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Research article

Mass spectrometry analysis of environmental pollutants in breast and artificial milk for newborns

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A B S T R A C T

Environmental toxins, particularly liposoluble compounds that accumulate in adipose tissues, present a risk for newborns, not only through breastfeeding but also through artificial milks. These compounds pass into breast milk, potentially exposing infants to harmful substances. In a monocentric observational study carried out in the Charleroi region, we employed liquid chromatography coupled with mass spectrometry to analyze the presence of environmental toxins in milk for newborns. Out of 39 breast milk and 12 artificial milk samples analyzed, 15 and six contained at least one pesticide, respectively, with nine different pesticides identified from a panel of 54 substances tested. The study found an association between the consumption of fresh produce and a higher presence of pesticides in breast milk. This.

highlights the broader issue of environmental toxin exposure for infants, regardless of the feeding method. The results underline the need for a comprehensive approach when considering the establishment of breast milk banks and the safety of artificial milk, especially in the context of potential risks to premature newborns. Our findings not only validate the analysis technique for detecting toxins in breast milk but also suggest the necessity for a larger prospective study to explore these risks in the future.

1. Introduction

The Stockholm convention on persistent organic pollutants (POPs) is designed to protect human health and the environment from the harmful effects of long-lasting chemical substances ([1,2]). They include, for example, polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and organochlorine pesticides (OCPs), which have been demonstrated to increase the incidence of obesity, Type 2 diabetes, hypertension, and cancer ([3–6]). POPs, which are toxic and persist for years before breaking down into less hazardous forms, can be found in industrial and pesticide use, spreading through soil, air, and water, often accumulating far from their original source ([7]).

Women store the lipophilic POPs in adipose tissues after ingestion of contaminated foods rich in fat. It has been shown that the lipids present in breast milk result from the mobilization of fat reserves accumulated in the body rather than the use of nutrients consumed during breastfeeding ([8]).

In light of these concerns, the convention highlighted the importance of monitoring breast milk as a key indicator of POP exposure. Breast milk sampling is favored because it is non-invasive and more feasible compared to other organic products ([9,10]).

Despite the presence of POPs in breast milk, the World Health Organization (WHO) continues to recommend breastfeeding as the

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List of abbreviations

BMI	Body mass index
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EPA	Environmental protection agency
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
IARC	International agency for research on cancer
OCP	Organochlorine pesticides
PBDE	Polybromodiphenyl ether
PCB	Polychlorinated biphenyls
POP	Persistent organic pollutant
UNEP	United nations environment program
USEPA	United States environmental protection agency
WHO	World health organization

ideal source of nutrition for infants ([11]). Breastfeeding provides essential nutrients (thiamin, riboflavin and other vitamins, oligo-elements, amino acids, oligosaccharides, lipids, etc.) which reduce infant mortality by different mechanisms including active immunoglobulin transfer, help prevent chronic diseases such as atopic diseases, obesity, Type 2 diabetes, and hypertension ([12]). However, the detection of POPs in breast milk has led to numerous studies focusing on controlling and monitoring POP exposure in different regions. These efforts aim to evaluate the long-term health risks associated with these toxins ([2,11]).

Belgium, actively participating in this global health initiative, has been involved in all WHO-led breast milk campaigns, with the latest campaign conducted in 2014 ([13]). The present study, an exploratory and prospective effort carried out in the Charleroi region of Belgium, focuses on assessing pesticide exposure in newborns through both breast milk and infant formula, in line with the prevalent pesticides used in both conventional and organic agriculture in the Wallonia region. Moreover, pesticides were selected in relation to their suspected or proven toxicities.

This analysis has become a major concern in the current debate about breast milk bank establishment, particularly regarding the exposure of premature newborns to these toxins. The findings are pivotal in understanding the potential risks and ensuring the safety of these vital resources for vulnerable infants.

2. Materials and methods

2.1. Donor selection and ethical considerations

The approval of the local ethics committee (Marie Curie Hospital) has been obtained prior to study initiation.

Donor selection occurred post-childbirth at the maternity of the Marie Curie Civil Hospital in Charleroi between January and March 2023. Typical postpartum hospitalization lasts 2–3 days, and mothers were enrolled in the study during this period.

To be included, donors needed to fulfill the following criteria: mother older than 18 years old projecting to breastfeed her child, the mother and the child had to be in good health, with the pregnancy proceeding normally, and the mother had to have had a stable residence in Charleroi or the surrounding countryside for at least 10 years.

2.2. Data collection process

Once the mother qualified under the inclusion criteria, she was informed about the survey context and objectives. If informed consent was obtained, she completed a questionnaire aligned with the UNEP protocol (9). The questionnaire covered: confirmation of inclusion criteria, biometrics, parity, urban or rural residence, dietary patterns, and medication use.

2.3. Sampling and storage

Collection methods involved manual expression or the use of a breast pump, resulting in at least 5 mL of milk per sample. Proper storage required refrigeration at 4 °C for a maximum of 72 h or prolonged freezing at −20 °C. Additionally, 12 samples of infant formula milk used in maternity and pediatric settings were gathered for comparative analyses.

2.4. Sample processing

The samples underwent preparation using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique, a widely accepted method for pesticide residue analysis in fruits, vegetables, cereals, and processed products ([14]).

In this process, 500 μL of milk was combined with 500 μL of acetonitrile, 0.2g of anhydrous magnesium sulfate, 0.05g of sodium chloride, and 1 % acetic acid in an Eppendorf tube. After mixing and treatment for 15 min in an ultrasonic bath, the tube underwent a 15-min centrifugation, and 200 μL of the resulting supernatant were collected and stored in a freezer at −80 °C until analysis.

2.5. Analysis of samples

The prepared samples were subjected to analysis using liquid chromatography coupled with mass spectrometry (LC1290 Infinity II – QqQ 6490, Agilent Technologies) at the Analytical Platform of the Faculty of Pharmacy of the Free University of Brussels (ULB, *Université Libre de Bruxelles)*. The analysis targeted around 50 environmental toxins (Supplementary Table 1). Briefly, 54 pesticides were screened according to one transition optimized for its collision cell energy and after separation by reverse phase chromatography on a ZORBAX Eclipse Plus C18, Rapid Resolution HD column (100 × 2.1 mm, 1.8 μm, Agilent Technologies) with an upstream ZORBAX Eclipse Plus C18 guard column (2.1 × 5mm, 1.8 μm, Agilent Technologies) at 40 °C. The flow rate was 0.5 mL/min and the injection volume 2.0 μL. Mobile phase A consisted of 0.1 % formic acid and 10 mM ammonium formate in MilliQ water, while mobile phase B consisted of 0.1 % formic acid and 10 mM ammonium formate in methanol. The gradient program for mobile phase B at the start was 0 %, then 50 % 0.5 min later, 2 min later the percentage was increased to 55 % for 3 min, then to 75 % for 2 min, then to 85 % for 0.8 min,

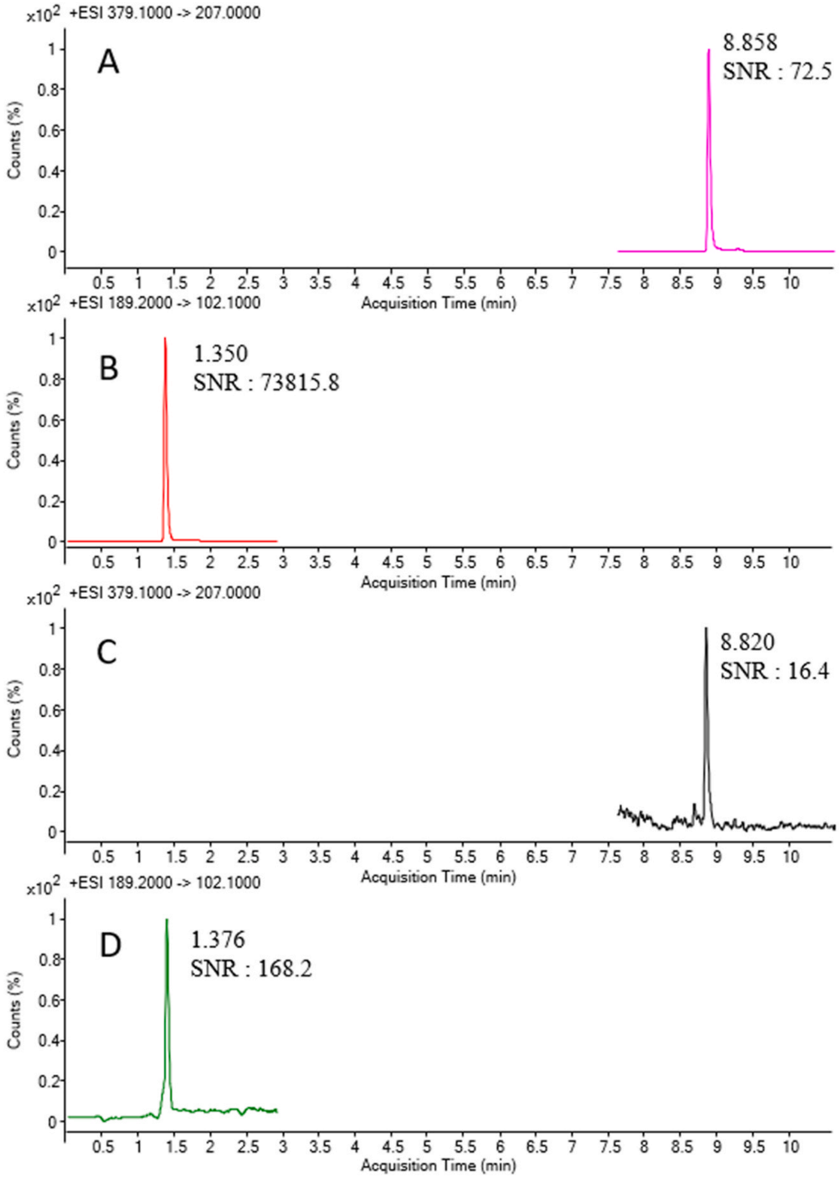

Fig. 1. Extract ion chromatogram of the quantifier ions of **A**: pyridate standard at 0.1 μg/mL; **B**: propamocarb standard at 0.1 μg/mL; **C**: pyridate in breast milk sample; **D**: propamocarb in breast milk sample. SNR: signal-to-noise ratio.

then to 100 % for 3.8 min, and finally abruptly back to 0 % until the end of the run. Total run time was 15 min ([15]).

The compounds were measured based on their transitions that have been optimized thank to Optimizer for Mass Hunter (Agilent Technologies). Then, a stock solution of standard at a concentration of 0.1 μg/mL has been injected. Finally, the samples have been injected and the compounds identified based on the transitions and the retention time in the same run. The acquisition was performed on a triple quadrupole, the gold standard to detect and quantify traces of pesticides. This machine is a low-resolution mass analyzer with high acquisition rate to ensure sensitivity. Chromatograms of standards and samples are presented on Fig. 1. The limit of quantification for these pesticides were measured at 0.1 ng/mL at a signal to noise (S/N) of 20–30 and the limit of detection was estimated at 0.033 ng/mL. In the supplementary data, we can see that propamocarb is over the limit of quantification while pyridate is between the limit of detection and the limit of quantification when looking at the signal to noise ratio (S/N).

2.6. Statistical data evaluation

Statistical analyses were carried out with GraphPad 9.5.1 (Prism®). Student's t-test was applied to continuous variables with Welch's correction for different variances, and the Fischer non-parametric test was employed for discontinuous variables. Significance was established at $p \leq 0.05$.

3. Results

3.1. Population and collection of breast milk samples

Between January and March 2023, 495 patients gave birth at Marie Curie Civil Hospital. Out of these, 160 were contacted, and 63 met the inclusion criteria. Four of them declined to participate in the study. Seventeen mothers ultimately did not provide a sample for analysis. Extraction issues were encountered with three samples. Therefore, analyses were conducted on 39 breast milk samples obtained at the maternity ward or at home after their return.

The mothers, on average, were 29.6 years old, with an average height of 164.7 cm, an average weight of 70.3 Kg, and an average body mass index (BMI) of 25.85 kg/m^2. Among them, 23 (59 %) were first-time mothers and 16 (41 %) were having their second child. Additionally, 15 (38.5 %) resided in rural areas, while 24 (61.5 %) lived in urban areas.

In terms of diet, 37 (94.9 %) followed a varied diet, while two (5.1 %) adhered to a vegetarian or pesco-vegetarian diet. Dietary preferences included a preference for fresh products in 16 (41 %) women, local products in 11 (28.2 %) women, and organic products in nine (23 %) women.

3.2. Analysis of breast milk samples

Breast milk samples were analyzed for 54 pesticides, revealing the presence of six compounds. Among them, pyridate ($n = 9$), piperonyl butoxide ($n = 5$), and fenpropidin ($n = 2$) recurred multiple times. Three pesticides were detected in a single sample each: propamocarb, flonicamid, and tribenuron methyl.

In 15 out of the 39 samples, at least one pesticide was identified. Eleven samples contained a single pesticide, while four samples contained two pesticides. Notably, pyridate was consistently found in all four samples, in combination with one of the other pesticides.

In order to avoid making donors feel guilty when answering the questionnaire in immediate postpartum, the ethics committee did not approve the inclusion of questions regarding tobacco consumption or other substances in this exploratory study. Consequently, the statistical analysis was limited. Age, BMI, parity, place of residence were not statistically different between samples containing pesticides and the others.

In terms of diets, no notable distinctions were observed between mothers favoring organic products or local products compared to those with other preferences. Nevertheless, a noteworthy finding indicated that pesticides were more commonly detected in the group of mothers who prioritized fresh products in their diet ($p = 0.0022$).

3.3. Analysis of artificial milk samples

Among the 12 analyzed samples of artificial milk, six were found to contain pesticides. Three pesticides were identified, with some appearing in multiple samples: thiabendazole ($n = 3$), matrine ($n = 2$), and isoxaben ($n = 1$). Opposite to breast milk samples, no pesticide association has been observed.

4. Discussion

In the present work, we have addressed the exposure of newborns to persistent organic pollutants during their first days of life because of its long-term potential impact on public health before further larger studies.

The study did not find a substantial disparity in pesticide presence between breast milk and industrial milk samples. This suggests that, despite existing regulations and monitoring efforts, industrial milk still contains detectable levels of pesticides. The presence of toxic substances in industries is probably due to the use of rapeseed and sunflower oils from regional production, but also to the cow's milk used. Nevertheless, contaminations in the production process cannot be excluded. In order to refine the results and characterize the origin of these toxic environments, analyses should be carried out on the different constituents of these milks before packaging.

POPs are fat-soluble compounds that can accumulate in adipose tissue and persist over an extended period. These adipose tissues are mobilized during breastfeeding. Approximately two-thirds of the fats in breast milk originate from the mother's adipose reserves, while one-third is derived from the mother's dietary intake ([16]). Consequently, dietary habits are considered a relevant variable and were included in the questionnaire for investigation.

Pesticides were more frequently detected in the group of mothers who prioritized fresh products in their diet, preferring fresh fruits and vegetables over processed foods (canned, jarred, industrial dishes, etc.). This finding can be attributed to the pesticides used in agriculture, which are employed to safeguard crops from insects, weeds, and fungi, making them more prevalent in fresh produce.

In 2014, the Belgian results of the sixth survey on POPs in breast milk were published. Milk was collected from all Belgian provinces (between eight and 30 donors per province). Concentrations of HCB, HCH, DDE, DDT, and PBDE were high for more than half of the samples. This study highlighted various factors that could contribute to a higher concentration of pesticides in breast milk, including the consumption of locally sourced fish and eggs, the mother's breastfeeding history, her age, and rural living. The toxins analyzed in 2014 were not part of the current laboratory testing panel ([13]). Interestingly, a Swedish study on organochlorine compounds in breast milk had shown that the levels of organochlorine compounds decreased between 1972 and 1997, whereas PBDE levels had increased ([17]).

A comparative review, encompassing 65 studies on pollutants found in breast milk and artificial milk worldwide, was published in 2023 ([18]). The main types of contaminants mentioned included metals (arsenic, aluminum, lead, and mercury), nitrogen compounds, chemical compounds from heat treatments, acrylamide, furan, medications, mycotoxins, pesticides (organochlorines, carbamates, pyrethroids, glyphosate, and triazines), and packaging materials. The presence of different pollutants varied by continent and living environment. Higher levels of toxins (metals, pesticides, and packaging materials) were found in industrial areas ([19]). Moreover, heavy metals can lead to health problems in children such as allergies, endocrine disorders, and neurodevelopment delay and disorders, which have recently been reviewed ([20]).

The chemical pesticide families identified in our study (Supplementary Table 2) have not been extensively studied in the existing scientific literature. Carbamates were referenced in studies from Australia ([21]) and Turkey ([22]). A study carried out in the United States found carbamates and piperonyl butoxide in breast milk ([23]). This discrepancy may be attributed to the fact that prior studies predominantly concentrated on a limited set of toxins and did not specifically include these substances. Published research has frequently prioritized the examination of more widely recognized and prevalent POPs, including PCBs, dioxins, and organochlorine pesticides.

These results underline the importance of diversifying analyses to include a broader range of pollutants in order to better understand real exposure to chemical substances and assess the risks to human health.

The impact of exposure to chemical agents on health depends on several factors: the timing, duration, and extent of exposure. The embryonic and fetal stages as well as early childhood are periods during which the body is particularly sensitive to toxins. In adults, the intestinal microbiota plays a role in detoxifying harmful chemicals, but the immunity and metabolism of a newborn are still immature, making them more vulnerable to toxins ([24]). Moreover, the ingestion of these toxins could impact microbiota development. Early exposure to pesticides and endocrine disruptors has several harmful consequences, including obesity, low birth weight, premature birth, neurological development disorders, reproductive disorders, and certain hormone-dependent cancers ([24]). Some of these toxins cross the placental barrier, amplifying the risk to fetal development.

As shown in this article and in other studies which have found environmental toxicants in breast milk, "toxicovigilance" measures that are much stricter than the current ones should be created and applied. This could be done by strengthening the pre-marketing control rules and careful post-marketing monitoring, by integrating more or complementing the rules underlying the drug industry and pharmacovigilance.

A few limitations must be mentioned. First, the questionnaire used only included a limited number of variables that could influence pesticide content. Questions about diet (*e.g.,* egg and fish consumption, tap water or bottled water), mother's profession, smoking habits, and domestic use of pesticides or household products could have been added for a more comprehensive detection of factors influencing pesticide presence. Of note, the reliance on self-reported dietary habits is prone to recall bias.

Moreover, the method used did not provide quantitative values for the detected pesticides. Therefore, it was not possible to assess the quantity of toxins present in the samples to compare them with reference doses or the quantitative results of other studies. The risk of cumulative effects in the presence of several toxins in the same sample could not be evaluated. This analysis targeted a predefined panel of substances, increasing sensitivity and precision, but other toxins were potentially present and were not detected.

Finally, given the limited number of samples in the present work, further studies with larger participant samples would be more representative of the region and could reveal other toxins or contributing factors.

Pesticide exposure assessment can extend to alternative matrices, including blood, umbilical cord blood, urine, hair, or meconium to address antenatal and postnatal exposure. Urinary matrices primarily indicate recent exposure, while meconium analysis enables the measurement of prenatal exposure as toxins crossing the placental barrier accumulate during pregnancy ([25]).

Many Belgian neonatal ICUs are advocating for the establishment of breast milk banks for premature infants. While the risks of infectious contamination are well understood and anticipated, our findings indicate that the issues related to environmental toxins require significant consideration and analysis before these milk banks can be systematically used. Additionally, it is crucial to adequately inform the parents of children benefiting from these banks about the potential risks of pesticide exposure.

5. Conclusion

This study, carried out in the Charleroi region, investigated the presence of toxins in breast milk and artificial milk during early infancy, examining potential environmental links. Our results identified nine different pesticides in both breast milk and artificial milk samples, with more than a third of breast milk samples and half of infant formula samples containing at least one pesticide. A notable correlation was found between pesticide presence in breast milk and maternal consumption of fresh products.

These findings highlight the urgent need for ongoing vigilance and research to understand and mitigate the long-term effects of pesticide exposure on newborn health. They emphasize the importance of implementing measures to reduce this exposure, reflecting a significant public health concern. This study calls for proactive efforts from health authorities and the community to protect infants from environmental toxins.

CRediT authorship contribution statement

Alicia Goutelle: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Julian Viseur:** Writing – review & editing, Methodology, Investigation. **Vincent Nuyens:** Methodology, Writing – review & editing, Methodology. **Eric Cavatorta:** Writing – review & editing, Conceptualization. **Pierre Van Antwerpen:** Writing – review & editing, Methodology, Conceptualization. **Yoann Maréchal:** Writing – review & editing, Validation, Methodology, Data curation, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32350.

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