL}$ ), heptachlor (100  $\mu\text{g/mL}$ ), and heptachlor epoxide (100  $\mu\text{g/mL}$ ).

Individual PCBs at 100  $\mu\text{g/mL}$  in isoctane were acquired from Absolute Standards: PCB 18 (2,2',5-trichlorobiphenyl, catalog no. 66018), PCB 28 (2,4,4'-trichlorobiphenyl, catalog no. 66028), PCB 31 (2,4',5-trichlorobiphenyl, catalog no. 66031), PCB 44 (2,2',3,5'-tetrachlorobiphenyl, catalog no. 66044), PCB 52 (2,2',4,5,5'-tetrachlorobiphenyl, catalog no. 66052), PCB 101 (2,2',4,5,5'-pentachlorobiphenyl, catalog no. 66101), PCB 118 (2,3',4,4',5-pentachlorobiphenyl, catalog no. 66118), PCB 135 (2,2',3,3',5,6'-hexachlorobiphenyl, catalog no. 66135), PCB 149 (2,2',3,4',5',6-hexachlorobiphenyl, catalog no. 66149), PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl, catalog no. 66153), PCB 170 (2,2',3,3',4,4',5-heptachlorobiphenyl catalog no. 66170), PCB 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl, catalog no. 66180), and PCB 194 (2,2',3,3',4,4',5,5'-octachlorobiphenyl, catalog no. 66194).

Hexachlorobenzene (catalog no. 79152) at 1,000  $\mu\text{g/mL}$  in methylene chloride and pentachloronitrobenzene (catalog no. 79012) at 1,000  $\mu\text{g/mL}$  in hexane were purchased from Absolute Standards. Pentachloronitrobenzene was used as the surrogate standard for OCPs, and PCB 170 was used as the surrogate standard for PCBs.

Other chemicals used in the analyses and sample extractions were purchased from Sigma Aldrich (St. Louis, MO), including sodium chloride, formic acid, sodium sulfate anhydrous, *n*-pentane, *n*-hexane, isooctane, and dichloromethane. The anhydrous sodium sulfate was heated at 400°C for 16 h and stored in a 130°C oven.

**Analytical methods.** Breast milk sample extraction was done in duplicate following the procedure documented by the United Nations Environment Programme (38). The frozen breast milk samples were allowed to thaw at 4°C for 4 h and then were homogenized manually for 1 min. A 5-mL sample aliquot was weighed, fortified with 100 µL of internal standards pentachloronitrobenzene and PCB 170, and mixed with 2 mL of formic acid.

Liquid-liquid extraction was performed twice on the milk samples by adding 12 mL of hexane-dichloromethane (5:1, v/v). After the liquid-liquid extraction, 1 mL of isooctane was added to the extract, which was then concentrated to 1 mL in a water bath (40 ± 2°C) under a continuous nitrogen flow. The extract was purified in two steps. The first involved passing the extract over an Al<sub>2</sub>O<sub>3</sub> glass column with glass frit (22 cm by 20 mm inside diameter) packed with 15 g of 8% deactivated Al<sub>2</sub>O<sub>3</sub> followed by 1 cm of Na<sub>2</sub>SO<sub>4</sub>. The extract was eluted with 210 mL of pentane, and the eluate was then evaporated and concentrated to 1 mL with a rotatory evaporator. The second step involved fractionation over a 1.5% (w/w) deactivated silica glass column (15 cm by 11 mm inside diameter) filled with 1.8 g of deactivated silica with 1.5% water followed by 1 cm of Na<sub>2</sub>SO<sub>4</sub>. The first fraction collected was eluted with 11 mL of hexane, and the second fraction was eluted with 10 mL of diethyl ether in hexane (15%, v/v). One milliliter of isooctane was then added to both fraction tubes, and the extracts were concentrated to 500 µL in a water bath (40 ± 2°C) under a continuous nitrogen flow. The eluates were then used for gas chromatography (GC).

**Instrumental analysis.** High-resolution GC (67890 series GC system, Agilent Technologies, Santa Clara, CA) was used to separate OCPs and PCBs from milk samples. Detection was performed by a <sup>63</sup>Ni electron capture detector and a DB-608 capillary column (30 m by 0.32 mm inside diameter, 0.5-µm film thickness). The GC-mass spectrometry (MS) analyses were performed on a gas chromatograph (7890, Agilent) connected to a mass spectrometer (5975, Agilent). An SLB-5ms P/N 28471-U capillary column (30 m by 0.25 mm inside diameter, 0.25-µm film thickness) was used to determine the OCPs and PCBs. The GC injector temperature was set at 250°C, with a sample injection volume of 1 µL, splitless injector mode, a purge flow of 2 mL min<sup>-1</sup>, purge time of 2 min, helium as the carrier gas at a constant flow rate of 1 mL min<sup>-1</sup>, and nitrogen as the make-up gas.

**Quality assurance and quality control.** Both the GC and MS equipment were checked for adequate performance before the analysis. Rigorous quality control was used throughout the analyses. The samples were processed in three batches, and every batch included a sample blank, sample duplicate, spiked sample (10 ppb of PCBs and other OCPs, 20 ppb of DDE and dieldrin, and 60 ppb of DDD and DDT) in addition to a standard curve.

For the GC-electron capture detection, 1 ppm of degradation mix (DDT and endrin) was run before every sequence to check the degradation of these compounds and the column's condition. Regular tuning was performed on the GC-MS equipment to ensure proper operation. For both instruments, solvents were used as blanks to clean the system for every sequence and after each sample.

TABLE 1. Spike recoveries for various POPs

Sample	Mean (SD) spike recovery (%)
Hexachlorobenzene	98.9 (7.5)
PCB 18	76 (45.3)
Lindane	63.9 (26.6)
β-BHC	42.8 (74.1)
Heptachlor	89.5 (16.7)
PCB 31	79.4 (72.9)
PCB 28	65.1 (58.2)
PCB 52	343.7 (416)
DDE	54.9 (23.7)
Dieldrin	67.4 (47.9)
PCB 118	82 (49.4)
PCB 149	90 (36.1)
PCB 153 + endrin	79.6 (83.1)
DDD	62.3 (9.3)
DDT + PCB 138	69.7 (13.5)
PCB 180	95.7 (15.1)
PCB 194	99 (4.6)

The calculated mean (standard deviation [SD]) recoveries are shown in Table 1. At least three extracted curves were used, and the correlation coefficients of the calibration curves were >0.995. Surrogate standards were also used.

**Statistical analysis.** STATA v. 13 was used for the statistical analysis. Study variables were summarized and checked for outliers using descriptive analysis. Categorical variables were described using frequencies and percentages. Means and SDs were used to represent continuous variables. The Shapiro-Wilk test was used to assess data normality. Spearman's rank correlation coefficient (ρ) was used to measure the association between the nonparametric variable POP, age, and BMI. The analysis of the total milk sample was categorized as (i) detectable amounts of POP and (ii) no POP. Chi-square tests were used to assess the difference between the two groups in terms of consumption of cereals and grains, dry and canned beans, potatoes, dairy products (pasteurized milk, yogurt, labneh, and white cheeses), and fresh fruits and vegetables.

## RESULTS AND DISCUSSION

Fifty-four lactating mothers took part in our study and completed the surveys. The demographic characteristics of the sample are listed in Table 2. The mean (SD) age was 28.0 (5.0) years and BMI was 23.3 (3.1). The primary governorates of residence were Mount Lebanon (27.8%), Beirut (27.8%), and South (25.9%). The majority of the nursing mothers had university degrees (74.1%), but only 44.4% engaged in paid employment. Concerning smoking, 33.3 and 14.8% of mothers reported that they smoked before and after pregnancy, respectively.

Among the screened POPs (hexachlorobenzene, PCB 18, lindane, β-BHC, heptachlor, PCB 31, PCB 28, PCB 52, DDE, dieldrin, PCB 118, PCB 149, PCB 153 plus endrin, DDD, DDT plus PCB 138, PCB 180, and PCB 194), DDE was the only POP detected in breast milk samples. This lack of POPs can be attributed to the fact that Lebanon is not an industrialized country. Van den Berg et al. (39) also reported that POPs were significantly less prevalent in the Southern Hemisphere, where fewer industrialized countries are

TABLE 2. Demographic characteristics of study participants

Characteristic	Value
Age (yr), mean (SD)	28.0 (5.0)
BMI (preconception), mean (SD)	23.3 (3.1)
Governorate, <i>n</i> (%)	
North	4 (7.4)
Bekaa	3 (5.6)
Nabatiyeh	3 (5.6)
South	14 (25.9)
Mount Lebanon	15 (27.8)
Beirut	15 (27.8)
Education, <i>n</i> (%)	
Illiterate	1 (1.9)
Primary	2 (3.7)
Intermediate	3 (5.6)
Secondary	4 (7.4)
Technical	4 (7.4)
University	40 (74.1)
Work, <i>n</i> (%)	
Yes	24 (44.4)
Medication use, <i>n</i> (%)	
Yes	11 (20.4) <sup>a</sup>
Supplement use, <i>n</i> (%)	
Yes	45 (83.3) <sup>b</sup>
Complications during pregnancy, <i>n</i> (%)	
Yes	17 (31.5) <sup>c</sup>
Alcohol consumption, <i>n</i> (%)	
No	52 (96.3)
Smoking before pregnancy, <i>n</i> (%)	
Yes	18 (33.3)
Smoking during pregnancy, <i>n</i> (%)	
Yes	8 (14.8)
Smoking in household, <i>n</i> (%)	
Yes	21 (38.9)
Exposed to smoke randomly, <i>n</i> (%)	
Yes	35 (64.8)
Smoking exposure at work, <i>n</i> (%)	
Yes	3 (5.6)

<sup>a</sup> Medical conditions reported included anemia, hyperthyroidism, bacteremia, and venous insufficiency.

<sup>b</sup> Mainly multivitamins, folic acid, iron, calcium, and vitamin D.

<sup>c</sup> Complications reported included iron deficiency anemia (35.7%), hyperemesis gravidarum (28.6%), and gestational diabetes (28.6%).

located. DDE contamination was found in 17.9% of the breast milk sample, with a mean (SD) concentration of 11.6 (5.0) µg/L and a range of 5.7 to 21.4 µg/L.

Both maternal age ( $\rho = 0.34$ ,  $P = 0.0143$ ) and prepregnancy BMI ( $\rho = 0.41$ ,  $P = 0.0155$ ) were positively associated with DDE contamination in breast milk (Table 3). This finding is consistent with those of previous reports, in which age was one of the most significant factors affecting POP concentrations in human milk (14, 17, 37). A

TABLE 3. Spearman correlations coefficients ( $\rho$ ) between DDE contamination in breast milk and maternal age and prepregnancy BMI

Variable	$\rho$	<i>P</i> value <sup>a</sup>
Age	0.34	0.0143*
Prepregnancy BMI	0.41	0.0155*

<sup>a</sup> \*  $P < 0.05$ .

higher BMI is typically associated with higher body fat, and POPs are known to accumulate in adipose tissues. Thus, because lactating women use fat reserves when secreting milk, a portion of the POPs will be transferred to breast milk (9).

Women who consumed cereals at least two times per week had detectable DDE contamination in their breast milk ( $P = 0.015$ ) versus those who consumed cereals less frequently. Consumption of potatoes ( $P = 0.026$ ), dry beans ( $P = 0.036$ ), and canned beans ( $P = 0.002$ ) at least once weekly also was significantly associated with DDE contamination (Table 4). An association between exposure to DDE from diet and DDE concentrations in breast milk was also reported in Spain (30) and Tunisia (3). Unofficial reports from Lebanon have confirmed the illegal import of some banned pesticides, including DDT, from bordering countries where their use is legal. Therefore, tubers, pulses, and cereal crops consumed in Lebanon might be highly contaminated with these illegal pesticides and can contribute to *p,p'*-DDE exposure. Although no studies on DDE in tubers, pulses, and cereals have been conducted in Lebanon, significant contamination in these crops was reported in Nigeria (1) and India (21). Irrigation water in Lebanon tends to be contaminated with DDE at concentrations far exceeding the maximums set by the U.S. Environmental Protection Agency (2, 4, 43). Cultivation of fruits and vegetables does not involve direct exposure to irrigation water, unlike cereals, potatoes, and beans, which may explain why consumption of fresh produce was not significantly associated with DDE contamination of breast milk samples (Table 4).

Defined tolerable daily intake concentrations are not applicable for infants because the food intake of infants per kilogram of body weight is higher than that of adults. Because of their dynamic growth, infants also differ from adults in their susceptibility to toxins (7). Proposed interventions should involve a quantitative estimate of risk-based reference values for consumption of contaminants in human milk and take into account socioeconomic factors and the recognized benefits of breastfeeding. The benefits of breastfeeding clearly compensate for the potential risks from exposure to DDE. Although breast milk is one of several means of infant exposure to POPs, particularly DDE, breastfeeding should always be encouraged. Our findings indicate the crucial need to establish and implement good agricultural practices, education programs, and monitoring policies for breastfeeding mothers to decrease the exposure of infants to POPs and other contaminants. Future studies should be conducted to quantify the POPs in the food supply in Lebanon and to

TABLE 4. Cross-tabulation of food groups and POP contamination in breast milk

Food group consumption	Overall samples ( <i>n</i> )	Mean (SD) (µg/L)	Detectable DDE in breast milk			Test of independence, $\chi^2(1)$	<i>P</i> value <sup>a</sup>
			No. of positive samples	% of overall samples	% of positive samples		
Cereals						5.9341	0.015*
<Twice per week	26	9.28	1	3.8	11.1		
≥Twice per week	28	12.65 (5.01)	8	28.6	88.9		
Potatoes						9.8182	0.026*
<Once per week	17	0	0	0	0		
At least once per week	37	12.27 (4.82)	9	24.3	100		
Canned beans						9.8182	0.002*
<Once per week	44	10.35 (1.64)	4	9.1	44.4		
At least once per week	10	13.82 (6.14)	5	50	55.6		
Dry beans						4.3776	0.036*
<Once per week	23	12.53	1	4.3	11.1		
At least once per week	31	12.24 (5.15)	8	25.8	88.9		
Fresh vegetables						0.0002	0.989
<Twice per week	23	12.69 (4.24)	4	17.4	44.4		
≥Twice per week	29	11.94 (5.71)	5	17.2	55.6		
Fresh fruits						2.1600	0.142
<Twice per week	24	15.59 (4.33)	2	8.3	22.2		
≥Twice per week	30	11.33 (4.80)	7	23.3	77.8		
Pasteurized milk						0.5400	0.462
≤Twice per week	30	13.50 (5.61)	6	20	66.7		
Daily	24	9.82 (0.75)	3	12.5	33.3		
White cheeses						0.3787	0.538
≤Twice per week	31	13.66 (5.11)	6	19.4	66.7		
Daily	23	9.49 (3.16)	3	13	33.3		

<sup>a</sup> \* *P* < 0.05.

determine the sources of exposure to these contaminants through the diet.

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