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Differences in mirex [dechlorane] and dechlorane plus [*syn*- and *anti*-] concentrations observed in Canadian human milk

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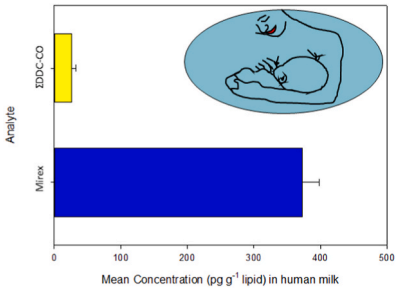
HIGHLIGHTS

- First national study of dechlorane plus in Canadian human milk.
- Higher detection frequency for *anti*-dechlorane plus than *syn*-dechlorane plus.
- Mirex was detected in 100% of the samples analyzed, a decrease in levels observed.
- Higher concentrations of mirex were observed than *syn*- and *anti*-dechlorane plus.
- Age was correlated with both mirex and ΣDDC-CO concentrations, but parity was not.

GRAPHICAL ABSTRACT



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ABSTRACT

As part of the pan-Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) study, human milk samples were collected between 2008 and 2011, and analyzed for mirex, an organochlorine insecticide and flame retardant, in addition to dechlorane plus (*syn*- and *anti*-DDC-CO), the flame retardant replacement for mirex. Mirex was analyzed separately, using a method for the analysis of existing organochlorine insecticides, while the presence of DDC-CO isomers was determined using a method developed for the detection of emerging flame retardants. Mirex was detected in all samples analyzed (n = 298), while *syn*- and *anti*-DDC-CO were present in 61.0% and 79.5% of the samples, respectively (n = 541). Mirex concentrations have declined in human milk since the 1990s. Since this is the first pan-Canadian dataset reporting DDC-CO concentrations in human milk, no temporal comparisons can be made. Maternal age was correlated with concentrations of both compounds although parity did not impact concentrations of either analyte. Given the presence of this relatively recently

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identified flame retardant (DDC-CO) in human milk from women across Canada, studies to identify dominant sources of this compound are critical. Despite low concentrations of environmental chemicals in human milk from Canadian women, Health Canada supports breastfeeding of infants because of the important health benefits to both the mothers and their infants.

1. Introduction

Mirex is a fully chlorinated organic compound that has been widely used in the control of fire ants and other insects globally (Environment Canada, 2013). In addition to its insecticidal properties, mirex had applications as an additive flame retardant in paints, electrical goods, plastics and rubber between 1959 and 1972 (Feo et al., 2012; National Center for Biotechnology Information, 2022). Although mirex was never registered for use as an insecticide in Canada, it was manufactured in Niagara Falls, New York, USA by the company then named Hooker Chemical and Plastics Corp., currently OxyChem, and was imported into Canada (Kaiser, 1978; PSPC, 2019; Wang et al., 2013). Between 1959 and 1975, the reported sales of mirex for non-insecticidal uses was primarily as a flame retardant. Annual usage ranged from 100 to 541, 691 pounds, totalling 2,481,374 pounds (1126 tonnes), representing approximately 75% of company sales over this 17 year period (Kaiser, 1978). While the insecticidal uses of mirex were largely within the US, the application as a flame retardant, under the name dechlorane, was not limited to the US (World Health Organization, 1990).

Following the removal of most uses of mirex as an insecticide in the US in the late 1970s, due to toxicity concerns, other highly chlorinated compounds were developed as replacement flame retardant products, including dodecachlorodimethanodibenzocyclooctane (DDC-CO, dechlorane plus or DP), present as *syn*- and *anti*-isomers (Bergman et al., 2012; Hoh et al., 2006; Kaiser, 1978). DDC-CO has similar fire retardant properties to those of mirex and it is used in electronics, wires and roofing materials (Hoh et al., 2006; Shen et al., 2010). Globally, there are two main DDC-CO producers, one being OxyChem in Niagara Falls, NY which had an estimated production rate of 450–5000 tonnes annually since 1986 and the other, Anpon, in Jiangsu Province, China where 300–1000 tonnes annually were estimated to have been produced since 2003 (Hansen et al., 2020).

Broad spectrum flame retardant use in buildings, consumer products, electronics and vehicles has occurred in recent decades to address fire safety regulations (Dreyer et al., 2019), which has resulted in the identification of indoor environments as important sources of human exposure to flame retardants (Abou-Elwafa Abdallah and Harrad, 2022; Akinrinade et al., 2021; Chen et al., 2019). DDC-CO concentrations have been reported in dust from a variety of locations with elevated levels being observed in public microenvironments (hotel/-supermarket/classroom/lobby), similar to concentrations measured in cars and offices (He et al., 2018).

Although DDC-CO was first reported relatively recently in environmental matrices (Hoh et al., 2006), it has been increasingly of research interest. The initial estimates of DDC-CO physical-chemical properties were consistent with long-range transport potential (Sverko et al., 2011). Additionally, the high bioaccumulation potential has resulted in global investigations to determine DDC-CO in environmental matrices and biota (Smythe et al., 2020; Wang et al., 2010; Zafar et al., 2020), food (Sun et al., 2019; Tao et al., 2016; Zacs et al., 2021) and human tissues (Fromme et al., 2015; Qiao et al., 2018; Yin et al., 2020). Both *syn*- and *anti*-dechlorane plus have been observed in all investigated environmental compartments. Although the ubiquitous nature of DDC-CO has been increasingly confirmed, its toxicity has not yet been well studied. DDC-CO was not found to be acutely toxic and had low potential for mutagenicity in studies with bacteria (Dou et al., 2015). Oxidative stress has been associated with oral exposure to DDC-CO in mice and the liver appears to be the target organ (Li et al., 2013; Wu et al., 2012).

DDC-CO has been reported in human tissues (Xian et al., 2011; Brasseur et al., 2014; Kim et al., 2016; Qiao et al., 2018; Fromme et al., 2020; Martinez et al., 2021; Bao et al., 2022; Wielsøe et al., 2022), although investigations into the concentrations in human milk are relatively limited (Siddique et al., 2012; Ben et al., 2013; Zhou et al., 2014; Čechová et al., 2017; Pan et al., 2020). The present work describes the determination of DDC-CO (*syn*- and *anti*-) and mirex, the compound it replaced, in human milk from the pan-Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) study.

The MIREC study was established to investigate maternal exposure to many chemicals. Samples of blood and urine were collected as part of the MIREC study, during trimester visits to the study clinics (Arbuckle et al., 2013; Arbuckle et al., 2014; Arbuckle et al., 2016; Fisher et al., 2016), in addition to human milk samples collected postpartum.

2. Materials and methods

2.1. Study population and sampling

The MIREC study was developed to examine multiple classes of environmental contaminants in pregnant women across Canada, rather than focussing on individual cities. This pan-Canadian approach was adopted to provide insight into the levels of chemical contaminants in women during pregnancy and their children on a national scale for a period between 2008 and 2011. Those clinics with existing research frameworks were the focus for identification of sites as recruitment centres. Before any centre was confirmed as a research centre for the MIREC study, approval from the research ethics boards of the research centre itself, that of the MIREC coordination centre (Centre hospitalier Universitaire [CHU] Sainte-Justine, Québec) and Health Canada was required. Participant recruitment occurred during maternal visits to the clinics (Arbuckle et al., 2013) and allowed the MIREC program to maintain contact with participants throughout their pregnancy, following recruitment. Each participant provided written consent, in advance of participation in the MIREC study.

Ultimately, 10 research centres were established in six provinces (British Columbia, Alberta, Manitoba, Ontario, Québec and Nova Scotia), with the majority of sites located in Ontario, which is the most populous province (Rawn et al., 2022). The research centres in Ontario represented different population densities and cultural backgrounds. Participation in the study was voluntary and, hence participants are not a representative random sample of the population.

Eligibility requirements to participate in the study included the following: women had to be 18 years of age or older, <14 weeks gestation and capable of communicating in either official language (i.e., English or French) (Arbuckle et al., 2013). Among the women approached regarding the study, approximately 59% were eligible and 39% of those agreed to participate in the study. A total of 2001 women enrolled in the study between 2008 and 2011. During each trimester, participants answered questions related to lifestyle, demographic and personal status, including pre-pregnancy body mass index (BMI), age and parity.

Among the 2001 participants in the MIREC study, approximately half of them (n = 1017) provided human milk samples, while the remaining participants contributed to other components of the study, they did not provide human milk samples (Fig. 1). All human milk collection was performed by participants between two and 10 weeks following the birth of their babies (Arbuckle et al., 2013).

Hand expression of both fore- and hind-milk was considered ideal for

the investigation; however, if participants experienced difficulty expressing the milk, a pump was provided for their use and retention. Participants were not required to collect milk for the samples at one sitting, but could collect it over a period of time for this study. If collection was completed within a few days, samples were kept at 4 °C between collections, but if it took a longer time, participants were asked to retain them in the freezer (−20 °C). All samples were shipped frozen to the study coordination centre in CHU Sainte-Justine, Québec, followed by transfer to the designated laboratory for aliquot development. Samples selected for the determination of mirex and DDC-CO were collected in glass jars and aliquots were taken into glass containers for storage at −80 °C until the laboratory was ready for analysis.

2.2. Sample distribution

Once research collection centres were established, data from the Canadian Community Health Survey (CCHS) (Statistics Canada, 2013) were used to develop a sampling framework for human milk. The sampling framework was developed to allow for sample distribution from all collection centres in a representative manner. Factors considered during the development of the distribution plan included estimates of the number of participants who would continue to breastfeed their infants beyond two weeks from the birthdate of their baby, maternal age and parity. The number of samples capable of being analyzed in the required timeframe by individual labs was also considered during this process, to ensure that samples from across all sampling centres were included (Table 1).

Separate aliquots of human milk received from participants were prepared for each type of analysis. Individual samples were coded with the same coding retained for all analyses, regardless of analyte class. All samples measured for one group of analytes were prepared for analysis within the same laboratory (i.e., samples for organochlorine insecticides, including mirex/dechlorane determination were analyzed in one lab, while samples used for DDC-CO measurement were analyzed in the lab responsible for novel halogenated flame retardants). The lab responsible for mirex analysis received 298 samples, and the lab involved in DDC-CO analysis received 554 human milk samples.

2.3. Extraction and Clean up

As mirex was considered exclusively as an organochlorine insecticide through the analytical work in the MIREC program, it was quantified

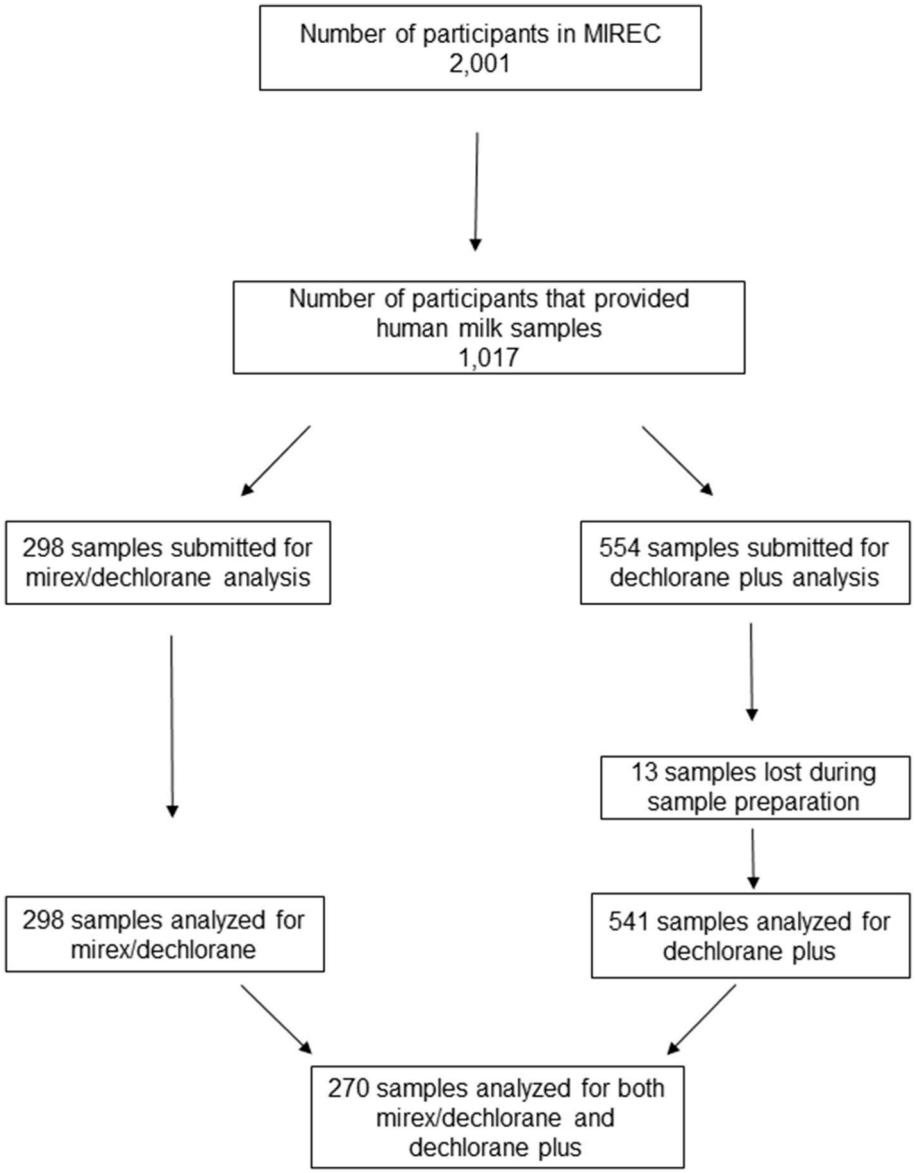

Fig. 1. Study population, sample distribution for analysis.

Table 1
Participant summary information corresponding to the human milk samples analyzed for mirex and DDC-CO.

Characteristic	Summary Statistics			
Mirex analyses	N = 298			
Age	Range: 21–46 years; mean 33.6			
Parity	Range: 1–4			
Age Group (years)	<30 years		≥30 years	
	Primiparous	Multiparous	Primiparous	Multiparous
Maternal Country of Birth				
Canada	38	21	89	104
Other	2	2	17	25
Total	40 (13%)	23 (8%)	106 (36%)	129 (43%)
DDC-CO analyses	N = 541			
Age	Range: 20–44 years; mean 32.6			
Parity	Range: 1–5			
Age Group (years)	<30 years		≥30 years	
	Primiparous	Multiparous	Primiparous	Multiparous
Maternal Country of Birth				
Canada	88	44	129	198
Other	11	5	25	41
Total	99 (18%)	49 (9%)	154 (29%)	239 (44%)

following the method used for pesticide analyses. In contrast, DDC-CO analyses were performed using a method developed for emerging flame retardants. The methods used for sample preparation and analysis for each of the methods described below were performed by the Food Research Division authors.

2.3.1. Mirex

Milk samples (25 g) were weighed into Erlenmeyer flasks and 1.35 ng ^{13}C labelled mirex (Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA) was added as a surrogate standard to each sample. Samples were extracted with 300 mL acetone: hexane (2:1) (Omnisolv, EMD Science, Mississauga, ON) with homogenization using a Polytron. Homogenized samples were transferred through a funnel fitted with glass wool (pre-cleaned by rinsing with dichloromethane) to a separatory funnel. The lower, water layer was discarded and the organic layer was treated with aqueous sodium chloride (EMD anhydrous, ACS grade, Ottawa, ON) and mixed. Following the dispersal of any emulsion, the water layer was discarded. The extract was dried over heat treated sodium sulfate (EMD anhydrous Tracepur, 10–60 mesh, 675 °C for 18 h in a muffle oven) and collected in a 500 mL round bottomed flask using a funnel with pre-cleaned glass wool. Samples were then evaporated to a small volume using rotary evaporation and dried for a second time over sodium sulfate and collected in a pre-weighed 125 mL round bottomed flask. Samples were set aside in the fume hood until constant weight was achieved. Lipid content was determined gravimetrically.

Samples were then diluted in 1:1 dichloromethane: cyclohexane (Omnisolv), filtered through 0.45 μm polytetrafluorethylene (PTFE) filters (Sigma Aldrich, St. Louis, MO, USA) and cleaned up using gel permeation chromatography (Autoprep 2000; OI Analytical, College Station, Texas) using 60 g S-X3 biobeads (200–400 mesh, Bio-Rad Laboratories, Mississauga, ON) with dichloromethane: cyclohexane (1:1, v/v) as the mobile phase with a flow rate of 5 mL min^{-1}. Following gel permeation chromatography, samples were concentrated to 2 mL in hexane for column chromatography using 6 g 2% water deactivated Florisil (EMD 60–100 mesh, Ottawa, ON) topped with anhydrous sodium sulfate and the extract was eluted with 60 mL hexane and collected for mirex analysis. Approximately 200 μL iso-octane (Omnisolv) was added as a keeper and extracts were concentrated to approximately 500 μL using rotary evaporation, transferred to 5 mL graduated centrifuge tubes and taken to less than 200 μL under a gentle stream of nitrogen in a water bath set to 29 °C. ^{13}C PCB 141 (Cambridge Isotope Laboratories, Tewksbury, MA, USA) (256 pg) was then added as a performance standard and samples were brought to a final volume of 200 μL with isooctane. Samples were then transferred to amber glass autosampler vials with glass inserts and stored at −80 °C until ready for analysis.

2.3.2. DDC-CO

Human milk samples were prepared for DDC-CO analysis using the method developed for novel halogenated flame retardants in cow milk (Rawn et al., 2016). Briefly, milk (10 g) was transferred into polypropylene centrifuge tubes, fortified with 625 pg ^{13}C *syn*-DDC-CO (Cambridge Isotope Laboratories), allowed to sit for 30 min on the laboratory bench and 1 mL HCl (ACS grade, J.T. Baker, Fisher Scientific) was added prior to sonication for 15 min. Following sonication, samples sat on the laboratory bench for 15 min and then 10 mL deionized purified water (minimum resistivity 18.2 MΩ) and 20 mL acetone: hexane (2:1, v/v), were added to the centrifuge tube. Samples were homogenized using an Omni Tissue Homogenizer for 1 min, capped and mixed for 10 min using a Roto-torque variable speed rotator (Cole-Parmer, Montréal, QC, Canada). Samples were centrifuged for 10 min at 12, 857×*g* in an Eppendorf centrifuge 5810R (Eppendorf, Mississauga, ON), set at 10 °C and the supernatant was transferred to a 15 mL graduated glass centrifuge tube and set aside while the sample was re-extracted using an additional 10 mL acetone: hexane (2:1) and a third extraction using 8 mL acetone: hexane (2:1), and the supernatants of the additional extractions were combined with the first. The extract was concentrated to 2.5 mL in a water bath set to ~40 °C with a gentle stream of nitrogen. Sample volumes were adjusted to 5 mL with dichloromethane and vortexed. Lipid determination was performed gravimetrically by removing an aliquot of the extracts (0.5 mL) and transferring them to pre-weighed aluminum dishes placed in the fume hood overnight to allow the solvent to evaporate and lipid determination was performed the following day.

Following the removal of 0.5 mL for lipid determination, similar to the mirex analysis protocol, DDC-CO samples were cleaned up using gel permeation chromatography (Gilson GX-271, Middleton, WI, USA) using 35 g of S-X3 biobeads (200–400 mesh) (Bio-Rad). The mobile phase was 1:1 dichloromethane: hexane (v/v) with a flow rate of 5 mL min^{-1} and extracts were concentrated to 5–10 mL using rotary evaporation. Additionally, samples were further cleaned up using columns of 8 g 3% water deactivated Florisil (60–100 mesh) with anhydrous sodium sulfate on the top and bottom of each column. DDC-CO was eluted using 50 mL of 1:1 dichloromethane: hexane and concentrated to ~1 mL using rotary evaporation, transferred to a 2 mL graduated centrifuge tube containing 50 μL toluene as a keeper and concentrated further to ~40 μL using a gentle stream of nitrogen in a water bath maintained at 40 °C. ^{13}C polybrominated biphenyl 194 (12.5 ng) purchased from Wellington Laboratories (Guelph, ON) was added as a performance standard and the final volume was adjusted to 50 μL with toluene. Samples were transferred into amber glass autosampler vials with a glass insert and samples were capped and stored at −80 °C until they were to be analyzed. Of the 554 samples included for analysis of DDC-CO, 13 were lost during sample preparation, resulting in the analysis of 541 human milk samples.

2.4. Analysis

2.4.1. Mirex

Mirex samples were analyzed using an Agilent 6890 (Agilent Technologies Canada, Mississauga, ON) gas chromatograph coupled to a MicroMass AutoSpec magnetic sector mass spectrometer. Separation of mirex from other analytes, surrogates and performance standards was achieved using a 30 m DB5 column (0.25 mm i. d. x 0.25 μm film thickness) coupled to a 3 m, 0.53 mm i. d. retention gap (Agilent Technologies). The injector was set to track the oven temperature. The initial temperature was 80 °C where it remained for 2 min, followed by an increase at a rate of 8 °C min^{-1} to 240 °C and a final increase to 280 °C at 15 °C min^{-1}, where it was held for 5 min. The head pressure was ramped from 28 kPa to 173 kPa throughout the analytical run. The carrier gas was helium. The mass spectrometer was operating in EI positive mode at 50 eV, the source temperature was 250 °C and the trap current was set to 650 μA. Injection volumes were 1 μL and the mass

resolution was 4000 for these analyses.

The quantification and qualifying ions for mirex were *m/z* 271.8102 and 269.8131, respectively. The surrogate and performance standard quantification and qualifying ions were 278.8240/280.8210 and 371.8817/373.8788, respectively. Retention times for mirex, ^{13}C mirex and ^{13}C PCB 141 were 31.67 min, 31.66 min and 26.31 min, respectively. Compounds were confirmed if the relative retention time matched with that of the corresponding analytical standard (±0.1 min) and the response ratio of the quantification/qualifying ions also agreed with the standard.

2.4.2. DDC-CO

DDC-CO samples were analyzed using an Agilent 7890A gas chromatograph (Agilent Technologies, Mississauga, ON) coupled to a Waters AutoSpec Premier high resolution mass spectrometer (Waters Corporation, Milford, Massachusetts, USA). Analyte separation was achieved using a 15 m DB-5MS column (0.25 mm i. d. x 0.10 μm film thickness) coupled to a 5 m, 0.53 mm i. d. deactivated fused silica retention gap (Agilent Technologies). The oven temperature was initially set to 80 °C and held for 2 min, followed by an increase to 170 °C at 20 °C min^{-1} and held for 5.5 min with a final increase to 320 °C at a rate of 25 °C min^{-1} and held for 10 min. Similar to the mirex analyses, the injector was set to track the oven temperature for these analyses, helium was used as the carrier gas at a constant flow of 1.2 mL min^{-1}. The mass spectrometer was operating in EI positive ion mode at 36 eV, the trap current was 600 μA and the source temperature was 250 °C. Sample volumes of 1 μL were injected and the mass spectrometer was operated at resolution 10,000 for DDC-CO analyses.

The quantification and qualifying ions of DDC-CO (*syn*- and *anti*-) were *m/z* 271.8102 and 269.8131, respectively. *Syn*-DDC-CO eluted first (retention time 17.91 min) followed by *anti*-DDC-CO (18.10 min). The surrogate and performance standards ^{13}C *syn*-DDC-CO and ^{13}C PBB194 quantification and qualifying ions were 278.8240/280.8210 and 637.5593/635.5618, respectively. As indicated for mirex, DDC-CO detection was confirmed based on relative retention time (±0.1 min) and response ratio of two characteristic ions to that of individual standards.

2.5. Quality assurance/quality control

With each set of samples analyzed for mirex, a reagent blank was included to correct results for any laboratory background contributions. In addition, an internal quality control human milk sample, was tested as unfortified and paired with a fortified sample, spiked at either 0.78 ng g^{-1} or 0.39 ng g^{-1} sample weight. Additionally, one of two standard reference materials (SRM 1953; non-fortified human milk or SRM 1954; fortified human milk; NIST, Gaithersburg, MD, USA) was run with each set of samples. The mean mirex concentration in the unfortified internal quality control human milk sample was 7.83 pg g^{-1}, with few results beyond ±2 standard deviations from the mean result. The mean mirex recovery from fortified human milk was 102%, ranging from 85.1% to 117%. The reference value for mirex in SRM 1953 was 68 pg g^{-1} and all analyses of this SRM were within one standard deviation of this result. The SRM 1954, had a certified mirex concentration of 515 pg g^{-1} and all aliquots of SRM 1954 tested were ±2 standard deviations of the certified value with the exception of a single aliquot tested. Mean recovery of the surrogate standard (^{13}C mirex) from human milk samples tested was 78.1%.

With each set of human milk samples analyzed as part of the MIREC study for DDC-CO, a reagent blank was included to correct for background levels in the laboratory. No reference materials with certified DDC-CO concentrations were identified throughout the duration of the human milk sample preparation and analysis. With each set, however, either an internal quality control sample of human milk, or cow milk known to be free of the compounds of interest, fortified at a concentration of 0.025 ng g^{-1} sample weight, was included. Mean concentrations in the human milk samples tested throughout the MIREC analyses were 34.4 pg g^{-1} lipid *syn*-DDC-CO and 52.0 pg g^{-1} lipid *anti*-DDC-CO. With few exceptions, concentrations remained within two standard deviations of the mean result. Recoveries of DDC-CO in fortified cow milk ranged from 80.4%–104% (mean = 90.8%) and 79.6%–119% (mean = 91.9%), *syn*- and *anti*-DDC-CO, respectively. The mean recovery of ^{13}C *syn*-DDC-CO from the MIREC samples tested in this study was 99.1%, although there were a few samples (n = 5 of 541) with very low recoveries (<10%). Each of the samples having low recovery were found to have DDC-CO concentrations below limits of detection.

2.6. Limits of detection

Method detection limits (MDLs) were established based on a 3:1 signal to background noise ratio and were determined for each sample individually, to account for instrument sensitivity and differences in sample size. The average mirex MDL was 0.024 pg g^{-1} sample; 0.776 pg g^{-1} lipid. Average detection limits for DDC-CO *syn*- and *anti*-were elevated compared to those for mirex, 0.107 pg g^{-1} sample and 0.076 pg g^{-1} sample; 2.93 and 2.72 pg g^{-1} lipid, respectively.

2.7. Statistical analysis

Statistical analyses were performed using SigmaPlot 12.5 (Systat Software Inc.). For those samples with DDC-CO concentrations < LOD, the concentration was set to ½ LOD (i.e., LOD/2) for statistical data analysis. Spearman correlations were developed to examine the relationship between mirex and DDC-CO for those samples that had been analyzed for both mirex and DDC-CO (n = 270), as well as between *syn*- and *anti*-DDC-CO concentrations in human milk samples (n = 541). Flame retardant concentrations were examined in defined groups (age, pre-pregnancy body mass index) to establish whether the concentrations were significantly different by grouping. Owing to the fact that the data were not normally distributed, one-way analysis of variance (ANOVA) tests were performed using Kruskal-Wallis ANOVA on ranks, followed by Dunn's method. Relationships were considered statistically significant if the p-value was less than 0.05.

3. Results

Mirex was detected in every sample analyzed from the present study with concentrations ranging from 44.9 pg g^{-1} lipid to 4540 pg g^{-1} lipid (Fig. 2). In contrast, *syn*-DDC-CO was detected in 61.0% of the samples analyzed while *anti*-DDC-CO was observed at concentrations above the

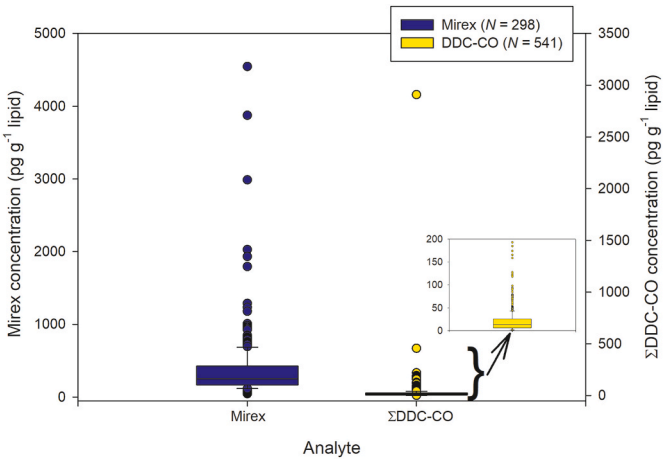

Fig. 2. Mirex and ΣDDC-CO concentrations in Canadian human milk collected from all sampling sites. Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th and 90th percentiles.

detection limit in 79.5% of the samples. The maximum *syn*-DDC-CO and *anti*-DDC-CO concentrations were observed in the same sample, with *syn*-being elevated over *anti*- (2380 and 530 pg g^{-1} lipid, respectively) (Table 2). While the mirex concentrations were distributed throughout the observed concentration range, DDC-CO concentrations were generally low, with <10% of the samples having greater than 50 pg ΣDDC-CO g^{-1} lipid (Fig. 2). The maximum ΣDDC-CO concentration was 2910 pg g^{-1} lipid, only 64% of the maximum concentration of mirex in the present study. The maximum mirex and ΣDDC-CO concentrations were present in different samples. Among the samples analyzed for both mirex (n = 298) and DDC-CO (n = 541), 270 were analyzed for both analytes. The mirex concentrations observed in these samples were significantly, but weakly correlated with ΣDDC-CO concentrations (Spearman correlation 0.182, ρ = 0.003).

Syn- and *anti*-DDC-CO concentrations were positively correlated (Spearman correlation 0.646, ρ < 0.001) with each other in the human milk collected in the present study. The fraction of ΣDDC-CO present as the *anti*-isomer in the samples, $f_{anti\text{-}}$, was calculated using only those samples where both *syn*- and *anti*-DDC-CO were present (n = 313). A*nti*-DDC-CO represents approximately 75% of the DDC-CO in the technical product (Sverko et al., 2011), although this value is thought to vary between lots and manufacturers (Ben et al., 2013). The $f_{anti\text{-}}$ varied widely among the Canadian human milk samples (0.182–0.951) in the present study (Table 2). The mean and median $f_{anti\text{-}}$ determined in the MIREC samples were 0.622 and 0.627, respectively. The lowest $f_{anti\text{-}}$ (0.182) was determined in the sample with maximum ΣDDC-CO concentrations (2910 pg g^{-1} lipid ΣDDC-CO).

Positive, but weak Spearman correlations were observed between maternal milk concentrations of both mirex and ΣDDC-CO, and maternal age (Spearman correlations 0.516, ρ < 0.001 and 0.104, ρ = 0.02, respectively) (Fig. 3). Mirex concentrations were significantly higher in participants ≥30 years (mean 292 pg g^{-1} lipid) than those <30 years (mean 169 pg g^{-1} lipid) (ANOVA p < 0.001). A weaker difference was observed between ΣDDC-CO concentration in women <30 years, mean 12.3 pg g^{-1} lipid relative to those ≥30 years, mean 14.3 pg g^{-1} lipid; p = 0.019). While relationships between parity, the number of previous viable pregnancies, and some legacy persistent organic contaminant concentrations in human milk have been reported in the literature (Klinčić et al., 2016; LaKind et al., 2004), this was not the case for either mirex (ANOVA, p = 0.740) or ΣDDC-CO (ANOVA, p = 0.883) in the present study.

Both mirex and DDC-CO are lipophilic compounds and, therefore, these flame retardant concentrations observed in human milk were examined in relation to the pre-pregnancy BMI. Mirex concentrations in human milk from individuals having lower pre-pregnancy BMI (<20 kg m^{-2}) were elevated (median concentration = 362 pg g^{-1} lipid) over those with higher pre-pregnancy BMI. Median mirex concentrations decreased with each BMI grouping (20–25, 260 pg g^{-1} lipid; >25–30, 227 pg g^{-1} lipid; >30–35, 209 pg g^{-1} lipid; >35, 119 pg g^{-1} lipid). There were some participants who did not provide the pre-pregnancy BMI and, as a result, those milk concentration data were compiled into a separate group; the median mirex concentration in this group was 227 pg g^{-1} lipid. Median mirex concentrations in milk from participants having a pre-pregnancy BMI <20 were significantly higher (p < 0.001) than found in the milk of women with pre-pregnancy BMI values in the ranges of >25 to 30, >30 to 35 and > 35 (Fig. 4). Additionally, median mirex concentrations in the milk of participants having pre-pregnancy BMI values between 20 and 25 were significantly higher than those with pre-pregnancy BMIs >35 (p = 0.019).

Similar statistical analyses were performed for ΣDDC-CO concentrations with respect to pre-pregnancy BMI. ΣDDC-CO concentrations in milk were significantly different (p = 0.007) among women belonging to the different pre-pregnancy BMI ranges. Significant differences in median ΣDDC-CO concentrations in milk were only found between pre-pregnancy BMIs of <20, 20–25 and > 25–30 (16.1 pg g^{-1} lipid, 14.3 pg g^{-1} lipid and 13.8 pg g^{-1} lipid, respectively) relative to concentrations in milk from individuals with pre-pregnancy BMIs >35 (4.02 pg g^{-1} lipid) (Fig. 5).

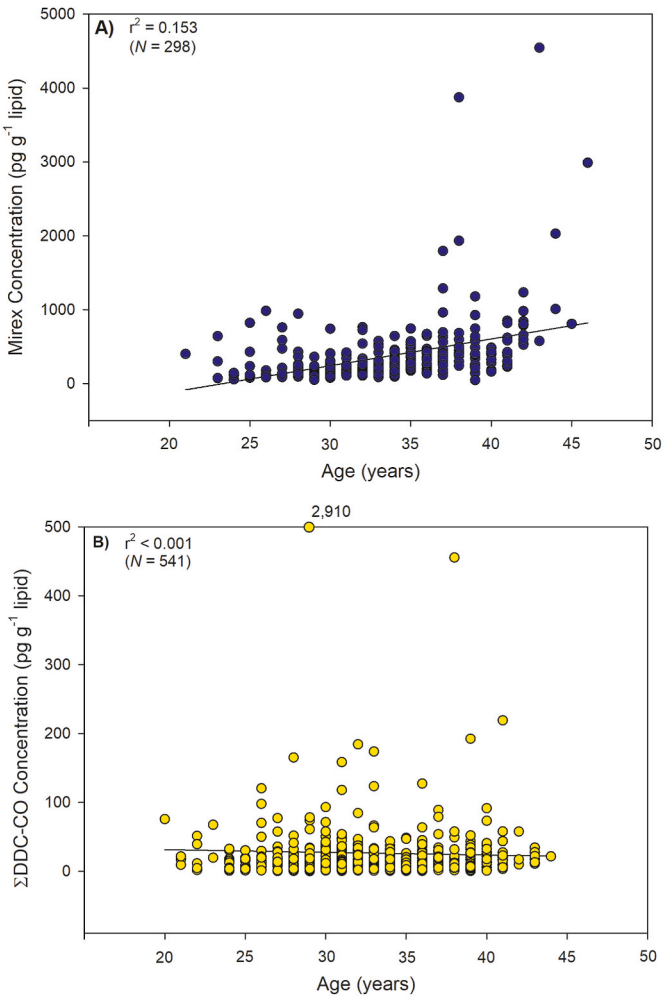

Fig. 3. Concentrations of A) Mirex and B) ΣDDC-CO in relation to maternal age (years) of MIREC participants.

Table 2
Mirex and DDC-CO concentrations (pg g^{-1} lipid) in Canadian human milk from the MIREC study. Concentrations < LOD were replaced with LOD/2.

Parameter	Mirex	*syn*-DDC-CO	*anti*-DDC-CO	ΣDDC-CO	$f_{anti\text{-}}$ DDC-CO
Detection frequency (%)	100	61.0	79.5	82.6	N/A
Minimum	44.9	0.098 (<LOD)	0.120 (<LOD)	0.239 (<LOD)	0.182
Maximum	4540	2380	530	2910	0.951
Mean	373	11.9	14.7	26.6	0.622
Median	251	4.21	8.59	13.7	0.627
Geometric Mean	273	3.45	6.65	11.6	0.602
Standard Deviation	445	103	32.9	128	0.148

4. Discussion

Mirex was identified as one of the original persistent organic pollutants (POPs) as part of the Stockholm Convention adopted in 2001 and was added to Annex A, ultimately identified for virtual elimination (Secretariat of the Stockholm Convention, 2019). In advance of this decision, mirex was included in the suite of analytes as part of Canadian

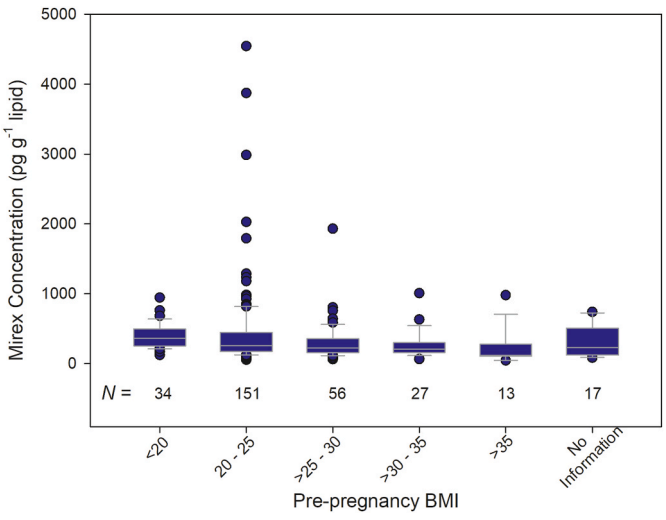

Fig. 4. Mirex concentrations in human milk in relation to pre-pregnancy BMI. Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th and 90th percentiles.

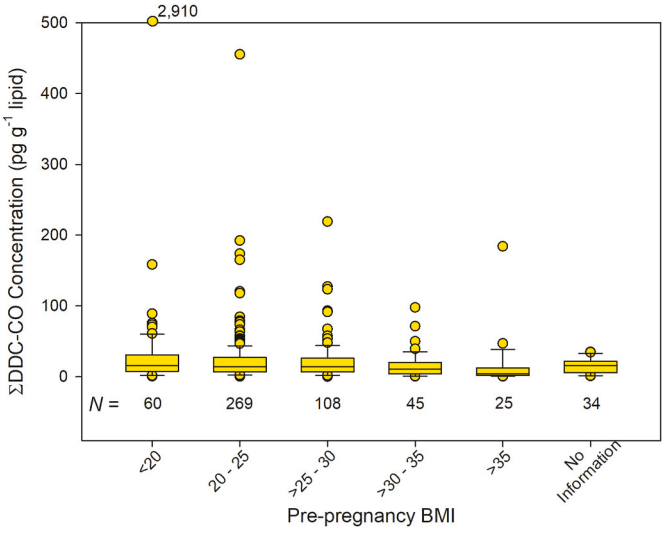

Fig. 5. ΣDDC-CO concentrations in human milk in relation to pre-pregnancy BMI. Box indicates 25th, 50th and 75th percentiles. Points indicate data outside of 10th and 90th percentiles.

human milk studies. During some of the early Canadian studies where mirex was considered, it was not observed at detectable concentrations (Mes et al., 1986; Mes and Davies, 1979). Mirex was, however, observed in Canadian human milk in the 1990s with detection frequencies of approximately 60% (Mes et al., 1993; Newsome et al., 1995). Median mirex concentrations in the milk from these studies were 2.3 ng g^{-1} lipid (Mes et al., 1993) and 1.55 ng g^{-1} lipid (Newsome et al., 1995). While the mirex detection frequency observed in the present study has increased relative to the earlier work, a corresponding decrease in concentrations has occurred over time (median 251 pg g^{-1} lipid/0.251 ng g^{-1} lipid; present study), approximately nine and six fold lower than the work reported in 1993 and 1995, respectively. The increase in detection frequency noted may be associated with the decrease in detection limit, rather than changes in the mirex profile within Canada. The temporal changes in mirex concentrations observed in Canadian human milk highlight the importance of continued biomonitoring to ensure understanding of human exposure to environmental chemicals and the effectiveness of regulatory action to reduce concentrations of hazardous chemicals.

Since DDC-CO has been identified as a chemical of concern relatively recently (Sverko et al., 2011), investigations into its presence in human tissues supports the work to understand DDC-CO distribution among environmental compartments, biota and the impacts of its exposure. DDC-CO has been reported in the literature in Canadian human milk from two cities; Kingston, ON, which is situated along Lake Ontario and Sherbrooke, QC, some distance from the Great Lakes (Siddique et al., 2012). Samples were collected from Kingston in 2003–2004 and from Sherbrooke in 2008–2009. While the only North American facility producing DDC-CO was in Niagara Falls, NY, USA, near Lake Ontario, DDC-CO detection frequencies were higher in Sherbrooke (77% and 96%, *syn*- and *anti*-, respectively) relative to Kingston (74% and 85%, *syn*- and *anti*-, respectively) (Siddique et al., 2012). The mean *anti*--DDC-CO (0.66 ng g^{-1} lipid) concentrations was slightly lower in Sherbrooke relative to Kingston (0.78 ng g^{-1} lipid, respectively), while the mean *syn*-DDC-CO was slightly higher (0.28 ng g^{-1} lipid) in Sherbrooke than observed in Kingston (0.26 ng g^{-1} lipid) (Siddique et al., 2012). In some additional work from the Sherbrooke area, Zhou et al. (2014) reported lower detection frequencies in human milk (40% and 50%, *syn*- and *anti*-, respectively) and lower median concentrations (*syn*-not detected, *anti*- 0.02 ng g^{-1} lipid) in human milk relative to serum samples (77%, 0.49 ng g^{-1} lipid *syn*- and 87%, 1.9 ng g^{-1} lipid *anti*-) from the same women.

The concentrations of *syn*- and *anti*-DDC-CO observed in human milk from this pan-Canadian MIREC study are low (Table 2), particularly when compared to concentrations observed in milk from women living near electronic-waste (e-waste) recycling activities. Mean ΣDDC-CO concentrations determined in milk from women who had been living in Wenling, China for more than five years, a location where recycling of both metals and electronic components has occurred since the 1970s, were 1.64 ng g^{-1} lipid (1640 pg g^{-1} lipid) *syn*- and 3.91 ng g^{-1} lipid (3910 pg g^{-1} lipid) *anti*-DDC-CO (Pan et al., 2020). Maximum concentrations observed in milk from this Chinese study were close to 10 times higher than the mean concentrations for both DDC-CO isomers (20.0 ng g^{-1} lipid, 31.5 ng g^{-1} lipid, *syn*- and *anti*-, respectively) and detection frequency of both isomers was 100% in the milk samples from this Wenling study (Pan et al., 2020). Additionally, an earlier study was performed to examine DDC-CO concentrations in human milk from women who had been living in Wenling, China for more than 20 years compared to those who had lived in the region for <3 years (Ben et al., 2013). Mean concentrations in milk from long-time residents were more than an order of magnitude higher (10.4 ng g^{-1} lipid *syn*- and 27.4 ng g^{-1} lipid *anti*-) than milk collected from women who had not lived in the region for many years (0.620 ng g^{-1} lipid *syn*- and 2.06 ng g^{-1} lipid *anti*-) (Ben et al., 2013). These data contrast with the results reported by Čechová et al. (2017) in human milk from European countries where detection frequencies were <10% *syn*-DDC-CO and <30% *anti*-DDC-CO. Mean reported concentrations from Slovakia, the Netherlands and Norway were 0.111 ng g^{-1} lipid, 0.278 ng g^{-1} lipid, 0.355 ng g^{-1} lipid *syn*- and 0.057 ng g^{-1} lipid, 0.155 ng g^{-1} lipid, 0.055 ng g^{-1} lipid *anti*-DDC-CO, respectively (Čechová et al., 2017). While the concentrations reported in European milk are low relative to similar samples from China, they remain above the mean concentrations of *syn*- (11.9 pg g^{-1} lipid) and *anti*-DDC-CO (14.7 pg g^{-1} lipid) observed in the present Canadian study.

While the range in $f_{anti\text{-}}$ observed (0.182–0.951) in the present study is wider than what was reported previously for Canadian human milk (0.64–0.93, N = 105) and serum (0.61–0.97, N = 102) (Zhou et al., 2014) from Sherbrooke, QC alone, the mean $f_{anti\text{-}}$ (0.622) determined in the present study is similar to that reported for Canadian human milk between 2003 and 2009 from Kingston, ON (0.68, N = 39) and Sherbrooke (0.67, N = 48) (Siddique et al., 2012). The $f_{anti\text{-}}$ in human sera and placental tissue from China ranged from 0.33 to 0.948 (Ben et al., 2014). The mean $f_{anti\text{-}}$ determined in the present study was similar to mean $f_{anti\text{-}}$ values reported in human serum from two communities in

China; one involving e-waste recycling (0.57) and the other largely involved in the fishing industry (0.63) (Ren et al., 2009). DDC-CO has been reported in samples of terrestrial and aquatic biota globally, resulting in a wide range in f_{anti-} (0.12–0.93) (Feo et al., 2012), indicating that f_{anti-} varies among organisms and regions sampled.

Maternal age has been associated with increased concentrations of numerous persistent organic chemicals (e.g., β-HCH, *p,p'*-DDE, hexachlorobenzene, PCBs) in human milk around the world (Aerts et al., 2019; Klinčić et al., 2016; Rawn et al., 2017; Rovira et al., 2022); however, this relationship appears to be inconsistent among the different organochlorine compounds, and may be a function of the narrow age range associated with this type of investigation (LaKind et al., 2004). In contrast to the observations of the present work where the relationship was confirmed, mirex concentrations considered on a whole weight basis, were not associated with maternal age in previous Canadian human milk surveys (Mes et al., 1993; Newsome et al., 1995). While there are limited studies to determine DDC-CO in human milk reported in the literature, additional investigations into these measurements will be important for temporal trend development and understanding what factors impact DDC-CO concentrations in humans. In addition, DDC-CO has been proposed for listing under the Stockholm Convention and should it be confirmed as a POP, data to follow changes in its concentrations will be important on a global scale (Secretariat of the Stockholm Convention, 2019).

A woman's parity, the number of children she has had, has been considered in terms of contaminant loads in the milk produced for several organochlorine compounds (Du et al., 2017; Klinčić et al., 2016; Lignell et al., 2016; Rovira et al., 2022). Interactions between age and parity were reported for polychlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs) in Canadian human milk from the MIREC study (Rawn et al., 2017). Previous Canadian human milk studies have shown that ΣPCBs were at higher concentrations in milk from primiparous women relative to milk from multiparous women, although it was not the case for mirex (Mes et al., 1993). Additionally, parity has been shown to impact ΣPCB, ΣDDT and ΣHCH concentrations in human milk from Croatia (Klinčić et al., 2016), but no relationship between parity and concentration was established for *p,p'*-DDE concentrations in human milk from Australia (Du et al., 2017). While an association between parity and ΣPCB concentration in human milk was identified in a Spanish study, it was not found for other organohalogen compounds analyzed as part of the study (Rovira et al., 2022), suggesting that this relationship is compound or compound class specific.

The lack of a relationship between participant parity and either mirex or ΣDDC-CO concentrations in the present study may be a function of the relatively low concentrations of these compounds across the majority of the samples collected, with only 10 of 298 samples having mirex at >1000 pg g^{-1} lipid and fewer than 10% of the samples analyzed for ΣDDC-CO having concentrations exceeding 50 pg g^{-1} lipid. Concentrations of mirex and DDC-CO were lower in the milk of women having higher pre-pregnancy BMIs (Figs. 4 and 5) and weak, but significant negative correlations were obtained between compound concentration and pre-pregnancy BMI (-0.259, $\rho < 0.001$ mirex; -0.141, $\rho = 0.001$ ΣDDC-CO) in the present study. Relationships between human milk concentration of organochlorine compounds and BMI are not clearly delineated, with some literature reporting no relationship while others do find them to be significant (LaKind et al., 2004). Weak positive correlations have been reported between BMI and HCH (β and Σ) concentrations in human milk from Chinese women (Lu et al., 2015). No associations, however, were found between BMI and other organochlorine compounds (ΣDDT, Σendosulfan, Σchlordane, etc.) (Lu et al., 2015). BMI has also been positively correlated with serum organochlorine concentrations from the Romanian population, with some associations related to age, gender, etc. (Luzardo et al., 2019).

Mirex concentrations are very low, when present in foods of animal origin (<10%), in retail food prepared as for consumption as part of the Canadian Total Diet Study (unpublished data), however, this compound was found in every human milk sample analyzed as part of the present study. Low concentrations of mirex have been reported in recent studies of fish and seafood samples globally (Ghelli et al., 2021), consistent with mirex concentrations observed in the Canadian Total Diet Study.

A review of fish data indicate that ΣDDC-CO concentrations may be in the range or extend beyond those reported for mirex (Ghelli et al., 2021). Initial analyses of DDC-CO isomers in Canadian Total Diet Study samples are similarly low, with concentrations in most samples tested remaining below detection limits, however, positive detections have been observed in processed meat products (e.g., organ meats, wieners; unpublished data). Dechlorane related compounds, including mirex and DDC-CO isomers, were reported in various food commodities (e.g., milk, meat, fish) collected in Belgium and it was determined that concentrations in food from Belgium were similarly quite low (L'Homme et al., 2015). The highest mirex concentration was observed in salmon (15.53 pg g^{-1} lipid) while the highest *syn*- (20.00 pg g^{-1} lipid) and *anti*-DDC-CO (6.61 pg g^{-1} lipid) concentrations were observed in eggs and vegetable oil, respectively (L'Homme et al., 2015). A survey of food from Korea found mirex to be elevated in fish with the maximum concentration reported in Spanish mackerel 107.30 ± 39.29 pg g^{-1} on a wet weight basis, but the highest concentrations of both *syn*- (43.02 ± 24.75 pg g^{-1} wet weight) and *anti*-DDC-CO (126.83 ± 79.81 pg g^{-1} wet weight) were observed in bovine liver (Kim et al., 2014).

Human milk samples were collected from across southern Canada for the present study and, therefore, indicate bioaccumulation of both mirex and DDC-CO across the country. Although food is known to be the primary source of legacy persistent compounds such as polychlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) to humans (Ryan et al., 2013), the current Canadian data suggest other important sources of dechlorane related compounds for the Canadian population. Both mirex and dechlorane plus were detected in human milk collected from across Canada, despite point sources being limited to near Lake Ontario, which suggests other potential routes of exposure. This aligns with the conclusions of L'Homme et al. (2015), who suggested that other routes of human exposure should be explored.

5. Conclusion

While human milk is known to be a source of exposure to environmental contaminants for infants and young children, the benefits of human milk consumption are generally considered to outweigh the potential impacts of chemical exposure. In addition to providing energy to the infants, human milk provides antibodies and a wide range of nutrients including, proteins, non-protein nitrogen, and carbohydrates (Andreas et al., 2015). The World Health Organization recommends that infants breastfeed exclusively for the first six months of life (World Health Organization, 2021).

Human milk collected from across six provinces in southern Canada was analyzed for mirex (n = 298) and DDC-CO (n = 541). These highly chlorinated flame retardant chemicals were both produced along the Canada: USA border, with DDC-CO being used as a replacement for mirex. Mirex was observed in all of the human milk samples analyzed while *syn*-DDC-CO and *anti*-DDC-CO were detected in 61.0% and 79.5% of the samples analyzed. The range of mirex concentrations (44.9 pg g^{-1} lipid – 4540 pg g^{-1} lipid) exceeded that of ΣDDC-CO (<method detection limits – 2910 pg g^{-1} lipid). These data represent the first pan-Canadian dataset describing DDC-CO in human milk. Concentrations of both mirex and DDC-CO (*syn*- and *anti*-) are low relative to concentrations reported from regions where e-waste recycling occurs. Mirex and ΣDDC-CO concentrations in the milk were positively, although weakly significantly correlated with maternal age, however, no relationship was found between parity and either compound. Higher BMI appeared to be negatively correlated with mirex and DDC-CO concentrations in human milk. The data appear to show a decline in mirex concentrations in Canadian human milk relative to previous work, although a paucity of data exist for DDC-CO in the Canadian situation,

and additional investigations into human exposure to DDC-CO are warranted.

Author contribution statement

Dorothea F.K. Rawn: Funding acquisition; Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Validation; Writing – original draft; Writing – review & editing. Sue C. Quade: Methodology; Investigation; Validation; Writing – review & editing. Catherine Corrigan: Methodology; Investigation; Validation; Writing – review & editing. Cathie Ménard: Methodology; Investigation; Validation. Wing-Fung Sun: Methodology; Investigation; Validation. François Breton: Methodology; Investigation; Validation. Tye E. Arbuckle: Funding acquisition; Conceptualization; Writing – review & editing. William D. Fraser: Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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References

Abou-Elwafa Abdallah, M., Harrad, S., 2022. Dermal uptake of chlorinated organophosphate flame retardants via contact with furniture fabrics; implications for human exposure. Environ. Res. 209, 112847. https://doi.org/10.1016/j.envres.2022.112847.

Aerts, R., Van Overmeire, I., Colles, A., Andjelković, M., Malarvannan, G., Poma, G., Den Hond, E., Van de Mieroop, E., Dewolf, M.-C., Charlet, F., Van Nieuwenhuyse, A., Van Loco, J., Covaci, A., 2019. Determinants of persistent organic pollutant (POP) concentrations in human breast milk of a cross-sectional sample of primiparous mothers in Belgium. Environ. Int. 131, 104979. https://doi.org/10.1016/j.envint.2019.104979.

Akinrinade, O.E., Stubbings, W.A., Abou-Elwafa Abdallah, M., Ayejuyo, O., Alani, R., Harrad, S., 2021. Concentrations of halogenated flame retardants and polychlorinated biphenyls in house dust from Lagos, Nigeria. Environ. Sci. Process. Impacts. 23, 1696–1705. https://doi.org/10.1039/D1EM00316J.

Andreas, N.J., Kampmann, B., Mehring Le-Doare, K., 2015. Human breast milk: a review on its composition and bioactivity. Early Hum. Dev. 91, 629–635. https://doi.org/10.1016/j.earlhumdev.2015.08.013.

Arbuckle, T.E., Davis, K., Marro, L., Fisher, M., Legrand, M., LeBlanc, A., Gaudreau, E., Foster, W.G., Choeurng, V., Fraser, W.D., 2014. Phthalate and bisphenol A exposure among pregnant women in Canada - results from the MIREC study. Environ. Int. 68, 55–65. https://doi.org/10.1016/j.envint.2014.02.010.

Arbuckle, T.E., Fraser, W.D., Fisher, M., Davis, K., Liang, C.L., Lupien, N., Bastien, S., Velez, M.P., Von Dadelszen, P., Hemmings, D.G., Wang, J., Helewa, M., Taback, S., Sermer, M., Foster, W., Ross, G., Fredette, P., Smith, G., Walker, M., Shear, R., Dodds, L., Ettinger, A.S., Weber, J.-P., D'Amour, M., Legrand, M., Kumarathasan, P., Vincent, R., Luo, Z.-C., Platt, R.W., Mitchell, G., Hidiroglou, N., Cockell, K., Villeneuve, M., Rawn, D.F.K., Dabeka, R., Cao, X.-L., Becalski, A., Ratnayake, N., Bondy, G., Jin, X., Wang, Z., Tittlemier, S., Julien, P., Avard, D., Weiler, H., Leblanc, A., Muckle, G., Boivin, M., Dionne, G., Ayotte, P., Lanphear, B., Séguin, J.R., Saint-Amour, D., Dewailly, E., Monnier, P., Koren, G., Ouellet, E., 2013. Cohort profile: the maternal-infant research on environmental chemicals research platform. Paediatr. Perinat. Epidemiol. 27, 415–425. https://doi.org/10.1111/ppe.12061.

Arbuckle, T.E., Liang, C.L., Morisset, A.-S., Fisher, M., Weiler, H., Cirtiu, C.M., Legrand, M., Davis, K., Ettinger, A.S., Fraser, W.D., 2016. Maternal and fetal exposure to cadmium, lead, manganese and mercury: the MIREC study. Chemosphere 163, 270–282. https://doi.org/10.1016/j.chemosphere.2016.08.023.

Bao, J., Hou, L., Wang, Y., Zhang, Y., Qiao, J., Su, M., Jin, J., Chen, L., 2022. Effect of affinity between dechlorane plus and human serum albumin on its serum concentration in a general population. Environ. Sci. Technol. 56, 2511–2518. https://doi.org/10.1021/acs.est.1c07525.

Ben, Y.-J., Li, X.-H., Yang, Y.-L., Li, L., Di, J.-P., Wang, W.-Y., Zhou, R.-F., Xiao, K., Zheng, M.-Y., Tian, Y., Xu, X.-B., 2013. Dechlorane Plus and its dechlorinated analogs from an e-waste recycling center in maternal serum and breast milk of women in Wenling, China. Environ. Pollut. 173, 176–181. https://doi.org/10.1016/j.envpol.2012.09.028.

Ben, Y.-J., Li, X.-H., Yang, Y.-L., Li, L., Zheng, M.-Y., Wang, W.-y., Xu, X.-B., 2014. Placental transfer of dechlorane plus in mother-infant pairs in an E-waste recycling area (Wenling, China). Environ. Sci. Technol. 48, 5187–5193.

Bergman, Å., Rydén, A., Law, R.J., de Boer, J., Covaci, A., Alaee, M., Birnbaum, L., Petreas, M., Rose, M., Sakai, S., Van den Eede, N., van der Veen, I., 2012. A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals. Environ. Int. 49, 57–82. https://doi.org/10.1016/j.envint.2012.08.003.

Brasseur, C., Pirard, C., Scholl, G., De Pauw, E., Viel, J.-., Shen, L., Reiner, E.J., Focant, J.-F., 2014. Levels of dechloranes and polybrominated diphenyl ethers (PBDEs) in human serum from France. Environ. Int. 65, 33–40. https://doi.org/10.1016/j.envint.2013.12.014.

Čechová, E., Vojta, Š., Kukučka, P., Kočan, A., Trnovec, T., Murínová, Ľ.P., de Cock, M., van de Bor, M., Askevold, J., Eggesbø, M., Scheringer, M., 2017. Legacy and alternative halogenated flame retardants in human milk in Europe: implications for children's health. Environ. Int. 108, 137–145. https://doi.org/10.1016/j.envint.2017.08.008.

Chen, Y., Cao, Z., Covaci, A., Li, C., Cui, X., 2019. Novel and legacy flame retardants in paired human fingernails and indoor dust samples. Environ. Int. 133, 105227 https://doi.org/10.1016/j.envint.2019.105227.

Dou, J., Jin, Y., Li, Y., Wu, B., Li, M., 2015. Potential genotoxicity and risk assessment of a chlorinated flame retardant, Dechlorane Plus. Chemosphere 135, 462–466. https://doi.org/10.1016/j.chemosphere.2014.12.066.

Dreyer, A., Neugebauer, F., Lohmann, N., Rüdel, H., Teubner, D., Grotti, M., Rauert, C., Koschorreck, J., 2019. Recent findings of halogenated flame retardants (HFR) in the German and Polar environment. Environ. Pollut. 253, 850–863. https://doi.org/10.1016/j.envpol.2019.07.070.

Du, J., Gridneva, Z., Gay, M.C.L., Trengove, R.D., Hartmann, P.E., Geddes, D.T., 2017. Pesticides in human milk of Western Australian women and their influence on infant growth outcomes: a cross-sectional study. Chemosphere 167, 247–254. https://doi.org/10.1016/j.chemosphere.2016.10.005.

Environment Canada, 2013. Toxic Substances List: Mirex. https://www.canada.ca/en/environment-climate-change/services/management-toxic-substances/list-canadian-environmental-protection-act/mirex.html. (Accessed 3 February 2022).

Feo, M.L., Barón, E., Eljarrat, E., Barceló, D., 2012. Dechlorane Plus and related compounds in aquatic and terrestrial biota: a review. Anal. Bioanal. Chem. 404, 2625–2637. https://doi.org/10.1007/s00216-012-6161-x.

Fisher, M., Arbuckle, T.E., Liang, C.L., Leblanc, A., Gaudreau, E., Foster, W.G., Haines, D., Davis, K., Fraser, W.D., 2016. Concentrations of persistent organic pollutants in maternal and cord blood from the maternal-infant research on environmental chemicals (MIREC) cohort study. Environ. Health Global Access Sci. Sour. 15, 59. https://doi.org/10.1186/s12940-016-0143-y.

Fromme, H., Cequier, E., Kim, J.-T., Hanssen, L., Hilger, B., Thomsen, C., Chang, Y.-S., Völkel, W., 2015. Persistent and emerging pollutants in the blood of German adults: occurrence of dechloranes, polychlorinated naphthalenes, and siloxanes. Environ. Int. 85, 292–298. https://doi.org/10.1016/j.envint.2015.09.002.

Fromme, H., Thomsen, C., Aschenbrenner, B., Haug, L.S., Weber, T., Kolossa-Gehring, M., Völkel, W., Schober, W., 2020. Time trend of exposure to dechloranes: plasma samples of German young adults from the environmental specimen bank collected from 1995 to 2017. Int. J. Hyg Environ. Health 229, 113593. https://doi.org/10.1016/j.ijheh.2020.113593.

Ghelli, E., Cariou, R., Dervilly, G., Pagliuca, G., Gazzotti, T., 2021. Dechlorane plus and related compounds in food—a review. Int. J. Environ. Res. Publ. Health 18, 1–16. https://doi.org/10.3390/ijerph18020690.

Hansen, K.M., Fauser, P., Vorkamp, K., Christensen, J.H., 2020. Global emissions of dechlorane plus. Sci. Total Environ. 742, 140677 https://doi.org/10.1016/j.scitotenv.2020.140677.

He, R.-W., Li, Y.-Z., Xiang, P., Li, C., Cui, X.-Y., Ma, L.Q., 2018. Impact of particle size on distribution and human exposure of flame retardants in indoor dust. Environ. Res. 162, 166–172. https://doi.org/10.1016/j.envres.2017.12.014.

Hoh, E., Zhu, L., Hites, R.A., 2006. Dechlorane plus, a chlorinated flame retardant, in the Great Lakes. Environ. Sci. Technol. 40, 1184–1189. https://doi.org/10.1021/es051911h.

Kaiser, K.L.E., 1978. The rise and fall of mirex. Environ. Sci. Technol. 12, 520–528. https://doi.org/10.1021/es60141a005.

Kim, J., Son, M.-h., Kim, J., Suh, J., Kang, Y., Chang, Y.-S., 2014. Assessment of Dechlorane compounds in foodstuffs obtained from retail markets and estimates of dietary intake in Korean population. J. Hazard Mater. 275, 19–25. https://doi.org/10.1016/j.jhazmat.2014.04.032.

Kim, J., Son, M.-h., Shin, E.-S., Choi, S.-D., Chang, Y.-S., 2016. Occurrence of Dechlorane compounds and polybrominated diphenyl ethers (PBDEs) in the Korean general population. Environ. Pollut. 212, 330–336. https://doi.org/10.1016/j.envpol.2016.01.085.

Klinčić, D., Herceg Romanić, S., Brčić Karačonji, I., Matek Sarić, M., Grzunov Letinić, J., Brajenović, N., 2016. Organochlorine pesticides and PCBs (including dl-PCBs) in human milk samples collected from multiparae from Croatia and comparison with primiparae. Environ. Toxicol. Pharmacol. 45, 74–79. https://doi.org/10.1016/j.etap.2016.05.002.

LaKind, J.S., Amina Wilkins, A., Berlin Jr., C.M., 2004. Environmental chemicals in human milk: a review of levels, infant exposures and health, and guidance for future research. Toxicol. Appl. Pharmacol. 198, 184–208. https://doi.org/10.1016/j.taap.2003.08.021.

L'Homme, B., Calaprice, C., Calvano, C.D., Zambonin, C., Leardi, R., Focant, J.-F., 2015. Ultra-trace measurement of Dechloranes to investigate food as a route of human exposure. Chemosphere 139, 525–533. https://doi.org/10.1016/j.chemosphere.2015.07.043.

Li, Y., Yu, L., Wang, J., Wu, J., Mai, B., Dai, J., 2013. Accumulation pattern of Dechlorane Plus and associated biological effects on rats after 90d of exposure. Chemosphere 90, 2149–2156. https://doi.org/10.1016/j.chemosphere.2012.10.106.

Lignell, S., Winkvist, A., Bertz, F., Rasmussen, K.M., Glynn, A., Aune, M., Brekke, H.K., 2016. Environmental organic pollutants in human milk before and after weight loss. Chemosphere 159, 96–102. https://doi.org/10.1016/j.chemosphere.2016.05.077.

Lu, D., Wang, D., Ni, R., Lin, Y., Feng, C., Xu, Q., Jia, X., Wang, G., Zhou, Z., 2015. Organochlorine pesticides and their metabolites in human breast milk from Shanghai, China. Environ. Sci. Pollut. Res. 22, 9293–9306. https://doi.org/10.1007/s11356-015-4072-z.

Luzardo, O.P., Badea, M., Zumbado, M., Rogozea, L., Floroian, L., Ilea, A., Moga, M., Sechel, G., Boada, L.D., Henríquez-Hernández, L.A., 2019. Body burden of organohalogenated pollutants and polycyclic aromatic hydrocarbons in Romanian population: influence of age, gender, body mass index, and habitat. Sci. Total Environ. 656, 709–716. https://doi.org/10.1016/j.scitotenv.2018.11.404.

Martinez, G., Niu, J., Takser, L., Bellenger, J.-P., Zhu, J., 2021. A review on the analytical procedures of halogenated flame retardants by gas chromatography coupled with single quadrupole mass spectrometry and their levels in human samples. Environ. Pollut. 285, 117476 https://doi.org/10.1016/j.envpol.2021.117476.

Mes, J., Davies, D.J., 1979. Presence of polychlorinated biphenyl and organochlorine pesticide residues and the absence of polychlorinated terphenyls in Canadian human milk samples. Bull. Environ. Contam. Toxicol. 21, 381–387. https://doi.org/10.1007/BF01685440.

Mes, J., Davies, D.J., Doucet, J., Weber, D., Mc Mullen, E.D., 1993. Levels of chlorinated hydrocarbon residues in canadian human breast milk and their relationship to some characteristics of the donors. Food Addit. Contam. 10, 429–441. https://doi.org/10.1080/02652039309374166.

Mes, J., Davies, D.J., Turton, D., Sun, W.F., 1986. Levels and trends of chlorinated hydrocarbon contaminants in the breast milk of Canadian women. Food Addit. Contam. 3, 313–322. https://doi.org/10.1080/02652038609373598.

Newsome, W.H., Davies, D., Doucet, J., 1995. PCB and organochlorine pesticides in Canadian human milk - 1992. Chemosphere 30, 2143–2153. https://doi.org/10.1016/0045-6535(95)00086-N.

Pan, H.-Y., Li, J.-F.-T., Li, X.-H., Yang, Y.-L., Qin, Z.-F., Li, J.-B., Li, Y.-Y., 2020. Transfer of dechlorane plus between human breast milk and adipose tissue and comparison with legacy lipophilic compounds. Environ. Pollut. 265, 115096 https://doi.org/10.1016/j.envpol.2020.115096.

PSPC, Public Services and Procurement Canada, 2019. Order Adding Toxic Substances to Schedule I to the Canadian Environmental Protection Act, 1999. vol. 2019 Canada Gazette Part I, 153, No. 26 (Accessed 4 February 2022).

Qiao, L., Zheng, X.-., Yan, X., Wang, M.-., Zheng, J., Chen, S.-., Yang, Z.-., Mai, B.-., 2018. Brominated flame retardant (BFRs) and Dechlorane Plus (DP) in paired human serum and segmented hair. Ecotoxicol. Environ. Saf. 147, 803–808. https://doi.org/10.1016/j.ecoenv.2017.09.047.

Rawn, D.F.K., Corrigan, C., Ménard, C., Breton, F., Sun, W.-F., 2016. A method for the analysis of multiple novel halogenated flame retardants in cow's milk. Food Addit. Contam. Part A Chem. Anal. Control Exposure Risk Assess. 33, 1207–1218. https://doi.org/10.1080/19440049.2016.1198049.

Rawn, D.F.K., Dufresne, G., Clément, G., Fraser, W.D., Arbuckle, T.E., 2022. Perfluorinated alkyl substances in Canadian human milk as part of the Maternal-Infant Research on Environmental Chemicals (MIREC) study. Sci. Total Environ. 831, 154888 https://doi.org/10.1016/j.scitotenv.2022.154888.

Rawn, D.F.K., Sadler, A.R., Casey, V.A., Breton, F., Sun, W.-F., Arbuckle, T.E., Fraser, W.D., 2017. Dioxins/furans and PCBs in Canadian human milk: 2008–2011. Sci. Total Environ. 595, 269–278. https://doi.org/10.1016/j.scitotenv.2017.03.157.

Ren, G., Yu, Z., Ma, S., Li, H., Peng, P., Sheng, G., Fu, J., 2009. Determination of dechlorane plus in serum from electronics dismantling workers in South China. Environ. Sci. Technol. 43, 9453–9457. https://doi.org/10.1021/es901672m.

Rovira, J., Martínez, M.Á., Mari, M., Cunha, S.C., Fernandes, J.O., Marmelo, I., Marques, A., Haug, L.S., Thomsen, C., Nadal, M., Domingo, J.L., Schuhmacher, M., 2022. Mixture of environmental pollutants in breast milk from a Spanish cohort of nursing mothers. Environ. Int. 166, 107375 https://doi.org/10.1016/j.envint.2022.107375.

Ryan, J.J., Cao, X.-L., Dabeka, R., 2013. Dioxins, furans and non-ortho-PCBs in Canadian total diet foods 1992-1999 and 1985-1988. Food Addit. Contam. Part A Chem. Anal. Control Exposure Risk Assess. 30, 491–505. https://doi.org/10.1080/19440049.2012.756988.

National Center for Biotechnology Information, 2022. PubChem Compound Summary for CID 16945, Mirex C10 Cl12 (Accessed 4 February 2022).

Secretariat of the Stockholm Convention, 2019. Listing of POPs in the Stockholm Convention (Accessed 7 July 2021).

Shen, L., Reiner, E.J., Macpherson, K.A., Kolic, T.M., Sverko, E., Helm, P.A., Bhavsar, S.P., Brindle, I.D., Marvin, C.H., 2010. Identification and screening analysis of halogenated norbornene flame retardants in the Laurentian Great Lakes: dechloranes 602, 603, and 604. Environ. Sci. Technol. 44, 760–766. https://doi.org/10.1021/es902482b.

Siddique, S., Xian, Q., Abdelouahab, N., Takser, L., Phillips, S.P., Feng, Y.-L., Wang, B., Zhu, J., 2012. Levels of dechlorane plus and polybrominated diphenylethers in human milk in two Canadian cities. Environ. Int. 39, 50–55. https://doi.org/10.1016/j.envint.2011.09.010.

Smythe, T.A., Mattioli, L.C., Letcher, R.J., 2020. Distribution behaviour in body compartments and in ovo transfer of flame retardants in North American Great Lakes herring gulls. Environ. Pollut. 262, 114306 https://doi.org/10.1016/j.envpol.2020.114306.

Statistics Canada, 2013. Canadian Community Health Survey - Annual Component (CCHS) (Accessed 25 October 2021).

Sun, J., Wu, Y., Tao, N., Lv, L., Yu, X., Zhang, A., Qi, H., 2019. Dechlorane plus in greenhouse and conventional vegetables: uptake, translocation, dissipation and human dietary exposure. Environ. Pollut. 244, 667–674. https://doi.org/10.1016/j.envpol.2018.10.094.

Sverko, E., Tomy, G.T., Reiner, E.J., Li, Y.-F., McCarry, B.E., Arnot, J.A., Law, R.J., Hites, R.A., 2011. Dechlorane plus and related compounds in the environment: a review. Environ. Sci. Technol. 45, 5088–5098. https://doi.org/10.1021/es2003028.

Tao, F., Matsukami, H., Suzuki, G., Tue, N.M., Viet, P.H., Takigami, H., Harrad, S., 2016. Emerging halogenated flame retardants and hexabromocyclododecanes in food samples from an e-waste processing area in Vietnam. Environ. Sci. Process. Impacts. 18, 361–370. https://doi.org/10.1039/C5EM00593K.

Wang, D.-G., Alaee, M., Byer, J.D., Brimble, S., Pacepavicius, G., 2013. Human health risk assessment of occupational and residential exposures to dechlorane plus in the manufacturing facility area in China and comparison with e-waste recycling site. Sci. Total Environ. 445–446, 329–336. https://doi.org/10.1016/j.scitotenv.2012.12.059.

Wang, D.-G., Yang, M., Qi, H., Sverko, E., Ma, W.-L., Li, Y.-F., Alaee, M., Reiner, E.J., Shen, L., 2010. An Asia-specific source of dechlorane plus: concentration, isomer profiles, and other related compounds. Environ. Sci. Technol. 44, 6608–6613. https://doi.org/10.1021/es101224y.

Wielsøe, M., Long, M., Bossi, R., Vorkamp, K., Bonefeld-Jørgensen, E.C., 2022. Persistent organic pollutant exposures among Greenlandic adults in relation to lifestyle and diet: New data from the ACCEPT cohort. Sci. Total Environ. 827 https://doi.org/10.1016/j.scitotenv.2022.154270.

World Health Organization, 2021. 5. Infant and Young Child Feeding. https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding. (Accessed 5 July 2021).

World Health Organization, 1990. For the International Programme on Chemical Safety. In: Mirex : Health and Safety Guide, vol. 39. ISSN 0259-7268.

Wu, B., Liu, S., Guo, X., Zhang, Y., Zhang, X., Li, M., Cheng, S., 2012. Responses of mouse liver to dechlorane plus exposure by integrative transcriptomic and metabonomic studies. Environ. Sci. Technol. 46, 10758–10764. https://doi.org/10.1021/es301804t.

Xian, Q., Siddique, S., Li, T., Feng, Y.-l., Takser, L., Zhu, J., 2011. Sources and environmental behavior of dechlorane plus - a review. Environ. Int. 37, 1273–1284. https://doi.org/10.1016/j.envint.2011.04.016.

Yin, J.-F., Li, J.-F.-T., Li, X.-H., Yang, Y.-L., Qin, Z.-., 2020. Bioaccumulation and transfer characteristics of dechlorane plus in human adipose tissue and blood stream and the underlying mechanisms. Sci. Total Environ. 700, 134391 https://doi.org/10.1016/j.scitotenv.2019.134391.

Zacs, D., Perkons, I., Abdulajeva, E., Pasecnaja, E., Bartkiene, E., Bartkevics, V., 2021. Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDD), dechlorane-related compounds (DRCs), and emerging brominated flame retardants (EBFRs) in foods: the levels, profiles, and dietary intake in Latvia. Sci. Total Environ. 752, 141996 https://doi.org/10.1016/j.scitotenv.2020.141996.

Zafar, M.I., Kali, S., Ali, M., Riaz, M.A., Naz, T., Iqbal, M.M., Masood, N., Munawar, K., Jan, B., Ahmed, S., Waseem, A., Niazi, M.B.K., 2020. Dechlorane Plus as an emerging environmental pollutant in Asia: a review. Environ. Sci. Pollut. Res. 27, 42369–42389. https://doi.org/10.1007/s11356-020-10609-2.

Zhou, S.N., Siddique, S., Lavoie, L., Takser, L., Abdelouahab, N., Zhu, J., 2014. Hexachloronorbornene-based flame retardants in humans: levels in maternal serum and milk. Environ. Int. 66, 11–17. https://doi.org/10.1016/j.envint.2014.01.010.