



Exposure to melamine and its derivatives in childcare facilities

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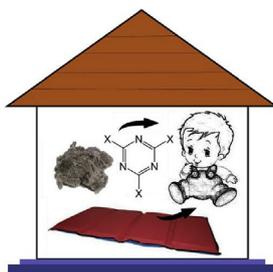
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HIGHLIGHTS

- Eleven melamine-based chemicals were measured in dust and nap mats from U.S. childcares.
- Dust concentrations were dominated by melamine, cyanuric acid, ammeline, and ammelide.
- Melamine concentrations in polyester mat covers were significantly higher than those in polyurethane mat foam.

GRAPHICAL ABSTRACT



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ABSTRACT

Melamine (MEL) and its derivatives are widely used in many consumer products, including furniture, kitchenware, and plastics. However, very limited knowledge exists on human exposure to MEL and its derivatives, especially in the indoor environment. Here, we determined the occurrence and distribution of 11 MEL derivatives in childcare facilities and estimated children's exposure through dust ingestion and dermal absorption. We analyzed dust and samples of nap mats, a commonly used item in many childcares, from eight facilities located in the United States. Eight MEL-based compounds were detected in dust, and total MEL concentrations ranged from 429 to 117,000 ng/g. The most abundant compounds found in the dust samples were MEL, cyanuric acid (CYA), ammeline (AMN), and ammelide (AMD), with median concentrations of 1620, 585, 1060, and 299 ng/g, respectively. MEL, CYA, AMN and 2,4,6-tris[bis(methoxymethyl)amino]-1,3,5-triazine (TBMMAT) were also detected in nap mats with median concentrations of 45.6, 19.8, 1510 and 2.5 ng/g, respectively. Σ MEL concentrations in mat covers (median 709 ng/g) were significantly higher than those in mat foam (median 15.1 ng/g). Estimated daily intakes (EDIs) of MEL and its derivatives via dust ingestion were two orders of magnitude higher than the EDIs through dermal absorption, but both were below the established tolerable daily intake levels. This is the first report on exposure to MEL and its derivatives in the childcare environment.

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1. Introduction

Melamine (MEL) drew worldwide attention following several food scandals when it was added to infant formula and pet food, which resulted in kidney damage, and in some cases, renal failure and the death of infants and pets (Dobson et al., 2008; Chen, 2009;

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Gossner et al., 2009). These food scandals led to a global focus on MEL occurrence in food, including infant formula and dairy (Cohen, 2008; Tittlemier et al., 2009; Filazi et al., 2012; Zhu and Kannan, 2018a), and the establishment of safety limits for MEL at 1.0–2.5 mg/kg in infant formula and dairy products by the World Health Organization (WHO, 2008).

However, in addition to food adulteration, MEL-based chemicals have also been used, and perhaps on a larger scale, in many commercial applications since the late 1930s (Lu et al., 2009). MEL and its derivative and metabolite, cyanuric acid (CYA), are high production volume chemicals with annual production volumes reaching 120,000 tons each in the United States (Chemview, 2016). MEL is used in the manufacturing of polymer resins and polymeric agents, such as laminates, coatings, polyamides, plastics, kitchenware, and dinnerware (An et al., 2017; Ritota and Manzi, 2018). It is also considered an excellent flame retardant because of its ability to interfere with combustion in all fire stages and in different ways (Ritota and Manzi, 2018). As a flame retardant, it is used in plastic, polyurethane foam, and paper (Thirumal et al., 2010; van der Veen and de Boer, 2012). In addition, MEL-based fibers are used in flame-resistant protective clothing due to their low thermal conductivity, high flame resistance, and self-extinguishing properties (Weil and Levchik, 2008). CYA is used along with MEL in many applications, as well as on its own as a disinfectant, chlorine stabilizer, sanitizer, and bleach agent (Cantu et al., 2000). Two other hydroxylated MEL analogues, ammeline (AMN) and ammelide (AMD), are impurities in the manufacturing of MEL (Rovina and Siddiquee, 2015). Some other compounds in the MEL family include: 2,4,6-tris[bis(methoxymethyl)amino]-1,3,5-triazine (TBMMAT), used as a cross-linking agent in coatings for cans, coils, automobiles, and nanoparticles for casting (U.S. production volume 23,000 tons/year) (Weiss, 1997; Weber et al., 2009); tris(3,5-di-*tert*-butyl-4-hydroxybenzyl) isocyanurate (TDTBHI), used as a plastic additive to improve flexibility and resistance to heat with a U.S. production volume of 460–4600 tons/year (Moreta and Tena, 2015; Chemview, 2016); 2,4-diamino-6-phenyl-1,3,5-triazine (DPT), used to increase the flexibility of MEL-urea-formaldehyde resins; 2,4,6-triallyloxy-1,3,5-triazine (TALT) and 1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TTT), used as cross-linking agents to improve the adhesion during the thermal copolymerization and lamination processes (Ang et al., 2000; Xin et al., 2017); and 2-amino-4-methoxy-6-methyl-1,3,5-triazine (AMMT), previously used as an herbicide and currently applied in production of fast-curing adhesive coatings (Holger Thiede, 2018). MEL, CYA, AMN, and AMD have been found in soil, water, sediment, and sludge (Qin et al., 2010; Zhu and Kannan, 2018b; Zhu et al., 2019a, Zhu et al., 2019b, Zhu et al., 2019c). TBMMAT has been detected in road runoff, urban creeks, and rivers (de Hoogh et al., 2006; Schwarzbauer and Ricking, 2010; Seitz and Winzenbacher, 2017; Peter et al., 2018). Limited information is available for the occurrence of other MEL-based chemicals.

There is limited knowledge on human exposure to MEL-based compounds. MEL was detected in urine of people who consumed MEL-adulterated food (Wu et al., 2010; Zhang et al., 2010; Liu et al., 2011; Panuwet et al., 2012; Lin et al., 2013). Recent studies found MEL and CYA in adult's (Wu et al., 2010; Zhu and Kannan, 2019c) and children's urine (Sathyanarayana et al., 2019), and breast milk (Zhu and Kannan, 2019b). The urinary levels of MEL and CYA in children were higher than those in adults, indicating higher exposure in children (Sathyanarayana et al., 2019; Zhu and Kannan, 2019c).

In air, MEL and its derivatives are likely to absorb to particulates due to their high octanol – air partition coefficients (log K_{oa} : 6.00–39.6; Table S1). A recent study reported MEL and three of its derivatives were frequently detected in house dust collected from

around the world (Zhu and Kannan, 2018b), suggesting household sources of these chemicals. This study found the highest levels of MEL in dust collected from U.S. homes (median 17,000 ng/g) (Zhu and Kannan, 2018b).

MEL and CYA alone have low acute toxicities; however, when combined, these two chemicals can form insoluble crystals in kidneys and induce renal damage and failure (Puschner et al., 2007; Filigenzi et al., 2008; Chang et al., 2014). In addition, MEL and CYA have been shown to induce neurological and reproductive toxicity in rats (An et al., 2011; Yang et al., 2012), and low-dose exposure to MEL has been associated with the increased risk of urolithiasis (Liu et al., 2011; Chu et al., 2018). Additionally, a recent study reported an association between MEL and CYA levels in children's urine with increased urinary markers of kidney injury (Sathyanarayana et al., 2019). However, understanding of the extent of exposure to MEL and related compounds remains very limited, especially among vulnerable populations.

The objective of this study was to determine the occurrence and distribution of MEL and its derivatives in the childcare environment. Children spend a significant amount of time in childcare (sometimes up to 10 h per day), and it is important to elucidate environmental exposures in this indoor environment. In this study, we analyzed dust and nap mats, a commonly used item in many childcare facilities, collected from eight childcare centers in the U.S. for eleven MEL derivatives in order to determine the occurrence and distribution of these compounds in childcares and estimate children's exposure through dust ingestion and dermal absorption.

2. Materials and methods

Sample collection. Floor and elevated surface dust samples were collected from seven childcare centers in Seattle, Washington ($n = 14$) and one childcare center in West Lafayette, Indiana ($n = 6$; across six rooms) in 2016. The building types of the recruited childcares included multiple classrooms, a former home, and a former church. The samples were collected using a pre-cleaned nylon collection sock inserted in a vacuum cleaner. Dust from elevated surfaces was collected along with floor dust (in the same sample) in order to collect enough sample because all centers vacuumed and mopped floors almost daily. Elevated surfaces consisted almost entirely of shelving and the tops of bookcases/storage cubbies. All collected samples were wrapped in aluminum foil, sealed, labeled, and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. In addition, twenty-six nap mat samples (a small piece randomly cut from a mat) were collected from the seven Seattle childcare centers, including polyurethane foam ($n = 20$) and vinyl and polyester cover ($n = 6$) samples.

Sample analysis. Samples of mats and dust were analyzed for eleven MEL derivatives as follows: ~ 100 mg of finely cut mat material or weighed dust sieved through a $500\text{ }\mu\text{m}$ mesh size sieve, was placed in a 15 mL polypropylene tube and spiked with surrogate standards ($^{13}\text{C}_3$ -MEL and $^{13}\text{C}_3$ - $^{15}\text{N}_3$ -CYA). The samples were equilibrated for 4 h at room temperature and then sonicated in 4 mL of methanol for 1 h. The mixture was centrifuged at 3000 rpm for 5 min, the supernatant was transferred to a clean tube, and the extraction was repeated twice with 4 mL methanol. Combined extracts were concentrated to dryness with nitrogen blow down, reconstituted in 500 μL acetonitrile with 5 mM ammonium formate buffer (95:5, v/v, pH = 4.0), filtered through a $0.2\text{ }\mu\text{m}$ nylon syringe filter, and spiked with internal standards ($^{15}\text{N}_3$ -MEL and $^{13}\text{C}_3$ -CYA).

Instrumental analysis. Both dust and mat samples were analyzed using ultra-performance liquid chromatograph coupled with a triple-quadrupole mass spectrometer (Agilent 1290 Infinity II UPLC – 6470 QQQ-MS) in the positive electrospray ionization (ESI+) and negative electrospray ionization (ESI-) modes for eleven

compounds: melamine (MEL), cyanuric acid (CYA), ammeline (AMN), ammelide (AMD), 2,4-diamino-6-phenyl-1,3,5-triazine (DPT), 2,4,6-tri-allyloxy-1,3,5-triazine (TALT), 1,3,5-tri-allyl-1,3,5-triazine-2,4,6-(1H,3H, 5H)-trione (TTT), 2,4,6-tris[bis(methoxymethyl)amino]-1,3,5-triazine (TBMMAT), 2-amino-4-methoxy-6-methyl-1,3,5-triazine (AMMT), 1,3,5-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)-s-triazine-2,4,6-(1H,3H, 5H)-trione (TDTBHI), and dichloroisocyanuric acid (DICASS). Fig. S1 shows chemical structures of the analytes. Chromatographic separation for MEL, CYA, AMN, and AMD was achieved on an Acquity UPLC HILIC column (50 mm, 2.1 mm i.d., 1.7 μ m thickness, Waters, Milford, MA). Column temperature was set at 40 °C, and ammonium formate buffer (A) (5 mM, adjusted to pH 4.0 using formic acid) and acetonitrile (B) were used as the mobile phase. The flow rate was 0.4 mL/min. The applied gradient was as follows: 0 min, 95% B; 5.6 min, 65% B; 9 min, 65% B; 9.1 min, 95% B; 15.1 min, 95% B. The instrument was equilibrated for 4 min after every run, and the nebulizer, gas flow, gas temperature, capillary voltage, sheath gas temperature, and sheath gas flow were set as 20 psi, 11 L/min, 260 °C, 3500 V, 300 °C, and 12 L/min, respectively. The injection volume was 2 μ L.

For the rest of the target analytes, chromatographic separation was done on an Acquity UPLC BEH C18 column (50 mm, 2.1 mm i.d., 1.7 μ m thickness, Waters, Milford, MA) with the column temperature set at 40 °C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B), and the flow rate was 0.4 mL/min. The applied gradient was as follows: 0 min, 30% B; 0.5 min, 30% B; 3.5 min, 70% B; 8 min, 74% B; 14 min, 100% B; 15.5 min, 100% B. The instrument was equilibrated for 4 min after every run, and the nebulizer, gas flow, gas temperature, capillary voltage, sheath gas temperature, and sheath gas flow were set as 40 psi, 10 L/min, 260 °C, 3500 V, 375 °C, and 12 L/min, respectively. The injection volume was 5 μ L. Multiple reaction monitoring (MRM) mode was used for data acquisition. The optimized MRM transitions, fragmentors, and collision energies are summarized in Table S2.

Quality assurance and quality control. Absolute matrix spike recoveries ranged from 54 to 133%. Surrogate standard recoveries were $111 \pm 8\%$ (mean \pm standard error) and $81 \pm 14\%$ for $^{13}\text{C}_3$ -MEL and $^{13}\text{C}_3$ - $^{15}\text{N}_3$ -CYA, respectively. Overall, blank levels constituted less than 1% of the levels in dust and mat samples. Blank concentrations were subtracted from all analyte concentrations. Method detection limits (MDLs) were set as three times the standard deviation of the target analyte levels detected in the blanks. For compounds not detected in the blanks, MDLs were based on a signal-to-noise ratio of three and ranged from 0.01 to 2.4 ng/g. Reproducibility of the analysis was achieved through the analysis of duplicate samples and relative standard deviations of MEL-based compounds in duplicate samples ranged from 5 to 18% ($n = 8$).

Data analysis. The estimated daily intakes (EDIs) of MEL and its derivatives via dust ingestion and dermal absorption were calculated based on median concentrations measured in dust using the average time spent in childcares (10 h), estimated children's dust ingestion rate and body surface area, the portion of dust adhered to skin, and the fraction of a contaminant absorbed by skin (EPA., 2011).

EDIs via dust ingestion were calculated using the eq (1):

$$\text{Dust ingestion EDI (ng/kg/d)} = \frac{(C_{\text{dust}} \times I_{\text{rate}}) \times T}{bw} \quad (1)$$

where C_{dust} is the concentration of an analyte in dust (ng/g), I_{rate} is the ingestion rate (0.06 g/day) (EPA., 2011), T is the time spent in the childcare environment (10 h) (Stubbings et al., 2018), and bw is the mean body weight (12 kg) (Stubbings et al., 2018).

EDIs via dermal absorption were calculated using the eq (2):

Dermal absorption EDI (ng/kg/d)

$$= \frac{(C_{\text{dust}} \times BSA \times DAS \times FA) \times T}{bw} \quad (2)$$

where C_{dust} is the concentration of an analyte in dust (ng/g), BSA is the exposed body surface area (2564 cm²) (EPA., 2011), DAS is the dust adhered to skin (0.01 mg/cm²) (EPA., 2011), and FA is the fraction of a contaminant absorbed by skin (0.007, unitless) (EPA., 2011), T is the time spent in the childcare environment (assumed to be 10 h) (Stubbings et al., 2018), and bw is the mean body weight (12 kg) (Stubbings et al., 2018).

Risks from MEL and CYA exposure were calculated as hazard quotients (HQ) using the eq. (3):

$$HQ = \frac{EDI}{TDI} \quad (3)$$

where TDI is the tolerable daily intake of MEL (3150 ng/kg/d) and CYA (2500 ng/kg/d) (Panuwet et al., 2010).

Basic and descriptive statistics were calculated using IBM SPSS Statistics 24, and Microsoft Excel 2016 software. Plots were generated using SigmaPlot 13 (Systat Software Inc.). Analysis of variance (ANOVA) was performed using log-transformed concentrations in Minitab 18. The letters representing the results of ANOVA indicate whether the concentrations are significantly different among groups. The concentrations sharing the same letter are not significantly different at $p < 0.05$.

3. Results and discussion

Table 1 shows the minimum, maximum, median, and mean concentrations (with their standard errors) for the 8 MEL derivatives (concentrations for TALT represent TALT + TTT values because these two compounds are isomers and their peaks were not chromatographically separated) measured in dust and nap mats. The complete concentration dataset for all the samples analyzed in this study is provided in Tables S3 and S4. DICASS, TDTBHI, and AMMT were not detected in any dust samples, and AMD, DPT, TALT, DICASS, TDTBHI, and AMMT were not detected in any mat samples, and are not included in the discussion. Total MEL concentrations (Σ MEL) represent the sum of the MEL derivatives included in Table 1 for dust and mats, respectively. Fig. 1 shows concentrations of MEL derivatives detected in dust (A) and mats (B) as box plots, and the letters included in the plots represent the results of the one-way analysis of variance (ANOVA) of log-

Table 1

Concentrations of MEL and its derivatives in dust and nap mats (ng/g) collected from the U.S. childcare centers. MDL: method detection limit; DF: detection frequency.

Compound	DF, %	Min	Max	Mean \pm SE	Median
Dust (N = 20)					
MEL	100%	159	66,600	6920 \pm 3470	1620
CYA	100%	93.4	1860	680 \pm 107	585
AMN	100%	87.6	48,500	5000 \pm 2510	1060
AMD	100%	57.3	2000	416 \pm 93.6	299
DPT	100%	4.49	187	60.5 \pm 11.8	46.1
TALT	55%	<MDL	29.8	3.10 \pm 2.67	0.450
TBMMAT	100%	5.19	69.1	22.5 \pm 3.81	16.4
Σ MEL		429	117,000	13,100 \pm 6030	3210
Nap mats (N = 26)					
MEL	85%	<MDL	16,700	1870 \pm 852	45.6
CYA	42%	<MDL	388	54.2 \pm 22.0	19.8
AMN	27%	<MDL	16,600	5460 \pm 1280	1510
TBMMAT	85%	<MDL	2380	241 \pm 131	2.51
Σ MEL		0.150	35,700	3280 \pm 1700	27.2

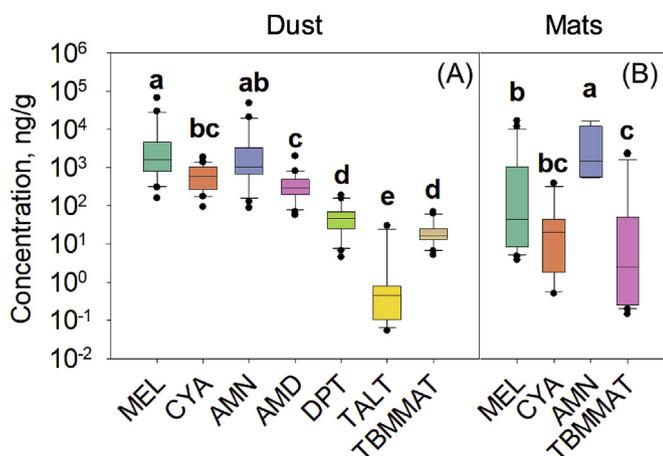


Fig. 1. Concentrations of MEL and its derivatives in dust (A) and nap mats (B) from the U.S. childcare centers (ng/g). Concentrations are shown as boxplots representing the 25th and 75th percentiles; black lines represent the median; and the whiskers represent the 10th and 90th percentiles. The letters represent the results of the analysis of variance (ANOVA); boxes sharing the same letters are not significantly different at $p < 0.05$. The ANOVA was done separately for the concentrations included in plots A and B.

transformed concentrations. The concentrations sharing the same letter are not significantly different at $p < 0.05$.

Concentrations in dust. Six MEL-based compounds were detected in all of the analyzed dust samples, while TALT was detected in 55% of the samples. Σ MEL concentrations ranged from 429 to 117,000 ng/g with a median concentration of 3210 ng/g. These MEL concentrations were an order of magnitude lower than the levels measured in U.S. house dust (median 17,000 ng/g), but higher than the concentrations measured in house dust collected from other countries, including China, Japan, and Korea (range 430–8400 ng/g) (Zhu and Kannan, 2018b). As shown in Fig. 1, MEL was the most abundant compound measured in these dust samples with a median concentration of 1620 ng/g, followed by AMN (median 1060 ng/g), CYA (median 585 ng/g), and AMD (median 299 ng/g). Interestingly, the levels of AMN measured in this study were higher than previously reported in U.S. homes (range 6.2–220 ng/g) (Zhu and Kannan, 2018b). Overall, MEL contributed 45% to Σ MEL concentrations, while AMN, CYA, and AMD contributed 29%, 16%, and 8%, respectively (Fig. 2). This pattern was different from that previously found in U.S. house dust with MEL constituting 79% of the Σ MEL concentrations (Zhu and Kannan, 2018b). This could be related to different products used in the home and childcare environments. Median concentrations of DPT, TALT, and TBMMAT

were 46.1, 0.45, and 16.4 ng/g, respectively, significantly lower than the levels of MEL ($p < 0.05$) based on the ANOVA of the log-transformed concentrations (Fig. 1). These compounds contributed less than 1% to Σ MEL concentrations. This is the first report on the occurrence of DPT, TALT and TBMMAT in indoor dust.

These dust samples were previously analyzed for brominated flame retardants (BFRs, including polybrominated diphenyl ethers [PBDEs]) and organophosphate esters (OPEs) (Stubbings et al., 2018). When compared with the levels of OPEs, BFRs, and PBDEs, median concentrations of Σ MEL were significantly lower ($p < 0.05$) than the levels of OPEs, but indistinguishable from the levels of BFRs and PBDEs in dust (Fig. S2A). These findings indicate that contamination of the indoor environment with MEL and related compounds is comparable to the contamination with widely used flame retardants and warrants further research.

Concentrations in nap mats. Only four out of 11 analyzed MEL-based chemicals were detected in nap mats. MEL and TBMMAT were detected in 85% of nap mats, while CYA and AMN were detected in 42% and 27%, respectively. Although AMN was detected in less than a third of nap mats, it was the most abundant chemical found in mats (both foam and cover) with a median concentration of 1510 ng/g. These concentrations were more than three orders of magnitude higher than MEL and CYA concentrations (median 45.6 and 19.8 ng/g, respectively). This is consistent with high AMN levels found in dust samples, suggesting that in some cases, mats may be a source of AMN in childcares. TBMMAT was found at a median concentration of 2.51 ng/g. Interestingly, high TBMMAT levels were found in two polyurethane-coated polyester cover samples (2380 and 2220 ng/g). Overall, Σ MEL concentrations in mat foam (median 15.0 ng/g) were significantly lower ($p < 0.05$) than those in mat cover (median 709 ng/g) (Table S3 and Fig. S3). Some of the mats analyzed here were new mats, purchased as flame-retardant free products. Comparison of Σ MEL concentrations in the used and new mats showed that Σ MEL concentrations in used foam ($n = 16$; median 12.8 ng/g) were similar to those in new foam ($n = 4$; median 17.2 ng/g) (Fig. S4A). However, the new mat cover ($n = 3$; mean $20,700 \pm 10,700$ ng/g) had Σ MEL concentrations that were up to two orders of magnitude higher than those in the used mat cover ($n = 2$; mean 113 ± 47.9 ng/g) (Fig. S4B), mainly dominated by MEL and AMN (Table S3 and Fig. 2). These new mats were covered by polyurethane-coated polyester (Fang et al., 2011). MEL and its derivatives are used as flame retardants to increase the heat tolerance in polyurethane coatings, and these findings suggest that MEL-based compounds maybe a component of polyester or polyurethane coating in these mats. In addition, MEL is widely used in the synthesis of water-soluble resin for the production of wrinkle-free textiles (Lacasse and Baumann, 2004; Zhang et al., 2008; Mecker et al., 2012; Rovina and Siddiquee, 2015), and in vinyl production as part of textile coatings to increase the stability of coatings (Salaun et al., 2009). The levels of MEL-based compounds in cover samples were higher than those of brominated flame retardants in car seat fabric (median 310 ng/g) (Wu et al., 2019), and those of bisphenols in infant clothing (median 12 ng/g) (Xue et al., 2017), suggesting nap mats can be a potential source of MEL-based contaminants in childcares. This is of concern given the extended time infants and young children spend sleeping (10–14 h per day) on nap mats, crib mattresses, and other bedding products (Boor et al., 2014). Overall, the levels of MEL-based compounds in mats were generally lower than the levels of brominated and organophosphate ester flame retardants measured in the same samples (Fig. S2B) (Stubbings et al., 2018). Σ MEL concentrations in mats (median 27.2 ng/g) were two orders of magnitude lower than those in dust (median 3210 ng/g). In addition, distribution profiles of MEL-based compounds were different in dust and mats (Fig. 2), suggesting other sources of these compounds contributing to high

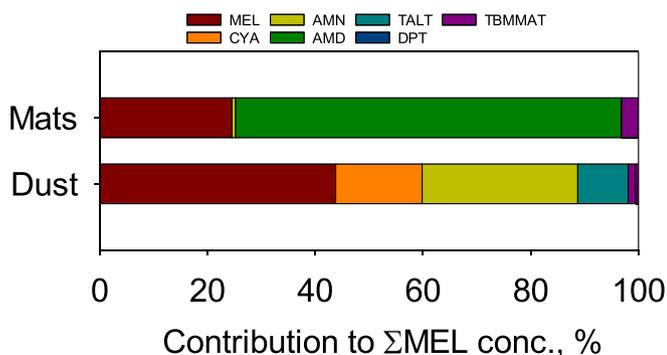


Fig. 2. Contributions of individual MEL-based compounds to dust and mat Σ MEL concentrations (%).

Table 2

Toddlers' estimated daily intakes (EDIs) of MEL and its derivatives through dust ingestion and dermal absorption (ng/kg/day) for the average (based on median dust concentrations) and high (based on the 95th percentile dust concentrations) exposure scenarios in the childcare environment. Hazard quotients (HQs) for MEL and CYA are also included.

Exposure scenario	Dust ingestion EDI		Dermal dust absorption EDI		Combined EDI		HQs	
	Average	95 th perc.	Average	95 th perc.	Average	95 th perc.	Average	95 th perc.
MEL	3.40	66.6	0.041	0.797	3.44	67.4	1.1×10^{-3}	2.1×10^{-2}
CYA	1.23	2.94	0.015	0.035	1.24	2.98	5.0×10^{-4}	1.2×10^{-3}
AMN	2.23	46.6	0.027	0.557	2.25	47.1		
AMD	0.63	1.83	0.008	0.022	0.64	1.85		
DPT	0.10	0.33	0.001	0.004	0.10	0.34		
TBMMAT	0.001	0.03	0.0001	0.0004	0.001	0.03		

concentrations of MEL and related compounds in dust from the childcare environment.

Toxicities of MEL and CYA increase significantly when these two chemicals are ingested at an 1:1 ratio, which can lead to formation of renal crystals (Puschner and Reimschuessel, 2011). The MEL/CYA concentration ratios in dust and mats are included in Figs. S5 and S6. MEL and CYA were simultaneously detected in all dust samples with MEL/CYA concentration ratios ranging from 0.5 to 48 (median 2.9). Only 9 mats had both MEL and CYA at detectable levels, and the relative MEL/CYA concentration ratios ranged from 0.4 to 840 (median 11.2). CYA was not detected in most of mats ($n = 15$) and 2 mats did not contain any MEL, suggesting that nap mats are a minor source of exposure to MEL and CYA and there are possibly other sources of these chemicals in the indoor environment.

Exposure assessment. Table 2 presents children's estimated daily intakes (EDIs) of MEL and its derivatives via dust ingestion and dermal absorption. Median EDIs of MEL and CYA estimated for toddlers through dust ingestion were 3.40 and 1.23 ng/kg/d, respectively, which were 10–30 times lower than those estimated for MEL (80.0 ng/kg/d) and CYA (19.3 ng/kg/d) in the U.S. homes (Zhu and Kannan, 2018b). These lower EDIs can be explained by relatively low concentrations of MEL and CYA found in childcares compared to homes and by the shorter exposure time (10 h) in childcare. The median EDIs of MEL and CYA through dermal absorption (0.041 and 0.015 ng/kg/d, respectively) were almost 2 orders of magnitude lower than the EDIs from dust ingestion. The EDIs from the combined dust ingestion and dermal absorption pathways for the average-exposure scenario (based on median dust concentrations) were 3.44 and 1.24 ng/kg/d for MEL and CYA, respectively, while for the high-exposure scenario (based on the 95th percentile dust concentrations), the EDIs were 67.4 and 2.98 ng/kg/d, respectively. HQs for MEL and CYA (based on tolerable daily intakes of 3150 and 2500 ng/kg/d, respectively) were 2.1×10^{-2} and 1.2×10^{-3} , respectively. Overall, the EDIs of MEL and CYA via dust ingestion and dermal absorption were well below the established tolerable daily intake for young children, but must be considered in context of exposure from other sources as well (Hsieh et al., 2009). Dietary MEL and CYA exposure for children in the U.S. and Canada was reported ranging from 420 ng/kg/d to 3200 ng/kg/d (Zhu and Kannan, 2018a, Zhu and Kannan, 2019a), which is 2–3 orders of magnitude higher than the intake estimated in this study. However, the tolerable daily intake dose was determined based on acute toxicity studies, and does not account for low dose chronic toxicity. This is concerning because the pathological pathway for chronic exposure to MEL was determined to differ from the acute pathway (Hsieh et al., 2012; Wu et al., 2015). Suggestive evidence of kidney damage has been found in U.S. children at current levels of exposure (Sathyanarayana et al., 2019).

Despite a small sample size and limited sampling locations, this

is the first study to report the widespread occurrence of several MEL-based compounds in the childcare settings. Future studies are needed to elucidate the sources of MEL contamination in the environment and potential adverse health effects of chronic exposure.

Authors statement

Guomao Zheng: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Writing. Brandon Boor: Writing - Investigation; Review & Editing. Erika Schreder: Conceptualization; Resources; Writing - Review & Editing; Funding acquisition. Amina Salamova: Conceptualization; Methodology; Investigation; Resources; Writing; Supervision; Project administration; Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2019.125505>.

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