Urinary metabolites of volatile organic compounds of infants in the neonatal intensive care unit

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BACKGROUND: Preterm infants (PTI) in the NICU are often placed in incubators that may increase their exposure to volatile organic chemicals (VOCs). To determine whether PTI in incubators have higher urinary concentrations of VOC metabolites compared with infants in cribs.

METHODS: Urine from 40 PTI in incubators and 40 infants in cribs was collected and analyzed for 28 urinary VOC biomarkers. Differences in metabolite concentrations between the two groups were compared.

RESULTS: Twenty two of the VOC metabolites were detected in at least one urine sample. All urine samples tested had measurable levels of six VOC metabolites. Biomarkers for acrolein, acrylonitrile, carbon disulfide, cyanide, *N*-dimethylformamide, ethylbenzene, ethylene oxide, propylene oxide, styrene, toluene/benzyl alcohol, vinyl chloride, and xylene were higher in the incubator group. The geometric means of five VOC metabolites were 2-fold higher than those reported for NHANES children 6–11 years of age in one or both of the groups with benzyl mercapturic acid being 7-fold and 12-fold greater than NHANES in the crib and incubator group, respectively.

CONCLUSION: All infants were exposed to VOCs. PTI in incubators have a different VOC exposure profile compared with infants in cribs. The health implications associated with these exposures require further study.

Within NICUs, plastic incubators can themselves serve as a source of VOCs and a microenvironment for entrapment and concentration of VOCs due to the decreased rate of air circulation compared with open-air beds (5). Analysis of the air within unoccupied incubators revealed that 2-heptanone and *n*-butyl acetate concentrations were higher within the incubators compared with room air in the NICU, with emission of these VOCs increasing when the incubators were in operational mode (6). Some VOCs have been directly linked to adverse health effects (7,8). These health effects differ for each chemical, but in general, given the inhalation route, respiratory irritation and central nervous system effects predominate (9). Some are also carcinogens, and a few may cause hypothyroidism and hearing loss. Health effects in children, in particular newborns, have not been studied. Little is known regarding the levels of VOCs in NICU environments or whether these levels may be associated with health effects.

Exposure to VOCs can be assessed using either air-monitoring approaches or by biomonitoring. The National Health and Nutrition Examination Survey (NHANES) has previously utilized urinary metabolites as quantifiable biomarkers to report the exposure of US populations to a variety of VOCs (10). An analytical method for measuring VOC metabolites in urine permits a simultaneous quantification of 20 metabolites that may indicate exposure to 18 parent compounds (11).

Whether infants are placed into incubators or cribs/bassinets (a crib is a bed surrounded by retaining slats; a bassinet is a plastic container with solid walls and open top) depends primarily on the weight of the infant, the gestational age of the infant, the need for phototherapy to treat hyperbilirubinemia, or the need for closer monitoring. In the University of Maryland Children's Hospital NICU, stable infants in incubators were in the same room as infants in open cribs. Infants in incubators routinely have the doors to the incubator open every 3 h when receiving feedings, diaper changes, and position changes by the bedside nurse. Airflow through the incubator ranges from 0.2 to 2.0 m/s and is calibrated annually.

Infants cared for in neonatal intensive care units (NICUs) represent a population with unique vulnerabilities to environmental exposures, including those from air. The infant's respiratory physiology results in a greater volume of inhaled air relative to body mass as compared with that of an adult (1). Combined with immature pathways of biotransformation and elimination, this can result in higher concentrations of compounds in infants relative to older humans (2,3). The NICU environment has many potential sources of volatile organic chemicals (VOC), including endotracheal tubes, intravenous solution bags, bottles, diapers, nasal cannulas, catheters, cleaning agents, and water vapor (4,5).

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In this study, we test the hypothesis that for infants in the NICU, the types and concentrations of VOC metabolites in urine collected from preterm infants (PTI) occupying incubators would be greater than those of infants in open cribs.

METHODS

Infant Inclusion Criteria

Urine was collected from infants in the NICU at the University of Maryland Children's Hospital between July and December 2014. The University of Maryland, Baltimore Institutional Review Board determined that this study was not human subjects research and therefore informed consent was not necessary. This is a non-randomized study, where infants in incubators ($n=40$) or open cribs ($n=40$) were enrolled who met the following criteria: The incubator group was comprised of infants having occupied an incubator for a minimum of 3 days before urine collection. We required eligible incubator infants to be breathing room air and/or receiving respiratory support via nasal cannula during those 3 days. Neonates included in the open-crib group occupied a crib or bassinet. Each infant in this control group was breathing room air, without respiratory assistance, for a minimum of 3 days before sample collection. Infants in both groups with any vascular access (IV, PICC line, and so on) during the 3 days before sample collection were excluded. Demographic data were collected from the medical record that was available for all infants.

Sample Collection

A urine sample was collected from each infant by placing a cotton gauze pad in the infant's diaper. It was retrieved 3 h later when it was saturated with urine. Absorbent pads soiled with stool were not collected. The urine was then squeezed out into a collection tube by placing the cotton pad into the barrel of a syringe and subsequently stored at -80°C within 1 h of collection; target analytes were stable under these conditions (11). The urine samples were shipped in batches on dry ice to the Centers for Disease Control and Prevention for analysis.

VOC Metabolite Quantification

Ultra-high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS) was used to quantify 28 urinary VOC metabolites from 18 parent chemicals according to Alwis *et al.* (11). Stable isotope analogs of each analyte were added so that analyte quantification was not impacted by ion suppression.

Statistical Analysis

Metabolite data for infants from both the incubator and crib groups were analyzed separately using R-studio statistical software, following the procedure outlined by the US Environmental Protection Agency (12). Medians and ranges were calculated using raw data for each metabolite. Samples with more than 50% of values with $<$ limit of detection (LOD) had insufficient data to calculate geometric means. To calculate geometric means, samples with values $<$ LOD were replaced in two ways: (1) metabolites with $<15\%$ of samples with $<$ LOD, and the value for LOD was replaced with LOD/2, and (2) metabolites with $15\text{--}50\%$ $<$ LOD, were winsorized by the following: For a data set of $n(1), n(2), \dots, n(40)$, and X of the values were $<$ LOD, then the values of $n(1), n(2), \dots, n(X)$ were replaced by $n(X+1)$, and the values of $n(40-X)$ to $n(40)$ were replaced by $n(40-X-1)$. The method of modification was used based on the group having the most number of values $<$ LOD. Therefore, both crib and incubator groups were winsorized, or both had $<$ LOD values replaced with LOD/2. Using these modified data sets, geometric means were calculated by taking the 40th root of the product of the values.

To determine the significance between the crib and incubator groups, metabolites with $>50\%$ of samples under the level of detection were evaluated as ordinal data (present/absent) using Fisher's exact test for proportions. The remaining modified metabolite data were tested for and failed normality. Therefore, they were normalized using standard log-transformation techniques. F -test variance comparison analyses were performed for incubator and crib groups in order to determine whether variances were equal or unequal. The corresponding two-tailed t -test was then utilized to determine the significance.

RESULTS

Demographic Comparison of Incubator and Open-Crib Groups

Demographics of the study population are shown in Table 1. The expected differences in gestational age at birth, post-conceptual age, day of life of obtaining samples, birthweight, number requiring feeding tubes, and nasal cannula were found as less mature and smaller babies are normally housed in incubators.

VOC Metabolite Concentrations

Urine was analyzed for 28 VOC metabolites corresponding to 19 parent compounds (Table 2). Of the 28 VOC metabolites measured, 22 (3MHA+4MHA, AAMA, AMCC, ATCA, BMA, CEMA, CYMA, DHBMA, GAMA, HEMA, 2HPMA, 3HPMA, HPMMA, MA, MHBMA3, MU, PHEMA, PGA, BPMA, MHBMA2, PMA, and TTCA) were quantified above the LOD in at least one urine sample. Six metabolites (AAMA, BMA, CEMA, DHBMA, 3HPMA, and MHBMA3) were present in all urine samples, regardless of group, corresponding to the parent compounds acrylamide, acrolein, 1,3-butadiene, and toluene. BPMA, MHBMA2, PMA, and TTCA were present in a minority of samples (3 (7.5%), 6 (15%), 1 (2.5%), and 9 (22.5%), respectively) from the incubator group, representing exposure to 1-bromopropane, 1,3-butadiene,

Table 1. Comparison of demographic variables of PTIs in cribs and incubators

	Crib n (%) or median (range)	Incubator n (%) or median (range)	P value
Male	26 (65.0)	18 (45.0)	0.11 ^a
African American	19 (48.7)	22 (55.0)	0.65 ^a
Gestational age at birth, weeks	36 (23.6–41)	31.4 (23.6–34.4)	$<0.001^b$
Post-conceptual age, weeks	38.5 (33.7–47)	33.6 (27.3–40)	$<0.001^b$
Age on sampling day, days	16 (3–99)	11 (2–81)	0.02 ^b
Weight at birth, kg	2.47 (0.69–4.67)	1.37 (0.56–2.62)	$<0.001^b$
Weight on sampling day, kg	2.78 (1.93–5.06)	1.64 (0.79–2.66)	$<0.001^b$
Feeding tube (%)	36 (90)	12 (30)	$<0.0001^a$
Nasal cannula (%)	0	9 (22.5)	0.002 ^a
Prenatal maternal smoking (%)	3 (7.7)	5 (12.5)	0.71 ^a

^aFisher's exact test.

^bMann-Whitney.

Table 2. Parent chemical, metabolites, and common names

Parent chemical ^a	VOC metabolite ^b	Common name ^b
Acrolein	<i>N</i> -acetyl-S-(2-carboxyethyl)-L-cysteine	CEMA
	<i>N</i> -acetyl-S-(3-hydroxypropyl)-L-cysteine	3HPMA
Acrylamide	<i>N</i> -acetyl-S-(2-carbamoyl-ethyl)-L-cysteine	AAMA
	<i>N</i> -acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	GAMA
Acrylonitrile	<i>N</i> -acetyl-S-(2-cyanoethyl)-L-cysteine	CYMA
Acrylonitrile, vinyl chloride, and ethylene oxide	<i>N</i> -acetyl-S-(2-hydroxyethyl)-L-cysteine	HEMA
Benzene	<i>trans, trans</i> -Muconic acid	MU
	<i>N</i> -acetyl-S-(phenyl)-L-cysteine	PMA
1-Bromopropane	<i>N</i> -acetyl-S-(<i>n</i> -propyl)-L-cysteine	BPMA
1,3-Butadiene	<i>N</i> -acetyl-S-(3,4-dihydroxybutyl)-L-cysteine	DHBMA
	<i>N</i> -Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	MHBMA3
Carbon disulfide	2-Thioxothiazolidine-4-carboxylic acid	TTCA
Crotonaldehyde	<i>N</i> -acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	HPMMA
Cyanide	2-Aminothiazoline-4-carboxylic acid	ATCA
<i>N,N</i> -Dimethylformamide, methyl isocyanate	<i>N</i> -acetyl-S-(<i>N</i> -methylcarbamoyl)-L-cysteine	AMCC
Ethylbenzene, styrene	Phenylglyoxylic acid	PGA
Propylene oxide	<i>N</i> -acetyl-S-(2-hydroxypropyl)-L-cysteine	2HPMA
Styrene	Mandelic acid	MA
Toluene, benzyl alcohol	<i>N</i> -acetyl-S-(benzyl)-L-cysteine	BMA
Xylene	3-Methylhippuric acid+4-methylhippuric acid	3MHA+4MHA

VOC, volatile organic chemical.
^aParent compounds with no detectable metabolites are not shown.
^bMetabolites that were not detected are not shown.

benzene, and carbon disulfide, but were not detected in any samples from the open-crib group. Metabolites of tetrachloroethylene and trichloroethylene were not detected in any samples. Thus, the metabolites of 17 parent compounds were present in at least one urine sample.

Eleven metabolites corresponding to 10 parent chemicals in the incubator group were increased significantly over the crib group (Table 3). These are ATCA, AMCC, BMA, CEMA, CYMA, HEMA, 2HPMA, PGA, MA, and 3+4 MHA corresponding to the parent VOCs acrolein, acrylonitrile, carbon disulfide, cyanide, *N,N*-dimethylformamide, ethylbenzene, ethylene oxide, propylene oxide, styrene, toluene/benzyl alcohol, vinyl chloride, and xylene.

Two metabolites, DHBMA and MHBMA3 corresponding to the parent compound 1,3-butadiene, were found to be significantly greater in the crib group compared to the incubator group (Table 4).

Six metabolites (AAMA, GAMA, MU, PMA, BPMA, and HPMMA) corresponding to acrylamide, benzene, 1-bromopropane, and crotonaldehyde, were not significantly different between the two groups (Table 5).

A comparison of the urine concentrations in the crib and incubator groups to the 2011–2012 NHANES survey among children 6–11 years of age (10) is shown in Table 6. We used NHANES as the comparison group despite the age and setting differences as no data for nonhospitalized infants were

identified. The geometric means of the incubator group were approximately twice that of levels reported in NHANES for CEMA, and HEMA. The geometric means of the crib group were twice that of NHANES for 3HPMA and MHBMA3. Of note, the geometric mean for *N*-acetyl-S-(benzyl)-L-cysteine (BMA), a metabolite of toluene and benzyl alcohol, was sevenfold greater for the crib group and 12-fold greater for the incubator group compared with data reported in NHANES.

DISCUSSION

Urinary metabolites of VOCs were detected in all infants in this study; despite the small sample size of this study, these results suggest widespread exposure of infants in the NICU to some VOCs. This is not surprising as VOCs are ubiquitous in indoor environments. Neonates in incubators showed increased levels of urinary metabolites consistent with higher levels of exposure to parent compounds such as acrolein, acrylonitrile, carbon disulfide, cyanide, *N,N*-dimethylformamide/methyl isocyanate, ethylbenzene, ethylene oxide, propylene oxide, styrene, toluene/benzyl alcohol, vinyl chloride, and xylene compared with infants in open cribs. These higher levels may be due to the lower gestational age/corrected age and slower metabolism in the less-mature infants in the incubators (2,13). In contrast, two urinary metabolites were elevated in the open-crib group, consistent with higher

Table 3. Metabolites significantly increased in PTI in incubators (I) compared with infants in cribs (C)

Parent compound	Urinary metabolite	LOD, ng/ml	Crib (C) or incubator (I)	# > LOD	Geometric mean (95% CI), ng/ml	Median (range), ng/ml	P value
Acrolein	CEMA	8	C	40	121.5 (92.2–160.0)	114 (16.2–708)	0.04
	3HPMA	13	C	40	179.8 (140.7–229.6)	192.5 (26.7–626)	0.07 ^a
Acrylonitrile	CYMA	0.5	C	40	573.7 (453.1–726.3)	576 (74–2910)	0.003
			I	34	401.6 (291.6–552.9)	326.5 (94.1–6430)	
Acrylonitrile, vinyl chloride, and ethylene oxide	HEMA	0.6	C	38	1.11 (0.82–1.52)	0.95 (<LOD–6.43)	0.003
			I	28	2.2 (1.6–3.0)	2.4 (<LOD–30.9)	
Carbon disulfide	TTCA	3.5	C	34	0.94 (0.81–1.08)	0.76 (<LOD–24)	<0.0001
			I	0	2.28 (1.71–3.02)	2.85 (<LOD–16.7)	
Cyanide	ATCA	15	C	9	N/A ^b	<LOD	0.003 ^c
	AMCC	5.5	C	39	N/A ^b	<LOD (<LOD–12.5)	0.002
Ethylbenzene, styrene	PGA	12	C	39	119.1 (84.7–167.6)	112.5 (<LOD–1900)	0.08 ^a
			I	30	255.6 (183.4–356.3)	274 (<LOD–1820)	
Propylene oxide	2HPMA	1.3	C	34	10.6 (9.04–12.33)	10.0 (<LOD–256)	<0.0001
	MA	12	C	30	12.8 (11.0–14.9)	12.6 (<LOD–107)	0.008
Toluene, benzyl alcohol	BMA	0.5	C	29	28.0 (22.2–35.4)	24.4 (<LOD–1370)	0.02
			I	35	217.9 (112.5–421.9)	255 (<LOD–16100)	
Xylene	3+4MHA	8.0	C	34	12.9 (8.1–20.3)	18.1 (<LOD–99.8)	0.013
			I	38	28.0 (19.6–40.9)	34.6 (<LOD–111)	0.009
			I	40	54.1 (37.2–78.4)	65.5 (<LOD–1030)	
			I	38	117.7 (70.2–197.3)	105.5 (<LOD–1900)	
			I	40	62.6 (46.3–84.6)	63.1 (5.3–479)	
			I	40	104.1 (79.4–136.5)	106 (18.7–388)	
			I	29	23.7 (19.2–29.3)	23.6 (<LOD–516)	
			I	36	38.8 (28.7–52.6)	45.3 (<LOD–182)	

CI, confidence interval; LOD, limit of detection.

^aTrend toward significance.^bInsufficient values to calculate the geometric mean.^cFisher's exact test.

Table 4. Metabolites significantly decreased in PTI in incubators (I) compared with infants in cribs (C)

Parent compound	Urinary metabolite	LOD, ng/ml	Crib (C) or incubator (I)	# > LOD	Geometric mean (95% CI), ng/ml	Median (range), ng/ml	P value
1,3-Butadiene	DHBMA	5	C	40	450.7 (360.7–563.1)	433 (118–2210)	0.011
			I	40	296.8 (234.9–375.0)	262.5 (87.5–2070)	
	MHBMA3	0.6	C	40	26.8 (16.9–42.6)	33.7 (1.1–969)	0.005
			I	40	10.6 (6.75–16.6)	8.1 (1.2–316)	

CI, confidence interval; LOD, limit of detection.

Table 5. Metabolites not significantly different in PTI in incubators (I) compared with infants in cribs (C)

Parent compound	Urinary metabolite	LOD, ng/ml	Crib (C) or incubator (I)	# > LOD	Geometric mean (95% CI), ng/ml	Median (range), ng/ml	P value
Acrylamide	AAMA	2.2	C	40	12.5 (10.1–15.3)	13.9 (2.9–32.3)	0.17
			I	40	9.7 (7.1–13.1)	8.5 (2.3–180)	
	GAMA	9.4	C	6	N/A ^a	<LOD (<LOD–15.4)	0.26 ^b
			I	2	N/A ^a	<LOD (<LOD–50.6)	
Benzene	MU	20	C	18	N/A ^a	<LOD (<LOD–904)	0.37 ^b
			I	23	N/A ^a	23.6 (<LOD–1180)	
	PMA	0.6	C	0	N/A ^a	<LOD (<LOD)	1.0 ^b
			I	1	N/A ^a	<LOD (<LOD–0.62)	
Crotonaldehyde	HPMMA	2	C	40	496 (324.5–757.8)	394.5 (125–61900)	0.92
			I	40	483.6 (354.79–659.3)	375.5 (107–9420)	

CI, confidence interval; LOD, limit of detection.

^aInsufficient data to calculate the geometric mean.

^bFisher's exact test.

Table 6. Comparison of urinary metabolites of crib and incubator infants to 2011–2012 NHANES survey among children 6–11 years of age

Parent compound	Analyte	NHANES 2011–2012		
		Crib	Incubator	6–11 years
		Geometric mean (95% CI), ng/ml	Geometric mean (95% CI), ng/ml	Geometric mean (95% CI), ng/ml
Acrolein	CEMA	121.5 (92.2–160.0)	179.8 (140.7–229.6)	77.5 (68.2–87.9)
	3HPMA	573.7 (453.1–726.3)	401.6 (291.6–552.9)	222 (199–248)
Acrylamide	AAMA	12.5 (10.1–15.3)	9.7 (7.1–13.1)	39.6 (36.0–43.5)
Acrylonitrile	CYMA	1.1 (0.82–1.52)	2.2 (1.57–2.97)	1.47 (1.35–1.60)
Acrylonitrile, vinyl chloride, and ethylene oxide	HEMA	0.94 (0.81–1.08)	2.28 (1.71–3.02)	0.94 (0.80–1.02)
1,3-Butadiene	DHBMA	450.7 (360.7–563.1)	296.8 (234.9–375.0)	255 (227–286)
	MHBMA3	26.8 (16.9–42.6)	10.6 (6.75–16.65)	8.29 (7.47–9.19)
Crotonaldehyde	HPMMA	496 (324.5–757.8)	483.6 (354.7–659.3)	318 (291–347)
Cyanide	ATCA	119.1 (84.7–167.6)	255.6 (183.4–356.3)	247 (227–270)
N,N-dimethylformamide, methyl isocyanate	AMCC	10.6 (9.0–12.3)	12.8 (11.0–14.9)	52.2 (47.1–57.9)
Ethylbenzene, styrene	PGA	28.0 (22.2–35.4)	217.9 (112.5–421.9)	161 (148–176)
Propylene oxide	2HPMA	12.9 (8.1–20.3)	28 (19.6–40.1)	45.2 (39.8–51.4)
Styrene	MA	54.1 (37.24–78.44)	117.7 (70.22–197.26)	111 (103–120)
Toluene, benzyl alcohol	BMA	62.6 (46.29–84.63)	104.1 (79.37–136.51)	8.78 (8.11–9.50)
Xylene	3MHA+4MHA	23.7 (19.2–29.3)	38.8 (28.7–52.6)	152 (134–172)

CI, confidence interval.

1,3-butadiene exposure. Potential sources of these 12 VOCs in a NICU include tobacco smoke (from parents' and caregivers' clothes and hair) (14), plasticizers from medical devices (5), excipients used in medications (15), and the cleaning/sterilizing agents used to clean the cribs and incubators in particular and the NICU in general (16,17).

Although certain VOC metabolites (CEMA, 3HPMA, AAMA, GAMA, CYMA, HEMA, DHBMA, MHBMA3, HPMMA, ATCA, AMCC, PGA, MA, and 3MHA+4MHA) identified in the incubator and/or open-crib groups share cigarette smoke as a common source (10), the precise source of potential exposure within the NICU was not investigated in the present study. A history of maternal cigarette smoking is routinely reported in the medical records in only 10% of the mothers of infants studied. The number of mothers who reported smoking was not found to be statistically significant between the crib and incubator groups. However, assessment of smoking using medical record data may be inaccurate, and our sample size may not be large enough to detect a difference in smoking between the crib and incubator groups. Also, the median day of life of urine sample collection was 11 and 16 days for infants in the incubators and cribs, respectively. This makes the detection of VOC metabolites related to prenatal maternal cigarette smoking unlikely. Although maternal smoking was not more common in the incubator group, it is possible that any cigarette smoke on the garments of a NICU caregiver or visiting parent may have off-gassed and becomes trapped within the enclosed incubator to cause increased concentrations of VOCs. On the other hand, infants in open cribs may have more physical contact with NICU staff and parents, potentially increasing exposure to VOC sources such as cigarette smoke on the clothing of handlers.

Plasticizers, acrylics, and synthetic rubber are common materials in the NICU. VOCs may be emitted from polyurethane foam in bedding materials (1,5,6,18).

Another possible source of VOCs inside the incubators is the cleaning and disinfecting agents, including ethylene oxide, which is commonly used as a disinfectant for respiratory equipment (16,17). There is also the possibility that VOCs inside the incubators originate from external sources, such as cleaning and medical care products that become part of the internal breathable air inside the incubators (19,20). However, as only a subset of VOCs was found to be higher in incubator infants compared with crib infants, other sources unique to individual VOCs must be considered.

Potential exposure to toluene deserves special mention. Toluene is a known teratogen and is associated with toluene embryopathy in infants whose mothers were highly exposed to solvents (occupationally or from solvent abuse). Although toluene exposure in these infants was orders of magnitude lower than that found in solvent abusers (21), the geometric means for the toluene metabolite BMA in both the crib and incubator groups were 7 and 12 times higher than that of NHANES for 6–11-year olds. Indeed, the geometric means were 1.9 and 3.1 times the NHANES that reported the 95th percentile for 6–11-year olds (95% CI 33.1 ng/ml (23.7–56.4

ng/ml)) (10). The highest concentrations measured in the crib group (479 ng/ml) and in the incubator group (388 ng/ml) indicate exposures of an order of magnitude higher than the 95th percentile of 6–11-year olds. However, BMA is a relatively nonselective biomarker; for example, benzyl alcohol can be metabolized to BMA (22). Benzyl alcohol, a known toxicant to preterm infants, is still used as an excipient in the NICU (15). The higher concentrations of BMA in the infants compared with NHANES children may be due to the benzyl alcohol used as an excipient in the medications that they receive while in the NICU. Additional work is needed to characterize toluene exposure for NICU infants, to disentangle the sources of the metabolite (i.e., toluene or benzyl alcohol), and to better understand whether the levels measured in this sample population are representative of infants at other locations.

The results of this study are presented with several caveats. First, parent VOCs may be broken down to form more than one metabolite and some metabolites may be derived from more than one parent VOC; not all biomarkers measured in this study are unique to an individual parent VOC, as shown in [Table 2](#). Thus, an elevated level of an individual metabolite may not represent exposure to a single compound. Second, we did not control for individual hydration since all babies in our NICU receive the same amount of fluid per day whether by parenteral or enteral nutrition. Further, interpretation of the sources of VOCs is difficult as unmeasured variables may have contributed to differences in VOC exposure, such as time between manufacturing and use of materials, and the smoking habits of hospital caregivers and visitors such as fathers and mothers. Another source of uncertainty that could impact urinary VOC levels is the time interval between sanitization of the incubators and cribs and placement of an infant into the incubator, with an off-gassing period ranging from 45 min to days. A 2-week period to allow for dissipation of these gases has been suggested (23).

In addition, we did not collect information needed to evaluate whether the differences in VOC levels between the two infant groups were related to the differences in metabolism rates between the groups. The incubator and open-crib groups have several demographic differences, including body weight and gestational age at sampling ([Table 1](#)), and these factors are related to the decision to place the infant in an incubator or open crib during their care in the NICU. The significantly lower gestational and post-conceptual age of the incubator group could contribute to lower excretion of the VOC metabolites in this group due to functional immaturity of the renal glomeruli and the excretion system of the smaller infants (13). However, other important differences are also present that we did not measure, including the presence or absence of bronchopulmonary dysplasia (a disease known to influence VOC production) (24), amount and type of medications (15), and dietary differences (breast milk vs. formula), all of which could influence the urinary excretion of VOCs. Therefore, we cannot directly extrapolate the quantity of parent compound

to which an infant was exposed from the quantity of urinary VOC metabolites measured from these infants. The proportion of parent compound VOC metabolized and excreted into the urine vs. the proportion distributed into the infant's tissues and the remaining bioreactive VOCs are unknown for the compounds analyzed here and may vary depending on an individual infant's metabolic characteristics (25). Finally, it is possible that some of the metabolites measured here could also be derived as a metabolic intermediate from an unknown VOC or an endogenous process.

In this study, we demonstrate the feasibility of measuring infant exposures to VOCs in a NICU setting. We further demonstrate that infants have exposures to a wide array of VOCs in both cribs and incubators and that exposures may vary depending on which type of unit an infant is placed. The findings indicate the need for a study with a larger sample size to increase the power of the study, multiple samples per infant to better characterize the variability in exposure, and more data on products used in the NICU to better understand the sources of VOC exposure. Such a follow-on study would help to determine the validity of these findings and to identify possible hospital practices that could be modified to reduce validated exposures. One such practice would be to increase the ventilation rate of the incubator. Current specifications for one incubator are an airflow of 0.2–2.0 m/s. Calibration could be increased to the higher end of this range. Further studies are needed to understand the clinical significance of VOC exposure within the NICU and the impact on long-term health, including both respiratory and neurodevelopmental outcomes.

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