


Elevated concentrations of quaternary ammonium compounds in childcare centers: A pilot study

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ABSTRACT

Quaternary ammonium compounds (QACs) are used as antimicrobials, preservatives, and antistatic agents in cleaning, disinfecting and personal care products, and textiles. High levels of QACs have been found in indoor dust in residential homes; however, there is limited information on QAC exposure in non-residential environments serving sensitive populations, such as childcare centers. In this study, we investigated the occurrence of QACs, including benzylalkyldimethyl ammonium compounds (BACs), dialkyldimethylammonium compounds (DADMACs), and alkyltrimethylammonium compounds (ATMACs), in dust from childcare centers and estimated daily intake of QACs by toddlers in childcare via dust ingestion. Nineteen QACs were detected in dust with a median total QAC concentration (Σ QAC) of 150 $\mu\text{g/g}$. BACs were the most abundant QAC group found at concentrations ranging from 2.67 to 1370 $\mu\text{g/g}$ (median 90.4 $\mu\text{g/g}$) and constituted 64 % of the Σ QAC concentrations. The QAC levels in dust from childcare centers were significantly higher than concentrations previously reported in homes. The EDIs for BACs, DADMACs, and ATMACs via dust ingestion calculated based on the 95th percentile concentrations in childcare dust were up to 30 times higher than those for toddlers in residential homes. These findings demonstrate high QAC exposure in childcares, posing significant early-life exposure for toddlers.

1. Introduction

Quaternary ammonium compounds (QACs) are salts of quaternary ammonium cations with hydrophobic alkyl or benzyl substitutes attached to the positively charged nitrogen (Arnold et al., 2023; Mahony et al., 2023). QACs are used as antimicrobials, preservatives, and antistatic agents in cleaning, disinfecting, and personal care products, and textiles (Morais et al., 2016; Zhang et al., 2015). The three most commonly used QAC groups include benzylalkyldimethyl ammonium compounds (BACs), dialkyldimethylammonium compounds (DADMACs), and alkyltrimethylammonium compounds (ATMACs), some of which are high production volume chemicals in the United States (Hora et al., 2020; Zhang et al., 2015). Certain QACs have been used as replacements of triclosan after it was banned in antibacterial soap in 2016

(Food and Drug Administration, 2016; Hora et al., 2020). In addition, the use of disinfecting, antibacterial, and antiviral products, including those containing QACs, has dramatically increased since the outbreak of the COVID-19 pandemic in 2020 and is expected to continue to rise (Ebrahimi and Akhavan, 2022; Hora et al., 2020).

QACs have been detected in the outdoor environment, including surface water, wastewater, sediment, soil, and sewage sludge (Arnold et al., 2023; Mahony et al., 2023; Pati and Arnold, 2020; Jansen et al., 2023; Yang et al., 2023). They have also been detected in the indoor environment, where QACs can adsorb onto hard surfaces after QAC-containing products are applied (Li et al., 2020). Our previous research has shown that QACs are ubiquitous in house dust and suggests that disinfecting products are a significant source of QACs in homes (Zheng et al., 2020b).

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There is a growing concern about the toxicity of QACs. QACs have been found in human blood, breast milk, urine, and feces (Hrubec et al., 2021; Zheng et al., 2021, 2022; Li and Kannan, 2024; Belova et al., 2024). However, there is limited data on the health effects of QACs. The existing studies primarily focus on the effects of occupational exposure in healthcare workers and cleaning staff, and have linked these exposures to exacerbation of asthma-related symptoms (Dumas et al., 2019; LaKind and Goodman, 2019; Walters et al., 2018). Recent studies have shown that QACs in human blood are associated with changes in health biomarkers including an increase in inflammatory cytokines (TNF α , IL-6, and IL-10), decreased mitochondrial function, and disruption of cholesterol homeostasis (zymosterol, 7-dehydrocholesterol, desmosterol, and lanosterol) in a dose-dependent manner (Hrubec et al., 2021). Animal studies have demonstrated that exposure to BACs and DADMACs impairs embryo development, causes neural tube defects, increases mouse embryonic death, and reduces pup body weight (Hostetler et al., 2021; Hrubec et al., 2017). Moreover, QACs disrupt human oligodendrocyte development, acting as developmental toxicants (Cohn et al., 2024). Because of these concerns, the Environmental Influence on Child Health Outcomes (ECHO) program has added QACs to the list of high-priority pollutants for bio-monitoring in children (Pellizzari et al., 2019).

Daycares and schools frequently clean and disinfect indoor surfaces and items to prevent the spread of illness (Frantz, 2023). Recent research indicates that school staff are often not instructed how to safely use disinfecting products and are concerned about student involvement in disinfection, inadequate ventilation, surface contact time, and potential health effects of these products (Hilbert et al., 2021). The improper use of disinfectants may pose adverse health risks, especially for children who spend a significant amount of their time in daycare or school, as children may have long-term consequences of early-life environmental exposures due to their rapid growth and physiologic development (Makri et al., 2004; Suk et al., 2003; Zhao et al., 2024). This emphasizes the importance of assessing QAC exposure in environments where children spend a significant amount of their time.

In this study, for the first time, we quantified 19 QACs, including seven BACs, six DADMACs, and six ATMACs, in indoor dust samples from childcare centers in the United States. We focused on indoor dust because it has been identified as a significant source of chemical exposures for children due to increased hand-to-mouth activity and normal play close to the ground where dust accumulates (Rudel et al., 2003). The objectives of this study are to: (1) determine QAC concentrations and distribution patterns in dust from childcare facilities; (2) compare QAC levels in childcare dust with those previously reported for other microenvironments; (3) estimate daily intake of QACs by toddlers in childcare.

2. Materials and methods

2.1. Sample collection and analysis

A total of 20 dust samples were collected from eight childcare centers in two cities of the United States: 14 samples from seven childcare centers in Seattle, Washington ($n = 14$) in 2016–2017 and six samples from one childcare center in West Lafayette, Indiana ($n = 6$, across six rooms) in 2016. The childcare facilities included a variety of building types, such as multi-classroom centers, a repurposed home, and a church building. The samples were collected using a nylon collection sock inserted in a vacuum cleaner as reported previously (Stubbings et al., 2018). Settled dust from elevated surfaces was collected along with floor dust because the centers vacuumed and mopped floors almost daily. Shelving and the tops of bookcases and storage cubbies made up most of the elevated surfaces.

A detailed description of sample pretreatment procedures has been published in our previous work (Zheng et al., 2020b). Briefly, ~100 mg of dust was sieved using a 500 μ m mesh size sieve to remove small debris and other coarse particles, transferred to a polypropylene tube, spiked with 50 ng of a surrogate standard (d_7 -C12-BAC), sonicated in 4 mL of acetonitrile for 1 hour and centrifuged at 3000 rpm for 5 min. The

supernatant was transferred to a clean tube, and the residues were extracted twice with 4 mL of acetonitrile. The combined extracts were concentrated to 1 mL using nitrogen gas and spiked with an internal standard (d_7 -C14-BAC) before the instrumental analysis.

2.2. Instrumental analysis

An ultra-performance liquid chromatograph interfaced with a triple-quadrupole mass spectrometer (Agilent 1290 Infinity II UPLC–6470 QQQ-MS) was used to analyze the samples. The mass spectrometer was equipped with a TurbolonSpray electrospray ionization (ESI) probe, which operated in positive ion mode for the analysis. An Acquity UPLC BEH C₁₈ column (1.7 μ m, 50 mm \times 2.1 mm, Waters, Milford, MA) was used for chromatographic separation. The column was maintained at 30 °C. A mobile phase of (A) water and (B) acetonitrile, both containing 0.1 % formic acid at a flow rate of 0.4 mL/min, was used to separate analytes. The gradient (linear) was programmed as follows: 0–0.5 min, 10 % B; 0.5–6 min, 100 % B; 6–10 min, 100 % B; 10–10.5 min, 10 % B; 10.5–14 min, 10 % B. A 5 μ L aliquot was injected into the LC system. Nitrogen (99.999 %) was used as a nebulizer gas and set at a pressure of 25 psi. The gas flow, gas temperature, capillary voltage, sheath gas temperature, and sheath gas flow were set at 10 L/min, 300 °C, 3500 V, 350 °C, and 12 L/min, respectively. The MS/MS data were acquired in multiple reaction monitoring (MRM) mode. Individual MS/MS parameters for each analyte are presented in Table S1.

2.3. Quality assurance and quality control

All glassware was baked at 400 °C for 4 h to reduce the potential contamination during the sample pretreatment. A procedural blank control was processed along with every batch of 6 samples to determine the background pollution level. Six spiked samples were extracted along the samples and the recoveries were evaluated by fortifying target analytes (50 ng of each) onto Ottawa sand. The absolute recoveries (mean \pm standard error) for BACs, DADMACs, and ATMACs were 113 ± 5 , 117 ± 3 , and 110 ± 4 %, respectively (Table S2). The recovery of the surrogate standard d_7 -C12-BAC was 118 ± 4 %. Method detection limits (MDLs) were defined as three times the standard deviation of the target analyte found in blank samples. For compounds not detected in blanks, MDLs were based on a signal-to-noise ratio of three. Blank levels and MDLs are included in Table S3.

2.4. Exposure assessment

Estimated daily intakes (EDIs, ng/kg body weight [bw]/day) via dust ingestion were calculated using Eq. (1):

$$EDI = \frac{(C_{dust} \times I_{rate}) \times T}{bw \times 1000} \quad (1)$$

where C_{dust} is the concentration of an analyte in dust (μ g/g), I_{rate} is the ingestion rate (0.1 and 0.2 g/day for the average and high dust intake scenarios, respectively) (EPA, 2011a), T is the time spent in the childcare environment (10 h) (Stubbings et al., 2018), and bw is the mean body weight (12 kg) (EPA, 2011b).

2.5. Data analysis

Statistical analysis was performed using IBM SPSS (ver. 24.0). All concentrations were blank corrected by subtracting average blank levels from sample levels. Concentrations below method detection limits (MDLs) were replaced with MDL/2 values. Pearson correlation analysis was used to investigate the associations among QAC concentrations (logarithmically transformed) in dust and an ANOVA test was used for the comparison of concentrations. The statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Dust concentrations

Table 1 shows the detection frequencies, minimum, median, 75th, 95th percentile, and maximum QAC concentrations in dust. QACs were detected in at least 95 % of the samples. The total QAC concentrations (Σ QAC, the sum of 19 QAC concentrations) ranged from 6.68 to 2650 $\mu\text{g/g}$ (median 150 $\mu\text{g/g}$). BACs were the most abundant QACs in these samples (median Σ BAC [the sum of 7 BAC concentrations] 90.4 $\mu\text{g/g}$) and constituted 64 % of the Σ QAC concentrations. DADMACs were the second dominant QAC group measured at a median Σ DADMAC concentration (the sum of 6 DADMACs) of 41.0 $\mu\text{g/g}$ and comprised 29 % of the Σ QAC concentrations. ATMACs were detected at relatively lower concentrations (median Σ ATMAC [the sum of 6 ATMACs] 9.54 $\mu\text{g/g}$) and comprised 7 % of the Σ QAC concentrations. C12- and C14-BACs were the most abundant QACs and contributed 30 and 24.8 % to the Σ QAC concentrations, respectively. These two BACs are generally the main ingredients in disinfecting products and the high levels found in dust may be due to disinfectant use in daycares. Figure S1 shows the Σ QAC concentrations in the childcare centers included in this study. Interestingly, centers 4 and 7 had the highest levels (1370 and 1000 $\mu\text{g/g}$, respectively). These concentrations were up to two orders of magnitude higher than those detected in the other centers in this study (8.90–629 $\mu\text{g/g}$). No significant seasonal variations were found between the QAC concentrations in samples collected in summer vs. winter (Figure S2).

The median Σ QAC concentrations were up to 4 orders of magnitude higher than those of organophosphate esters (median 10.5 $\mu\text{g/g}$) (Stubbings et al., 2018), brominated flame retardants (median 4.40 $\mu\text{g/g}$) (Stubbings et al., 2018), melamine and its derivatives (median 3.21 $\mu\text{g/g}$) (Zheng et al., 2020a) and per- and polyfluoroalkyl substances (median 0.270 $\mu\text{g/g}$) (Zheng et al., 2019) analyzed previously in these dust samples (Figure S3). However, the levels reported here were lower than those for phthalate esters (\sim 500 $\mu\text{g/g}$) found in daycare centers elsewhere (Fromme et al., 2016; Gaspar et al., 2014). These results further indicate the extent of QAC contamination in these childcare centers.

Table 1

Detection frequencies (DF, %), minimum (min), median, 75th, 95th percentile, and maximum (max) concentrations ($\mu\text{g/g}$) of QACs in dust collected from childcare centers. The contribution (Contr, %) of each QAC to the Σ QAC concentration (based on median concentrations) is also included

Compound	DF	Min	Median	75th	95th	Max	Contr
BACs							
C6-BAC	95	<MDL	0.363	0.026	4.34	4.34	0.01
C8-BAC	100	0.003	0.270	1.52	24.4	24.7	0.20
C10-BAC	100	0.002	0.174	2.04	21.3	21.6	0.13
C12-BAC	100	0.588	40.9	184	709	721	30.0
C14-BAC	100	0.882	33.8	138	389	391	24.8
C16-BAC	100	0.752	9.26	46.9	163	165	6.78
C18-BAC	100	0.434	2.79	8.08	45.3	45.5	2.05
Σ BAC		2.67	90.4	368	1350	1370	64.0
DADMACs							
C8-DADMAC	100	0.21	15.0	111	276	279	11.0
C10-DADMAC	100	0.394	13.7	120	791	805	10.1
C12-DADMAC	100	0.014	0.0684	0.209	7.79	7.93	0.05
C14-DADMAC	100	0.002	0.0175	0.104	5.98	6.11	0.01
C16-DADMAC	100	0.114	0.483	0.825	8.25	8.38	0.35
C18-DADMAC	100	1.56	14.3	25.4	102	104	10.5
Σ DADMAC		3.22	41.0	279	1010	1020	29.0
ATMACs							
C8-ATMAC	100	0.002	0.0842	1.25	34.0	34.7	0.06
C10-ATMAC	100	0.012	0.430	6.55	193	196	0.32
C12-ATMAC	100	0.033	0.797	2.98	11.0	11.1	0.58
C14-ATMAC	100	0.013	0.210	0.441	1.50	1.52	0.15
C16-ATMAC	100	0.413	2.21	3.69	112	117	1.62
C18-ATMAC	100	0.271	1.92	3.37	15.6	15.9	1.41
Σ ATMAC		0.788	9.54	19.9	255	260	7.00
Σ QAC		6.68	150	660	2610	2650	100

3.2. Correlations

Cluster analyses of the dust QAC data shows that all BACs, DADMACs, and ATMACs are clustered together, except C14- and C16-ATMACs (Figure S4). Specifically, concentrations of C10–C16 BACs, C8–C12 ATMACs, C8- and C10-DADMACs were strongly correlated (r : 0.53–0.99; $p < 0.05$), indicating common sources for these compounds. Previous studies suggest that the use of disinfecting products could be a potential source of QACs indoors (Arnold et al., 2023; Zheng et al., 2020b), providing a potential explanation for the significant correlations between certain BACs, DADMACs, and ATMACs. In addition, concentrations of C12-, C14-, C16-, and C18-DADMACs were significantly correlated with each other (r : 0.95–0.99; $p < 0.001$), even though they were minor contributors to the Σ QAC concentrations. DADMACs are used as active ingredients in fabric softeners due to their excellent antistatic properties (Arnold et al., 2023; Hora et al., 2020), which could potentially explain this clustering behavior. Interestingly, C14- and C16-ATMACs were significantly correlated with each other but not with the rest of the QACs. This could be due to the different partitioning behavior of these two QACs, as they are relatively more volatile than the rest of the QACs targeted here (Zheng et al., 2021). In addition, ATMACs are used as primary ingredients in stabilizers and preservatives present in various consumer products, such as air fresheners and hair care products, distinguishing them from the rest of the QAC analytes (Arnold et al., 2023).

3.3. Comparison with household dust

The Σ QAC dust concentrations in childcare centers were up to 4 times higher than those previously reported for the same QACs in house dust from the United States (Zheng et al., 2020b) collected before and after the COVID-19 pandemic (sampling years 2016 and 2020; medians 36.3 and 58.9 $\mu\text{g/g}$, respectively; $p < 0.05$) (Fig. 1). This can be attributed to the extensive use of the QAC-containing professional cleaners and sanitizers in childcare settings (Arnold et al., 2023; Holm et al., 2019; Dewey et al., 2022). The distribution of the three QAC groups in childcare dust was consistent with that found in household dust (64, 29 and 7 % vs. 56, 26, and 18 % for BACs, DADMACs, and ATMACs,

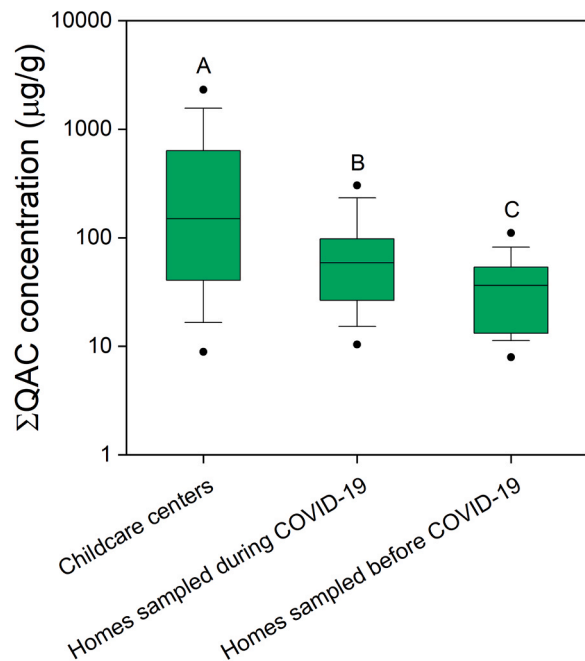


Fig. 1. The Σ QAC concentrations ($\mu\text{g/g}$) in dust collected from the childcare centers ($n = 20$) in this study and from homes sampled during ($n = 40$) and before the COVID-19 pandemic ($n = 21$) from our previous study (Zheng et al., 2020b). Concentrations are shown as box plots, representing the 25th and 75th percentiles; black lines represent the median, and whiskers represent the 10th and 90th percentiles. The letters represent the results of the one-way analysis of variance (ANOVA); the concentrations are ranked from the highest to lowest in alphabetic order, and concentrations marked with different letters are statistically different at $p < 0.05$.

respectively) (Zheng et al., 2020b), indicating common sources of QACs in both childcare and residential environments (Fig. 2). Moreover, QAC concentrations in childcare centers were 3–7 times higher than those in public settings in South China (Cheng et al., 2024) and 10 times higher than those reported in house dust from Belgium (Belova et al., 2023).

3.4. Exposure assessment

We assessed estimated daily intake (EDI) of QACs for toddlers in childcare settings through accidental dust ingestion considering two scenarios: the average dust intake (0.1 g/day) and high dust intake (0.2 g/day) (Table 2). The Σ QAC EDIs were 519 and 1040 ng/kg bw/day for the average and high dust intake scenarios, respectively, which was one order of magnitude greater than those estimated for adults in residential homes before and after the outbreak of the COVID-19 pandemic (17.5 and 38 ng/kg/d, respectively) (Figure S5) (Zheng et al., 2020b). The Σ QAC EDI via dust ingestion estimated here was 18100 ng/kg bw/day when considering the 95th percentile concentration and the high dust intake scenario. Although children spend only part of their day in childcare (up to 10 h/day) (Stubbings et al., 2018), the highest Σ QAC EDI for toddlers in daycare exceeded that estimated for toddlers in residential homes during the COVID-19 pandemic (444 ng/kg/d) by up to 40 times (Figure S5) (Zheng et al., 2020b). Particularly, the 95th percentile EDIs for BACs, DADMACs, and ATMACs under the high dust intake scenario were 9380, 7010, and 1770 ng/kg bw/day, respectively, up to 30 times higher than those for toddlers in residential homes during the COVID-19 pandemic reported in our previous study (Zheng et al., 2020b). EDIs based on the median concentrations for C12- and C14-BACs were 142 and 117 ng/kg bw/day for the low dust intake scenario and 284 and 235 ng/kg bw/day for the high dust intake scenario, respectively. These EDIs were two orders of magnitude lower than the tolerable daily intake of C10-DADMAC and

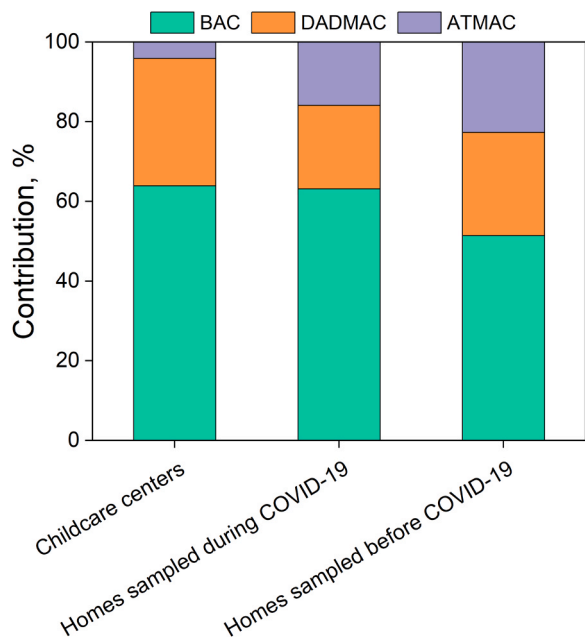


Fig. 2. Contribution of each QAC to the Σ QAC concentrations in dust collected from the childcare centers ($n = 20$) in this study and from homes sampled during ($n = 40$) and before the COVID-19 pandemic ($n = 21$) from our previous study (Zheng et al., 2020b).

Table 2

Estimated Daily Intake (EDI, ng/kg bw/day) of QACs via dust ingestion based on the median and 95th percentile concentrations for the average (dust intake of 0.1 g/day) and high (dust intake of 0.2 g/day) exposure scenarios

	Average dust intake		High dust intake	
	Median	95th	Median	95th
BACs				
C6-BAC	0.0294	15.1	0.0588	30.1
C8-BAC	0.938	84.7	1.88	169
C10-BAC	0.603	74.0	1.21	148
C12-BAC	142	2460	284	4920
C14-BAC	117	1350	235	2700
C16-BAC	32.1	566	64.3	1130
C18-BAC	9.69	157	19.4	315
ΣBAC	314	4690	628	9380
DADMACs				
C8-DADMAC	52.1	958	104	1920
C10-DADMAC	47.7	2750	95.5	5490
C12-DADMAC	0.238	27.0	0.475	54.1
C14-DADMAC	0.0607	20.8	0.121	41.5
C16-DADMAC	1.68	28.6	3.35	57.3
C18-DADMAC	49.7	354	99.4	708
ΣDADMAC	142	3510	285	7010
ATMACs				
C8-ATMAC	0.292	118	0.585	236
C10-ATMAC	1.49	670	2.99	1340
C12-ATMAC	2.77	38.2	5.54	76.4
C14-ATMAC	0.730	5.21	1.46	10.4
C16-ATMAC	7.67	389	15.3	778
C18-ATMAC	6.66	54.2	13.3	108
ΣATMAC	33.1	885	66.2	1770
ΣQAC	519	9060	1040	18100

C8-C18 BACs [1×10^5 ng/kg bw/day for both compounds] recommended by the European Food Safety Authority (EFSA) (European Food Safety Authority, 2014). However, tolerable daily intake thresholds are generally established based on acute toxicity studies and do not account for the risks of long-term low-dose chronic exposure (EPA, 2017). This is particularly alarming as C12- and C14-BACs are the primary active ingredients in many disinfecting products that are commonly used in

residential and public spaces.

Overall, these findings warrant further research on exposure to QACs in childcare facilities, along with action by childcare providers and government agencies to reduce exposure. Given the availability of safer alternatives and a United States Environmental Protection Agency certification for safer disinfectants, childcare providers can easily avoid the use of QACs while continuing to appropriately clean, sanitize, and disinfect. Government agencies can reduce exposure by mandating ingredient transparency for products, restricting the use of these chemicals in cleaning and disinfecting products, and by providing guidelines on safer practices and products.

4. Conclusions

This is the first study to report the occurrence of QACs in childcare settings. While our study has a limited sample size and the results cannot be generalized to all United States childcare facilities, our findings indicate that QACs are widespread in the childcare environment at levels greater than those in homes, resulting in significant early-life exposures. Considering the crucial developmental stages and the significant amount of time children spend in childcare, high exposure to QACs is of concern. Large scale exposure assessment studies and longitudinal health studies focused on QACs and their impacts on health are needed to understand the effects from acute versus chronic exposure, especially for susceptible populations such as children, pregnant women, and older adults. Childcare facilities can reduce exposure of children to QACs by choosing safer cleaning and disinfecting strategies and products, following guidelines such as those issued by Washington State Department of Health (Washington State, 2023) to disinfect only when needed and to use products with safer ingredients.

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CRedit authorship contribution statement

Yao Cheng: Writing – original draft, Visualization, Software, Methodology. **Zhong Lv:** Data curation. **Erika Schreder:** Writing – review & editing, Resources, Investigation, Conceptualization. **Min Hu:** Data curation. **Abby Mutic:** Writing – review & editing. **Guomao Zheng:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Amina Salamova:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.hazl.2024.100138](https://doi.org/10.1016/j.hazl.2024.100138).

Data availability

Data will be made available on request.

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