First detection of quaternary ammonium compounds in breast milk: Implications for early-life exposure

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BACKGROUND: Quaternary ammonium compounds (QACs), commonly used in cleaning, disinfecting, and personal care products, have recently gained worldwide attention due to the massive use of disinfectants during the COVID-19 pandemic. However, despite extensive use of these chemicals, no studies have focused on the analysis of QACs in human milk, a major route of exposure for infants.

OBJECTIVE: Our objectives were to identify and measure QACs in breast milk and evaluate early-life exposure to this group of compounds for nursing infants.

METHODS: Eighteen QACs, including 6 benzylalkyldimethyl ammonium compounds (BACs, with alkyl chain lengths of C8-C18), 6 dialkyldimethyl ammonium compounds (DDACs, C8-C18), and 6 alkyltrimethyl ammonium compounds (ATMACs, C8-C18), were measured in breast milk samples collected from U.S. mothers. Daily lactational intake was estimated based on the determined concentrations for 0–12 month old nursing infants.

RESULTS: Thirteen of the 18 QACs were detected in breast milk and 7 of them were found in more than half of the samples. The total QAC concentrations (ΣQAC) ranged from 0.33 to 7.4 ng/mL (median 1.5 ng/mL). The most abundant QAC was C14-BAC with a median concentration of 0.45 ng/mL. The highest median ΣQAC estimated daily intake (EDI) was determined for <1-month old infants based on the average (using the median concentration) and high (using the 95th percentile concentration) exposure scenarios (230 and 750 ng/kg body weight/day, respectively).

SIGNIFICANCE: Our findings provide the first evidence of the detection of several QACs in breast milk and identify breastfeeding as an exposure pathway to QACs for nursing infants.

IMPACT STATEMENT: Our findings provide the first evidence of QAC occurrence in breast milk and identify breastfeeding as one of the exposure pathways to QACs for nursing infants.

Keywords: Early-life Exposure; Emerging Contaminants; Biomonitoring; Child Exposure/Health

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INTRODUCTION

Quaternary ammonium compounds (QACs) are a large group of organic substances used as disinfectants and surfactants in a range of applications, including but not limited to cleaning, disinfecting, and personal care products, pharmaceuticals, pesticides, and biomedical materials [1]. The three most well-known QAC groups include benzylalkyldimethyl ammonium compounds (BACs), dialkyldimethyl ammonium compounds (DDACs), and alkyltrimethyl ammonium compounds (ATMACs), some of which were produced in volumes ranging from 10 to 50 million pounds in the United States in 2015 [1]. After the ban of triclosan use in antibacterial soap by the United States Food and Drug Administration in 2016, some QACs have been used as its replacements [1, 2]. Moreover, the use of QACs as disinfectants has increased since the outbreak of the coronavirus disease 2019 (COVID-19) due to their effectiveness against SARS-2-CoV [1]. This pattern of increased use may continue even beyond the pandemic as the global consumption of disinfecting products is predicted to increase at a rate of 10% per year from 2020 to 2027 [3].

QACs have been detected in the environment, including wastewater sludge, surface waters, sediments, and soil [4–10]. They have also been found in foodstuff (e.g., fruits, milk, and vegetables), dust, and air, suggesting that humans can be exposed to QACs via diet, dust ingestion, dermal absorption, and inhalation [11–18]. However, despite extensive use, there is very limited data on human exposure to and biomonitoring of QACs. The only two existing biomonitoring studies detected several QACs (e.g., C12- and C14-BACs) in blood and have demonstrated that these compounds can bioaccumulate in the human body [17, 19]. Moreover, the latest research indicates that human exposure to QACs has increased during the COVID-19 pandemic [16, 17]. However, there are no studies on the occurrence of QACs in human breast milk, a major nutritional source for infants.

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There is a growing concern regarding the toxicity of some QACs. Most studies on sub-chronic toxicity of BACs and DDACs have shown that exposure to these compounds can result in skin irritation [20–22] and adverse respiratory effects [23–26]. Recent in vitro, in vivo, and epidemiological studies on the chronic QAC exposure show that exposure to BACs and DDACs is associated with immunotoxicity [19], metabolic disorders [27, 28], and reproductive toxicity [29, 30]. Furthermore, BACs can cross the placenta and alter cholesterol and lipid homeostasis in neonatal mice brains after gestational exposure [31], suggesting that BACs are potential neurotoxicants.

Infants are more vulnerable to adverse effects of environmental exposures due to their rapid development and growth [32, 33]; therefore, it is critical to evaluate the early-life environmental exposures. Breastfeeding is recognized as a significant exposure pathway to many environmental contaminants for nursing infants [34–36]. For the first time, this study reports the levels of eighteen QACs, including 6 BACs (with alkyl chain lengths of C8-C18), 6 DDACs (C8-C18), and 6 ATMACs (C8-C18), in breast milk samples collected from U.S. mothers, as well as estimates the daily lactational intake of these compounds for nursing infants.

**MATERIALS AND METHODS**

**Recruitment and sample collection**

Forty-eight primiparous women pregnant and planning to breastfeed or currently breastfeeding and residing in Seattle, Washington, United States were recruited over social media channels and via parenting groups and paper flyers during March-October 2019 (before the COVID-19 pandemic) [36]. Breast milk was manually extracted into a provided empty glass jar pre-cleaned with water, isopropyl alcohol, and methanol (breast pumps were not used). After collection, the samples were taken from participants within 24 h and stored at −4 °C until shipment to Indiana University, where they were stored at −20 °C until analysis. Information on demographics and socioeconomic status, as well as the use of household cleaners, disinfectants, personal care products, and the household disinfection frequency was also collected (Table 1). Ingredient information for each product listed in surveys was searched online. The products were grouped based on the following categories: cleaning products without QACs, cleaning products with QACs, personal care products with ATMACs, and personal care products without ATMACs. The QACs-containing products included sprays (squirt bottles) and wipes.

**Sample analysis**

Two mL of breast milk (thawed at room temperature) were spiked with surrogate standards (d7-C12-BAC and d9-C10-ATMAC) and ultrasonicated in 4 mL of acetonitrile for 1 h. The supernatant was transferred to a new tube after the sample was centrifuged (3000 rpm, 5 min). The residue was re-extracted twice, and supernatants were combined, concentrated to 2 mL under N2 in a water bath at 40 °C and diluted with 4 mL of 5% ammonium hydroxide in water (v/v). The sample was loaded on an Oasis WCX cartridge (6 cc, 150 mg, 30 µm) preconditioned with 6 mL of methanol and 6 mL of water. After washing with 3 mL of 5% ammonium hydroxide in water (v/v) and 3 mL of 10% methanol in water (v/v), the cartridges were dried under vacuum. The target analytes were eluted with 6 mL of 2% formic acid in methanol (v/v).

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**Table 1.** Summary of demographic characteristics and the use of disinfecting and personal care products for participants in this study (n = 48).

| Characteristics | N  | Percentage, % | Characteristics | N  | Percentage, % |
|-----------------|----|---------------|----------------|----|---------------|
| **Demographic factors** |    |               | **Disinfecting habits** |    |               |
| Age (years)     |    |               | Yes            | 21 | 44            |
| <33             | 17 | 36            | No             | 26 | 54            |
| >33             | 29 | 60            | Missing        | 1  | 2             |
| Missing         | 2  | 4             | Disinfectant use |    |               |
| **Education completed** |    |               | More than once a week | 8  | 38            |
| College         | 17 | 36            | Less than once a week | 11 | 52            |
| Advanced degree | 29 | 60            | Missing        | 2  | 10            |
| Missing         | 2  | 4             | Disinfecting frequency (n = 21) |    |               |
| **Census tract median income** |    |               | Wipes          | 13 | 62            |
| Low-income      | 1  | 2             | Sprays         | 8  | 38            |
| Lower middle    | 8  | 16            | Missing        | 0  | 0             |
| Middle          | 20 | 42            | Personal care product use |    |               |
| Upper middle    | 18 | 38            | Using ATMAC- containing products (n = 48) |    |               |
| Missing         | 1  | 2             | Yes            | 10 | 21            |
| Residence status in Seattle (years) |    |               | No             | 38 | 79            |
| <20             | 33 | 69            | Frequency (n = 10) | 13 | 27            |
| >20             | 13 | 27            | 2-5 times a week | 7  | 70            |
| Missing         | 2  | 4             | Child’s age at time of collection |    |               |
| <6 months       | 19 | 40            | 5-10 times a week | 3  | 30            |
| >6 months       | 24 | 50            | Maternal BMI (kg/m²) |    |               |
|                 | 5  | 10            | Underweight, <18.5 | 0  | 0             |
|                 |    |               | Normal, 18.5–24.9 | 28 | 58            |
|                 |    |               | Overweight, 25–29.9 | 10 | 21            |
|                 |    |               | Obese, >30     | 6  | 13            |
|                 |    |               | Missing        | 4  | 8             |
dryness using N₂, redissolved in 500 μL of acetonitrile, filtered, and spiked with the internal standard (d₆-C14-BAC).

**Instrumental analysis**

The target compounds were identified and quantified on an ultra-performance liquid chromatography tandem mass spectrometer (Agilent 1290 Infinity II UPLC – 6470 QQQ-MS) in the positive electrospray ionization (ESI+) mode using a previously developed method [17]. An Acquity UPLC BEH C₁₈ column (50 mm, 2.1 mm i.d., 1.7 μm thickness, Waters, Milford, MA) was used for the UPLC separation of the target analytes. A delay column (ZORBAX RR Eclipse Plus C₁₈, 50 mm, 4.6 mm i.d., 3.5 μm thickness, Agilent, Palo Alto, CA) was set up to reduce the background contamination from the instrument. The mobile phase consisted of water (A) and acetonitrile (B), both containing 0.1% formic acid. The flow rate was 0.4 mL/min. The following gradient was employed: 10% B for 0.5 min initially, ramped to 100% B at 6 min and held for 4 min, returned to 10% B at 10.5 min and equilibrated for 3.5 min after every run. The injection volume was 5 μL. The nebulizer, gas flow, gas temperature, capillary voltage, sheath gas temperature, and sheath gas flow, were set at 25 psi, 10 L/min, 300 °C, 3500 V, 350 °C, and 12 L/min, respectively. The data acquisition was conducted under a multiple reaction monitoring (MRM) mode and the optimized MRM transitions, fragmentors, and collision energies are presented in Table S1.

**Quality assurance and quality control**

All glassware was heated at 500 °C for 8 h in a muffle furnace before use. Procedural blanks were used to monitor background contamination in the laboratory (n = 5). In addition, field blanks (n = 2) were collected to check background contamination during sampling. Method quantification limits (MQLs) were set as ten times the standard deviation of the target analyte levels detected in the blanks. For compounds not detected in the blanks, MQLs were based on a signal-to-noise ratio of ten. All data were blank corrected by subtracting average blank levels from the sample levels. The blank levels for some analytes (e.g., C14- and C18-DDACs and C14-ATMAC) were elevated; however, the levels in samples were still higher than in blanks, hence the detection frequencies did not significantly decrease after blank-correction. Still, the data for these analytes should be considered with caution. Procedural and field blank levels for all analytes are included in Table S2.

Quantification of the detected target analytes was performed by isotope dilution using calibration curves with concentration ranges of 0.1 – 10 ng/mL and correlation coefficients in linearity tests were all >0.99. The average absolute recoveries for the spiked samples ranged from 41 ± 0.5% (mean ± standard error) to 149 ± 5% and are given in Table S3. The average recoveries of the surrogate standards were 80 ± 0.7% and 73 ± 0.5% for d₆-C12-BAC and d₆-C10-ATMAC, respectively (Table S4). The concentrations were not corrected based on surrogate recoveries.

**Data analysis**

Lactational estimated daily intakes (EDIs, ng/kg body weight [bw] /day) of QACs were calculated using Eq. 1:

\[
\text{EDI} = C \times \text{FIR}
\]

where C is a median concentration of a QAC in breast milk in ng/mL and FIR is a food ingestion rate (mL/kg bw/day) representing the average daily intake of breast milk (150, 140, 110, and 83 mL/kg bw/day for <1, 1–3, 3–6, and 6–12 month old infants, respectively) [37].

Statistical analyses were conducted using IBM SPSS Statistics 24 and Minitab 13 and plots were generated using Sigma Plot 13. Correlation heatmaps and hierarchical clustering were done using Pearson correlation analysis in R studio. No significant correlation was found between the lipid content and QAC concentrations in breast milk (Table S5); hence concentrations are given in ng/mL. Concentrations below MQLs were replaced with MQL/2 values for the descriptive statistics and correlation analyses. The significance level was set at p < 0.05.

**RESULTS**

**Population characteristics**

A description of the participants’ demographic characteristics is presented in Table 1. All mothers were breastfeeding their first child. Most of the participants were Caucasian and lived in or around Seattle, Washington (the average [with standard error] residence time was 13 ± 11 years). Participants’ age ranged from 24 to 42 years old (average 34 ± 4.0 years). Ninety-six percent of the mothers had attained higher education and 80% lived in middle or upper-middle income neighborhoods. Fifty-eight percent had a normal BMI (18.5–24.9), while 34% were overweight or obese. No significant correlation was found between the QAC concentrations and demographic characteristics, possibly due to the limited sample size.

**Characterization of QAC levels in breast milk**

The detection frequencies and concentrations of the 18 QACs measured in breast milk are given in Table 2. Thirteen of the 18 QACs were detected in breast milk and 7 were found in more than half of the samples. All analyzed samples had at least one QAC detected above the limit of detection. The total QAC concentrations (ΣQAC, the sum of the 13 detected QACs) ranged from 0.33 to 7.4 ng/mL with a median concentration of 1.5 ng/mL. BACs were the most abundant QACs found in these samples with a median ΣBAC concentration (the sum of 5 detected BACs) of 0.92 ng/mL accounting for 71% of the ΣQAC concentration, followed by ATMACs detected at a median ΣATMAC [the sum of 6 detected ATMACs] concentration of 0.44 ng/mL (29% of the ΣQAC concentration). DDACs were detected in 15% of the samples and at lower concentrations (<2.2–2.8 ng/mL). C10-C16 BACs and C14- and C16-ATMACs were detected in the majority of the samples (67–88%). The most abundant QAC was C14-BAC with a median concentration of 0.45 ng/mL, followed by C12-BAC (median 0.38 ng/mL), C16-ATMAC (0.21 ng/mL), and C14-ATMAC (0.16 ng/mL). These four compounds accounted for 92% of the ΣQAC concentrations. C10- and C16-BACs and C18-ATMAC were detected at significantly lower concentrations (p < 0.05 based on a one-way analysis of variance [ANOVA], Table 2 and Fig. 1). Although C10-DDAC was only detected in 8% of the samples, high concentrations of C10-DDAC were exclusively found in the samples collected from the mothers who reported using QAC-containing disinfectants. C18-BAC, C8- and C10-DDACs, and C8- C12 ATMACs were found in less than half of the samples, and C8-BAC and C12-C18 DDACs were not detected.

The individual QACs with detection frequencies over 50% were clustered into two major groups based on the correlation heatmaps and hierarchical clustering (Fig. 1). The first group consisted of C10-C16 BACs and C14-ATMAC (r: 0.44–0.96, p < 0.05) and the other cluster included C16- and C18-ATMACs (r: 0.33–0.44, p < 0.05).

**Effect of product use**

Forty-two percent of mothers in this study indicated that they regularly used QAC-containing disinfecting products in their homes. Among those using such products, sixty-two percent regularly used QAC-containing disinfecting products in their homes. Forty-two percent of mothers in this study indicated that they used disinfecting sprays was almost 2 times higher compared to the mothers who used disinfectants without QACs (mean ± standard error [95% CI], 2.88 ± 0.63 [1.89–3.88] vs. 1.62 ± 0.29 [1.24–2.33] ng/mL, Fig. 2). The mean ΣQAC concentration in breast milk from mothers who used disinfecting sprays was almost 2 times higher compared to those who used wipes (2.88 ± 0.63 [1.89–3.88] vs. 1.52 ± 0.38 [0.74–2.30] ng/mL; p < 0.05). Mothers who disinfected more frequently (more than once per week) had a slightly higher ΣQAC concentration in their breast milk than those who disinfected less often (2.21 ± 0.60 [1.02–3.39] vs. 2.03 ± 0.56 [1.02–3.04] ng/mL). A similar trend was found for the ΣBAC concentrations. Higher ΣATMAC concentrations were found in the group of mothers who used QAC-containing disinfecting products, specifically in those who used disinfecting sprays (Fig. 2). No...
relationship was found between the ΣATMAC concentrations and the use of personal care products that list ATMACs as the main ingredients.

Exposure assessment
Lactational estimated daily intakes (EDIs) of QACs for infants of <1, 1–3, 3–6, and 6–12 month old are presented in Table 3. The highest median ΣQAC EDI was found for the <1 month old infants (230 ng/kg bw/day), followed by that for the 1–3 month old (210 ng/kg bw/day), 3–6 month old (170 ng/kg bw/day), and 6–12 month old (120 ng/kg bw/day) infants. This decline can be explained by the increase in the body weight and decrease in breast milk consumption with age. The median EDI for C14-BAC was the highest for all age groups (37–68 ng/kg bw/day), followed by the EDI for C12-BAC (32–57 ng/kg bw/day). In the high-exposure scenario (based on the 95th percentile concentrations), the ΣQAC EDI increased to 420–750 ng/kg bw/day.

DISCUSSION
Our findings indicate widespread detection of QACs in breast milk with at least one QAC found in each sample and 7 QACs detected in more than half of the samples. As this is the first study reporting the occurrence of QACs in breast milk, a direct comparison of our results with other studies is not possible. When comparing the QAC levels in this study to the previously reported levels of emerging

| Table 2. Detection frequencies (DF, %), the mean (with their standard errors), minimum, 25th percentile, median, 75th percentile, 95th percentile, and maximum concentrations of each QAC measured in breast milk (ng/mL; n = 48) and their contributions (contr, %) to the ΣQAC concentrations. |
|---|---|---|---|---|---|---|---|---|
| BACs | | | | | | | | |
| DF % | Mean ± SE ng/mL | Min ng/mL | 25th ng/mL | Median ng/mL | 75th ng/mL | 95th ng/mL | Max ng/mL | Contr. % |
| C8-BAC | 0 | <MQL | | | | | | |
| C10-BAC | 79 | 0.0047 ± 0.00035 | <MQL | 0.0031 | 0.0049 | 0.0054 | 0.0098 | 0.011 37 |
| C12-BAC | 81 | 0.51 ± 0.067 | <MQL | 0.14 | 0.38 | 0.69 | 1.5 | 2.2 29 |
| C14-BAC | 67 | 0.56 ± 0.064 | <MQL | 0.45 | 0.80 | 1.3 | 2.1 | 35 |
| C16-BAC | 69 | 0.12 ± 0.018 | <MQL | 0.088 | 0.17 | 0.32 | 0.52 | 6.7 |
| C18-BAC | 10 | 0.34 ± 0.18 | <MQL | <MQL | <MQL | <MQL | 0.87 | 0.95 71 |
| ΣBAC | 90 | 1.2 ± 0.14 | <MQL | 0.40 | 0.92 | 1.7 | 3.3 | 4.1 |

DDACs
| C8-DDAC | 8 | 0.25 ± 0.23 | <MQL | <MQL | <MQL | <MQL | 0.81 | 0.94 71 |
| C10-DDAC | 8 | 1.5 ± 0.44 | <MQL | <MQL | <MQL | <MQL | 2.6 | 2.8 |
| C12-DDAC | 0 | <MQL | | | | | | |
| C14-DDAC | 0 | <MQL | | | | | | |
| C16-DDAC | 0 | <MQL | | | | | | |
| C18-DDAC | 0 | <MQL | | | | | | |
| ΣDDAC | 15 | 1.0 ± 0.35 | <MQL | <MQL | <MQL | <MQL | 2.3 | 2.8 |

ATMACs
| C8-ATMAC | 6 | 0.054 ± 0.016 | <MQL | <MQL | <MQL | <MQL | 0.082 | 0.087 71 |
| C10-ATMAC | 6 | 0.093 ± 0.042 | <MQL | <MQL | <MQL | <MQL | 0.16 | 0.17 71 |
| C12-ATMAC | 44 | 0.062 ± 0.020 | <MQL | <MQL | <MQL | <MQL | 0.079 | 0.17 71 |
| C14-ATMAC | 85 | 0.21 ± 0.032 | <MQL | <MQL | <MQL | <MQL | 0.16 | 0.24 71 |
| C16-ATMAC | 88 | 0.39 ± 0.099 | <MQL | <MQL | <MQL | <MQL | 0.13 | 0.21 71 |
| C18-ATMAC | 65 | 0.031 ± 0.0059 | <MQL | <MQL | <MQL | <MQL | 0.013 | 0.027 71 |
| ΣATMAC | 98 | 0.67 ± 0.11 | <MQL | <MQL | <MQL | <MQL | 2.0 | 0.47 71 |
| ΣQACs | 100 | 2.0 ± 0.24 | 0.33 | 0.88 | 1.5 | 2.9 | 5.0 | 7.4 71 |

*Percent contribution to the ΣQAC concentration was not calculated because of DF < 50%. <MQL: concentrations below the method quantification limit; SE: standard error. Some concentrations are below the blank levels because the data was blank-corrected by subtracting the average blank levels from sample levels.

Fig. 1 Concentrations of QACs detected in more than 50% of the breast milk samples (ng/mL). Concentrations are shown as box plots, representing the 25th and 75th percentiles; black lines represent the median and the whiskers represent the 10th and 90th percentiles; the dots represent the 5th and 95th percentiles. The letters show the results of the one-way analysis of variance (ANOVA) and the concentrations are ranked from the highest to the lowest in alphabetical order (A stands for the highest and C for the lowest). The concentrations sharing the same letter are not statistically different at p < 0.05 (e.g., C12-BAC, C14-BAC, and C16-ATMAC).
contaminants, the median ΣQAC concentration (1.5 ng/mL) was lower than that of organophosphate esters (medians 3.5–3.9 ng/mL) [34], comparable to that of melamine derivatives (1.4 ng/mL) [38], but 10 times higher than the median concentration of per- and polyfluoroalkyl substances (PFAS) in breast milk from U.S. mothers (0.12 ng/mL) [17]. Although information about the route and magnitude of exposure to QACs is limited, these findings suggest that certain QACs may accumulate in breast milk at comparable or higher levels than some of the other ubiquitous environmental contaminants.

Two recent studies have reported a frequent detection of QACs in human blood, demonstrating widespread human exposure to QACs in the general U.S. population [17, 19]. The median ΣQAC concentration measured in breast milk was two times lower than that found in human blood collected before the pandemic (sampling year 2019; median 3.4 ng/mL) [17]. This may be explained by the strong binding affinity of QACs to blood proteins, thus allowing only a fraction of QACs to be transported to breast milk [17]. The QAC profiles in breast milk and blood were somewhat different: breast milk was more enriched with BACs, while blood had almost equal contributions from both BACs and ATMACs (73 and 27% vs. 48 and 50%, respectively) [17]. In both cases, DDACs constituted only a minor fraction of the ΣQAC concentrations. The different QAC profiles in blood and breast milk could be explained by a compound-specific lactational transfer. However, this comparison should be considered with caution because these blood and breast milk samples were not paired, thus these differences could be confounded by other factors, such as sampling time, demographic characteristics, and exposure levels.

The most abundant QACs found in these breast milk samples were BACs and ATMACs. Previous studies have shown that BACs are the major ingredients in disinfecting products [16, 39], while ATMACs are mainly used in cosmetics and hair conditioners [1, 40, 41]. Interestingly, strong positive correlations were found between C10–C16 BACs and C14-ATMAC concentrations in breast milk. Similarly, C14-ATMAC was the most abundant ATMAC in some cleaning products analyzed in our previous study, and a strong correlation was observed among the dust levels of C14-ATMAC and C10–C16 BACs [16]. These results suggest that cleaning products could be a potential source of ATMACs in breast milk.

Our results suggest that disinfecting practices may have an effect on the QAC levels in breast milk. The mean ΣQAC concentration in breast milk from mothers who used disinfecting products were higher than those who did not use such products or used disinfectants without QACs. Especially, higher levels of QACs were found in mothers who used disinfecting sprays.
compared to mothers who used wipes. This could be related to the previously reported higher content of QACs in sprays (1.66% by weight) compared to wipes (0.135%) [16]. In addition, mothers who disinfected more often had a somewhat higher mean ΣQAC concentration in their breast milk than those who disinfected less frequently. Taken together, these results suggest that the use of disinfecting products that contain QACs may affect the levels in breast milk. The lack of a significant relationship between the ΣATMAC concentrations and the use of personal care products that list ATMACs as the main ingredients, could be because C20–C22 ATMACs commonly found in hair conditioners were not analyzed in this study.

The QAC intake in the present study was more than 3 orders of magnitude lower than the intake through surface-to-hand-to-mouth transfer reported for 3-year-old toddlers, but higher than the estimated dermal absorption through handwashing estimated for the same age group [39]. The ΣQAC EDI was up to 5 times lower than that estimated based on the accidental dust ingestion for toddlers (210–620 ng/kg bw/day) [16]. Generally, the C12-BAC EDIs in both the average and high exposure scenarios were below the reference dose (10 ng/kg bw/day) established by the European Food Safety Authority (EFSA) [42]. However, this reference dose does not account for the potential adverse effects of the chronic exposure to QACs [42].

CONCLUSIONS

This is the first study reporting the detection of QACs in breast milk and identifying breastfeeding as an exposure pathway to QACs in nursing infants. The limitations of this study include a small sample size covering a limited geographic area and a quite homogenous population. Information on the specifics of milk collection (e.g., timing, cleaning of the breast and hands, etc.) was not collected. Nonetheless, our findings demonstrate widespread exposure to QACs: at least one QAC was found in each breast milk sample and 7 QACs were found in more than half of the samples. We also found higher QAC levels in breast milk from mothers who used QAC-containing disinfecting products and disinfected more often. Considering a significant increase in the use of QACs as common disinfectants during the COVID-19 pandemic, our findings warrant future research on QAC toxicity and human health effects.

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AUTHOR CONTRIBUTIONS
GZ: Conceptualization, Lab and Data Analysis, Writing - Original draft preparation; ES: Data collection, Writing - Reviewing and Editing; SS: Data evaluation, Writing-Reviewing and Editing; AS: Supervision, Conceptualization; Writing- Reviewing and Editing.

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