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Microplastics Contamination in Breast Milk and Infant Milk Products in Indonesia

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Microplastics contamination has been detected in milk consumed by infants, with estimates suggesting an intake of 106-113 microplastic particles per day. These particles may pose potential health risks. However, the microplastic contamination in breast milk and formula milk in Indonesia remain unclear. This study aims to address this gap. This study employed a descriptive observational design with a cross-sectional approach. Microplastic detection was carried out on four groups: fresh breast milk, breast milk stored in plastic bags, powdered formula milk, and liquid formula milk. Breast milk samples were collected from breastfeeding mothers at Puskesmas Mulyorejo, while formula milk was obtained from various market in Surabaya. The processed samples were filtered using filter paper, and the retained particles were examined. The number and shape of microplastic particles were identified using a binocular microscope, while the polymer characteristics were analyzed using micro-FTIR. The average number of microplastic particles was highest in powdered formula milk (15.34±4.74), followed by liquid formula milk (11.59±9.50), stored breast milk (6.07±5.46), and fresh breast milk (1.41±1.50). Microplastic contamination was not detected in 17 out of 46 breast milk samples. Fragmented microplastic particle shapes dominated all samples. Nylon and Polymethyl Methacrylate were the most common plastic polymers in breast milk, while Polyoxymethylene, Polyvinyl Chloride, and Polymethylpentene were prevalent in formula milk. In conclusion, microplastic contamination in breast milk was minimal compared to formula milk, making breast milk the best feeding option for infants.

Keywords: Breast Milk, Dangerous Contaminants, Food Safety, Formula Milks, Microplastics

INTRODUCTION

Plastic takes centuries to degrade into microplastic particles (Arifin et al, 2023). Imperfect plastic production, such as improper cutting and poor plastic quality, also leads to the abundance of microplastics as contaminants (Deng et al, 2022). Microplastics can enter the human body through ingestion, primarily via contaminated food and drinks that reach the digestive system (Donkers et al., 2022). It is predicted that the daily consumption of microplastics for boys and girls will be 113 and 106 particles per day, respectively (Cox et al, 2019; Sincihu, Elias, and Keman, 2022). As much as 0.61-0.89 mg of microplastics per day are consumed by infants due to the use of plastic packaging, including infant milk products (Liu et al, 2022). The vertical translocation from mother to breast milk is not yet well understood (Flores et al, 2023; Liu L, 2023). As much as 76.5% of breast milk is contaminated with microplastics (Ragusa, 2022). The contamination is higher in formula milk packaged in cartons compared to those in cans (Zhang et al, 2023). This finding has not yet been reported in Indonesia.

Microplastics can be absorbed through epithelial gaps in the intestine and enter the bloodstream (Karbalaei et al., 2018; Sincihu et al., 2022), after which they may be distributed to various tissues, including the breast. Particles smaller than 10 µm can penetrate breast adipose tissue (Wellnitz & Bruckmaier, 2021) and cross the blood–milk barrier via active transport (Ragusa, 2022). These particles often carry toxic additives or adsorbed pollutants, posing potential health risks (Li et al., 2020). Liu (2023) reported that storing liquids in plastic packaging can release particle clumps (<300 µm) and fragments (1–50 µm), which may contain harmful substances that can be transmitted from mother to infant during breastfeeding (Duale et al., 2022). Formula milk, as a substitute for breast milk, is also susceptible to microplastic contamination. Powdered formula is typically packaged in tinplate cans or plastic cartons with aluminum foil laminates, while liquid formula milk is stored in

containers with plastic laminate layers. Microplastics in formula milk may originate from the production process, packaging materials, transportation, and storage conditions (Li et al., 2020; Liu et al., 2022; Zhang et al., 2023). In children, exposure to these toxic substances may adversely affect their quality of life and contribute to the development of long-term health conditions, such as autism spectrum disorder (Kim & Yi, 2020; Zaheer et al., 2022). Microplastics may disrupt brain function through the actions of their metabolites, as well as via neuroendocrine and neuroimmune pathways (Lee et al., 2023).

The extent of microplastic contamination in breast milk and formula milk in Indonesia remains poorly documented, despite its potential to pose significant health risks to children. This gap highlights the urgency of investigating the microplastics contamination in infant milk sources. Given that milk serves as the primary source of nutrition during the early stages of life, its safety is vital for optimal infant growth and development. Microplastic exposure may pose long-term health risks, making this topic a critical focus for public health research. This study provides novel data on microplastic contamination in different types of milk consumed by infants in Indonesia. The purpose of this study is to describe and compare the presence of microplastics in four categories: fresh breast milk, breast milk stored in plastic bags, powdered formula milk, and liquid formula milk.

METHODS

Research Design

This is a quantitative study with a cross-sectional approach. The researcher only detects the quantity and characteristics (shape, polymer type, and additive materials) of microplastic particles as contaminants in breast milk and infant formula milk samples. Furthermore, the study describes the comparison of microplastic contaminant abundance without conducting statistical testing.

Population and Sample

This study includes four sample groups: fresh breast milk, breast milk stored in plastic bags, powdered formula milk, and liquid formula milk. Fresh breast milk and stored breast milk samples were collected from breastfeeding mothers visiting the Puskesmas Mulyorejo in Surabaya (n=46). Each participant manually expressed 5 cc of breast milk, which was then divided into a glass tube and a plastic bag. Powdered formula milk (n=26) and liquid formula milk (n=14) were sourced from markets in Surabaya, representing various commonly used brands. Each sample consisted of 5 grams of powdered formula and 5 cc of liquid formula. The sample size was determined based on Lameshow's formula, ensuring a minimum requirement with α = 5% and β = 80%. Prior to breast milk sample collection, respondents received detailed information and provided consent. For formula milk samples, the researchers anonymized the product labels to protect brand identities.

Preparation of Milk Samples

Fresh breast milk was stored in a glass tube, while breast milk kept in plastic bags was frozen at -4°C for 24 hours. To simulate standard storage and processing methods, the frozen milk was thawed by immersing it in 40°C water for 30 minutes. Powdered formula milk was placed in a beaker glass and mixed with 5 cc of distilled water, whereas liquid formula milk was directly poured into a beaker glass. To degrade the samples, 1 cc of 10% potassium hydroxide (KOH) solution was added, and the mixture was left at room temperature for 48 hours. This was followed by the addition of 1 cc of 67% nitric acid (HNO_3), allowing the reaction to continue for another 48 hours at room temperature. The next step involved heating the beaker glass containing breast milk or formula milk to 40°C until the fat clumps dissolved. In the second stage, the separation process was conducted by transferring all milk samples into 10 cc plain vacutainer tubes, followed by centrifugation at 2300 rpm for 5 minutes. In the third stage, the supernatant was filtered using a 0.45 µm Millipore® membrane filter. The filter paper was then stored in a covered Petri dish to prevent contamination and dried at 40°C overnight. The detection of microplastic follows the method of Flores et al. (2023) in their research. The use of 5 cc sample provides a balance between ease of handling and analytical sensitivity in microplastic quantification. Small volumes ranging from 1 to 10 cc in liquid biological and food-based matrices have been considered representative and appropriate for microplastic detection in previous studies, including those involving blood, beverages, and milk-based products (Leslie et al., 2022; Ragusa, et al., 2022).

Contamination Control

To prevent cross-contamination and ensure accurate detection of microplastics, strict contamination control procedures were applied throughout the processes of sample collection, preparation, and analysis. All materials used for sample collection and analysis were non-plastic, including glass tubes, glass breast milk pumps, cellulose-based filter papers, sterile water stored in glass bottles, and glass vacutainers. After filtration, the filter papers were placed in glass petri dishes during the heating process. Despite these efforts, airborne contamination during microscopic examination of the filter papers could not be completely avoided. It should be acknowledged that total contamination control against microplastic exposure is nearly impossible, even in internationally conducted studies.

Analysis Techniques

The quantification of microplastic particle count and shape on the filter paper was conducted using a Nikon Eclipse® E100 binocular microscope with 40x and 100x magnification. Observations were performed across five fields of view by three examiners to minimize errors, and the results were documented in a result form. This analysis was carried out at the Clinical Pathology Laboratory of Widya Mandala Surabaya Catholic University. Meanwhile, the identification of plastic polymers and additive substances as contaminants was performed using a micro-

FTIR (Fourier Transform Infrared) imaging tool at the Center for Food and Agriculture, Universitas Katolik Soegijapranata. All data were presented in the form of graphs, microscopic images, and distribution tables.

Statistical Test

This study used Kruskal-Wallis Test to determine whether there were significant differences in the mean number of microplastic particles among the four groups. A 95% confidence level was applied. Kruskal-Wallis Test was chosen because the data were not normally distributed ($p = 0{,}001 < 0.05$), as determined by the Shapiro-Wilk test.

Ethical Clearance

This study utilizes human biological samples, specifically breast milk, and has received ethical approval from the Health Research Ethical Clearance Commission at Universitas Airlangga with the reference number 947/HRECC.FODM/VIII/2023, following the 7 ethical principles outlined in the WHO 2011 declaration.

RESULTS AND DISCUSSION

Abundance of Microplastics in Breast Milk and Formula Milk

The detection of microplastic quantities was carried out on the four sample groups. Quantification was performed on five fields of view under an Epson Eclipse® binocular microscope at 40x and 100x magnification to ensure accuracy (Figure 1). The results showing the number of microplastics in each milk sample group are presented in Figure 2.

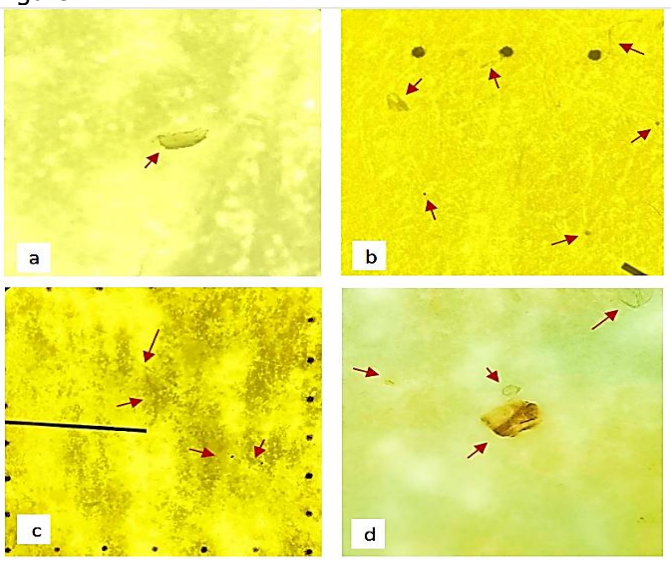

Figure 1. Microscopic Detection of Microplastic Particles in Breast Milk and Formula Milk

Description: → Red arrow is Microplastic Particle. a) Microplastics in fresh breast milk under 100x magnification, b) Microplastics in breast milk stored in plastic bags under 40x magnification, c) Microplastics in powdered formula milk under 40x magnification, d) Microplastics in liquid formula milk under 100x magnification

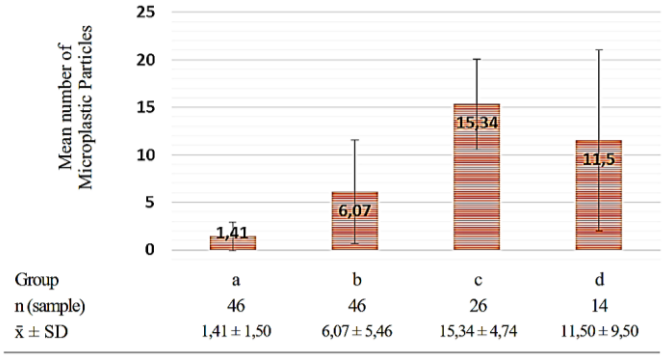

Figure 2. Number of Microplastics in Each Group

Description: a) Microplastics in fresh breast milk group, b) Microplastics in breast milk stored in plastic bags group, c) Microplastics in powdered formula milk group, d) Microplastics in liquid formula milk group.

Based on Figure 2, it is clear that all breast milk and formula milk samples were detected with microplastic particles in varying amounts. The highest average number of microplastic particles was found in the powdered formula milk group (c), with 15.34 particles per 5 grams of milk, followed by liquid formula milk in packaging group (d) with 11.5 particles per 5 mL of milk. The lowest average number of microplastic particles was found in fresh breast milk group (a), with 1.41 particles per 5 mL of milk. The finding of a higher average number of microplastic particles in breast milk stored in plastic bags (b), with 6.07 particles per 5 mL of milk, compared to fresh breast milk, indicates contamination or fragmentation of the packaging during the processing of breast milk at a temperature of 40°C. This finding aligns with the views of Jones et al. (2015) and Deng et al. (2022), who stated that the higher the temperature exposed to plastic bags, the more likely surface cracks will form in the plastic, leading to the release of plastic particles that cause contamination of the contents inside the food packaging.

Additionally, it appears that the number of microplastics increases with the longer contact time with plastic packaging and the duration of milk processing. This is evident in the powdered formula milk group (d), which underwent longer processing, showing the highest average number of microplastics compared to the fresh breast milk group (a). This finding is consistent with the study by DaCosta-Filho et al. (2021), which mentions that the presence of microplastics in milk samples occurs after the milk is processed using machines, tools, and storage containers made of plastic.

Table 1
Statictical Test

Group		N	Mean Rank	Normality Test	Kruskal Wallis Test
Microplastic Particles	Fresh breast milk	46	30.96	*Asymp. Sig. 0,001*	*Asymp. Sig. 0,001*
	Breast milk stored in plastic bags	46	70.13		
	Powdered formula milk	26	110.04		
	Liquid formula milk	14	90.50		

The Kruskal-Wallis Test yielded an Asymptotic Significance value of 0.001 (<0.05), indicating that there was a statistically significant difference in microplastic particle counts among the four groups of milk samples. The difference was illustrated in Figure 2 and described in detail in Figures 3, 4, 5, and 6 below.

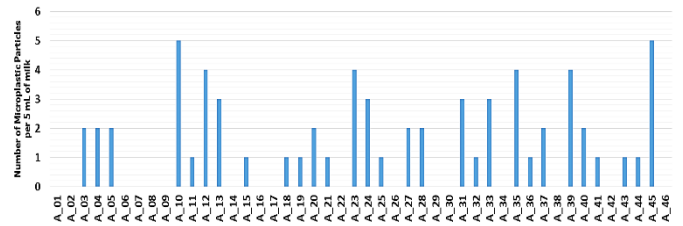

Figure 3. The Number of Microplastic Particles in Each Fresh Breast Milk Sample

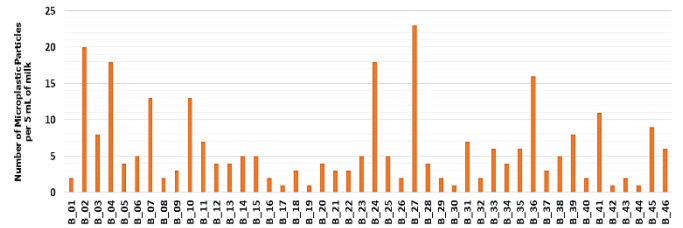

Figure 4. The Number of Microplastic Particles in Each Breast Milk Stored in Plastic Bags Sample

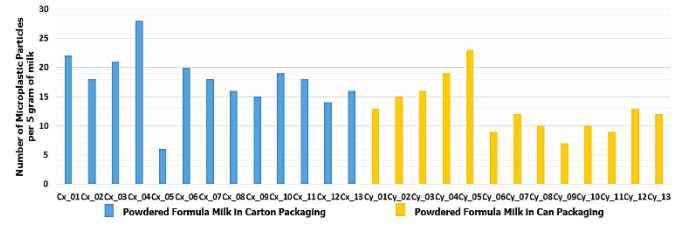

Figure 5. The Number of Microplastic Particles in Each Powdered Formula Milk Sample

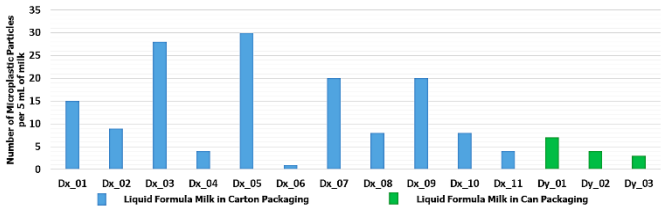

Figure 6. The Number of Microplastic Particles in Each Liquid Formula Milk Sample

Based on Figure 3, 4, 5, and 6, it can be observed that the number of microplastic particles found in each sample from all groups varies greatly, with a range from zero contamination up to a maximum of 30 microplastic particles. In the fresh breast milk sample group (Figure 3), it was found that the majority of samples did not show microplastic contamination, with 16 samples (34.8%) being free of contamination. Meanwhile, no samples in the other groups (Figure 4, 5, and 6) were free from microplastic contamination. The findings of a range from 0 to 5 microplastic particles in the fresh breast milk samples indicate the need to identify the determinant factors of microplastic contamination in breast milk through inhalation or ingestion pathways. The research by Flores et al. (2023) and Ragusa et al. (2022) mentions that the determinant factors include environmental pollution in residential areas, consumption patterns, contamination in livestock or vegetables, drinking water, and the use of plastic materials in food processing and storage. Meanwhile, in the breast milk samples stored in plastic bags (Figure 4), a higher number of microplastic particles were found due to contamination from the plastic storage bags, which resulted from fragmentation during the heating process at 40°C in the preparation of the frozen milk samples.

In the powdered formula milk samples (Figure 5) and liquid formula milk samples (Figure 6), it was found that the number of microplastic particles varied greatly. However, further analysis showed that the average number of microplastics in powdered formula milk with carton packaging (refill) was higher compared to can packaging (Image 9), with 17.8 and 12.9 particles per sample of powdered milk, respectively. A similar description is also shown by the liquid formula milk group (Figure 6), with 14.3 particles per liquid formula sample in carton packaging and 4.7 particles per liquid formula sample in can packaging. These findings are consistent with the research by Zhang et al. (2023), which explains that the contamination of microplastic particles is more likely during the processing of powdered formula milk compared to liquid formula milk. A similar explanation also applies to the processing of milk into carton packaging. However, the findings of this study differ from the research by Basaran et al. (2023) on formula milk, which reported microplastic contamination ranging from 62 to 142 particles in each milk sample. Similar to breast milk, some formula milk samples showed a lower number of microplastic particles compared to other formula milk samples. This indicates the need for identifying the

determinant factors of microplastic particle contamination abundance in formula milk preparations. This presents an opportunity for future research.

Shape of Microplastic Contaminants in Breast Milk and Formula Milk

Table 2
Microscopis Observation of Microplastic Shapes

Group	Microplastic Shapes (Average particles per sample group)			
	fragments	filaments	granules	pellets
Fresh Breast milk	1,28 (90,8%)	0,13 (9,2%)	0 (0,0%)	0 (0,0%)
Breast milk stored in plastic bags	4,5 (74,1%)	1,57 (25,9%)	0 (0,0%)	0 (0,0%)
Powdered formula milk	11,9 (77,6%)	2,4 (15,6%)	0 (0,0%)	1,04 (6,8%)
Liquid formula milk	8,78 (76,3%)	2,72 (23,7%)	0 (0,0%)	0 (0,0%)

Based on Table 2, it can be seen that the fragment particle shape is the most dominant contaminant across all breast milk and formula milk sample groups. Meanwhile, the filament shape is the second most abundant. No granule-shaped particles were found in any of the samples, while pellet-shaped particles were only present in the powdered formula milk sample group, and in very small quantities. The results of this study align with the research conducted by Muniasamy et al. (2020), which reported that microplastic particles exhibited a variety of colors (blue), shapes (fibers and fragments), and sizes (0.1–5 mm) as the most common. Similar observations were also noted in the findings of Flores et al. (2023), who mentioned that fragment were most prevalent in human breast milk and infant milk, while the study by Chakraborty et al. (2024) highlighted that filaments were the most dominant.

Polymer Types and Additives Materials of Microplastic Contaminants in Breast Milk and Formula Milk

The identification of polymer types of microplastic contaminants in the samples was conducted using the micro-FTIR (Fourier Transform Infrared) imaging tool at the Center for Food and Agriculture, Universitas Katolik Soegijapranata. The identified microplastic polymers and additive materials in the breast milk and formula milk samples are shown in the following Table 3 and 4.

Table 3
Characteristics pf Microplastic Polymer Types

Polymer Types	Percentage of Microplastics Polymer Types in Breast Milk	Percentage of Microplastics Polymer Types in Formula Milk
Polypropylene (PP)	-	2,5%
Polyethylene (PE)	-	3%
Polyvinyl Chloride (PVC)	5%	15,5%
Nylon	57.09%	4%
Polymethyl Methacrylate (PMMA)	9.17%	0,5%
Polyamide (Amorphous Nylon)	2.08%	-
Polyethylene Terephthalate (PETE)	0.83%	5%
Polyurethane / Polyacetylene (PU/PA)	2.92%	7,5%
Polyoxymethylene (POM) / Resin	2.08%	20%
Polymethylpentene (PMP)	3.75%	15%
Polybutylene Terephthalate (PBT)	3.33%	5%
Ethylene Vinyl Acetate (EVA)	0.42%	2,5%
Ethylene Vinyl Alcohol (EVOH)	-	1,5%
Fluorinated Ethylene Propylene (FEP)	-	0,5%

Table 4
Characteristics of Additive Materials

Additives Materials	Percentage of Additive Materials Found in Breast Milk	Percentage of Additive Materials Found in Formula Milk
Aromatic Polyamide (ARAMID)	0.42%	-
Methylcellulose	-	2,5%
Polyacrylamide Flocculant (PAF)	-	2%
Polymer Additive (Irganox 1076; DLTDP)	0.84%	0,5%
Polyvinyl Alcohol (PVA)	-	2%
Hydroxypropyl Cellulose (HPC)	-	0,5%
Ethylcellulose	-	0,5%
Nitrocellulose	9.58%	0,5%
Others	2.51%	9%

Based on Table 3, the most common plastic polymer contaminants in breast milk stored in plastic bags are Nylon and Polymethyl Methacrylate (PMMA). These two materials originate from the plastic bags. Meanwhile, the plastic polymer contaminants in powdered formula milk are Polyoxymethylene (Resin), Polyvinyl Chloride (PVC), and Polymethylpentene (PMP). These three polymers are commonly used in the processing equipment for formula milk production and commercial packaging. Table 4 shows that the most commonly found additives as contaminants in breast milk stored in plastic bags and powdered formula milk are Nitrocellulose and Methylcellulose. There are other additives present, accounting for 9% in powdered milk and 2.51% in breast milk samples, indicating that powdered formula production involves more additives compared to breast milk contamination by plastic bags.

However, the results of this study differ from the findings of Muniasamy et al. (2020), who mentioned that polyethersulfone and polysulfone were common types of microplastics found in milk samples. Meanwhile, the research by Chakraborty et al. (2024) highlighted that polyethylene polymer was the most dominant in both milk sample groups. This may occur because the types of plastic polymers involved in the production process differ from those in Mexico and Bangladesh, where their research was conducted. Similarly, the study by Basaran et al. (2023) mentioned that plastic polymers such as Ethylene vinyl acetate, polyethylene terephthalate, polypropylene, polyurethane, and nylon were the dominant polymers found in milk in Turkey.

The findings indicate that infants in Indonesia are exposed to Nylon and Polymethyl Methacrylate microplastics through breast milk stored in plastic storage bags. This exposure may contribute to intestinal inflammation, such as chronic diarrhea, act as an endocrine disruptor that interferes with growth and development, and lead to hormonal and metabolic imbalances (Kannan and Vimalkumar, 2021; Ullah et al, 2022; Wang et al, 2024). Excessive accumulation of these microplastics could potentially increase the risk of cancer or immune system disorders in humans (Zhi et al, 2024). The presence of Nylon and Polymethyl Methacrylate in breast milk is suspected to come from plastic storage bags used for breast milk. These materials are widely utilized in commercial products due to their lightweight, strength, flexibility, heat resistance, and chemical durability, which may lead mothers to reuse breast milk storage bags.

Meanwhile, infants who consume formula milk are exposed to an even greater amount of more hazardous plastic materials, namely Polyoxymethylene, Polyvinyl Chloride, and Polymethylpentene. These three plastic polymers are likely introduced from various sources during the preparation of infant formula. Polyoxymethylene may come from the formula measuring spoon and parts of automatic formula-making machines, Polyvinyl Chloride from the inner lining of formula packaging and manufacturing equipment, and Polymethylpentene from measuring spoons and production tools. Under normal conditions, Polyoxymethylene is generally non-toxic. However, when exposed to high temperatures during formula preparation, it can release formaldehyde, a substance associated with an increased risk of cancer due to prolonged exposure. Furthermore, Polyoxymethylene and Polymethylpentene, as microplastics, generally have negative effects as endocrine disruptors, interfering with hormone regulation in infants and potentially affecting growth and brain development (Arman et al, 2022; Ullah et al, 2022; Solleiro-Villavicencio et al, 2020). Polyvinyl Chloride poses a greater health risk because it contains phthalates, Bisphenol A, and dioxins. These chemicals are not only endocrine disruptors and carcinogens, but even in small amounts, they can lead to toxicity, severe hypersensitivity reactions, reproductive organ abnormalities, impaired brain development, behavioral disorders, diabetes, and childhood obesity (Lieshchova et al, 2019).

One of the key findings of this study is the detection of microplastic particles in both breast milk and powdered infant formula. This indicates a potential route of early-life exposure to microplastics among infants. The

presence of these particles suggests not only environmental contamination but also possible leaching from packaging and storage materials. These findings have important implications for breastfeeding mothers and public health, emphasizing the need for increased caution in milk handling practices. Furthermore, this study supports the urgency of reviewing and improving current policies on breast milk storage and infant formula packaging, particularly in promoting the use of safer, non-plastic alternatives to reduce the risk of microplastic exposure in infants.

The limitations of this study include the wide standard deviation, which indicates the need to identify the factors determining the abundance of microplastic contamination in breast milk and formula milk samples. There are also factors of contamination during the sample processing, such as distilled water in plastic bottles, rubber gloves, fiber-based apron, and contamination from the air. Additionally, the lack of observation during the formula milk production process means that the source of the microplastic particle contaminants found in the samples could not be further explained.

CONCLUSIONS

Fresh breast milk was the safest type of milk, with the lowest level of microplastic contamination, followed by breast milk stored in plastic bags. Powdered formula milk from various brands in Indonesia showed the highest level of risk. These findings underscore the urgent need for stricter regulation and quality control of plastic materials used in the production, packaging, and storage of infant milk products. There is also a critical need to educate breastfeeding mothers on safe breast milk storage practices, preferably using glass bottles. Promoting direct breastfeeding should be a public health priority to minimize microplastic exposure in infants.

SUGGESTION

For future research, it is important to observe the environmental conditions during the formula milk production process, particularly the use of plastic materials and airborne contamination. Additionally, factors influencing the abundance of microplastics in breast milk need to be identified, such as parental personal hygiene, environmental sanitation, the use of plastic kitchen utensils, plastic feeding utensils for infants, the use of plastic bags for food storage, and the cleanliness of food processing. Furthermore, sample preparation free from contamination should be a primary concern for microplastic researchers, such as designing contamination-free rooms specifically for sample preparation. These research directions also have broader implications, particularly for breastfeeding mothers and public health policy. The findings support the need to reassess the use of plastic-based storage materials for breastmilk and formula packaging. Promoting safer, non-plastic alternatives may be crucial in reducing microplastic exposure during infancy.

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