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Pilot Study of Sources and Concentrations of Size-Resolved Airborne Particles in a Neonatal Intensive Care Unit

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Abstract

Infants in neonatal intensive care units (NICUs) are vulnerable to environmental stressors. Few studies have reported on airborne particles in the NICU environment. During a four-day pilot study in a private-style NICU, we measured size-resolved particle number (PN) concentrations with 1-min resolution. The investigation included simultaneous sampling in an unoccupied baby room and in an incubator of an otherwise normally functioning NICU. Background submicron (0.3-1 μm) particle levels in the room were 3-4 orders of magnitude lower than outdoors, owing to high-efficiency particulate filtration of supply air. Airborne supermicron particles were detected in the room; their presence was attributed primarily to emissions from occupant movements. The fraction of in-room PN detected within an infant incubator ranged from 0.2 for particles >10 μm to 0.6 for particles with diameter 0.3-0.5 μm. The incubator humidifier was a strong additional source of particles smaller than 5 μm. Activities by researchers, designed to simulate caregiver visits, were associated with elevated particle concentrations across all measured size ranges, and were particularly discernible among larger particles. Concentrations increased with the number of occupants and with the duration and vigor of activities. The highest levels were observed when fabrics were handled. Against the low background in this environment, even small occupancy-associated perturbations – such as from a brief entry – were discernible. Measurements from a second NICU in a different US region were found to be broadly similar. A notable difference was higher submicron particle levels in the second NICU, attributed to elevated outdoor pollution.

Keywords: NICU environment, Incubator, Indoor air quality, Airborne particles, Human activities, Particle size distribution.

1. Introduction

The inhalation of airborne particles is known to be associated with risks of increased mortality, respiratory illnesses and other adverse health effects of infants and children [1-3]. Preterm infants in neonatal intensive care units (NICUs) are particularly vulnerable to health harm resulting from environmental exposures [4]. Moreover, the airborne route may play a role in the dispersal of microbes indoors [5]. However, limited published information is available on the sources and concentrations of airborne particles in the NICU environment.

In the most detailed prior description of air quality in a NICU in the United States, Domanico et al. [6] compared levels of environmental parameters and patient progress in an open-style vs. private- (or bay-) style NICU. The authors reported that the private-style facility was associated with "fewer apneic events, reduced nosocomial sepsis and mortality, as well as earlier transitions to enteral nutrition" and better infants' progress. Though specific causal factors were not isolated, the study showed that staff activity was correlated with higher levels of airborne coarse particles (> 2 μm), carbon dioxide and noise, and that exposure to these agents was reduced in the private-style configuration. In a few other studies where NICU air quality parameters were monitored, microbes in airborne particles were assessed using culturing methods, and the mechanical ventilation system and renovation activities were assessed as potential sources. Ryan et al. [7] found that enhanced ultraviolet germicidal irradiation of HVAC cooling coils significantly decreased the number of culturable microbes in the HVAC system and in the NICU, in air and on surfaces. The reduced environmental loads were linked, in turn, to decreases in tracheal microbial colonization of infants, and reductions in ventilator-associated pneumonia. A study in Belgium by Mahieu et al. [8] found that renovation activities in a NICU were linked to increases in airborne concentrations of *Aspergillus* spp. but with no corresponding

increases in nasopharyngeal colonization of neonates. No relationship was demonstrated between patient occupant density and air concentrations in that study. The importance of surfaces and their cleaning for controlling outbreaks of specific organisms and eradicating sources of potential pathogens in NICUs has been highlighted in a few prior studies [9-11]. Other NICU environmental parameters previously studied in relation to prevention of nosocomial infections have included noise, illumination, medical equipment hygiene, microbes on surfaces, overcrowding, and the importance of inhibiting free mixing across rooms [11–16].

The study by Domanico et al. [6] provided important preliminary evidence in support of the hypothesis that human activity is a significant source of coarse particles in the NICU environment. That study left many questions open for future research. Particle levels were presented in relative terms without associated units, limiting the comparability of findings to other environments. The study did not differentiate among particle sizes, even though size can be associated with distinct sources, constituent materials, and potential health impacts. Particles smaller than 2 μm, though important for health, were not evaluated. The relative contributions of airborne particles from outdoor air versus occupancy were not probed, and the modulation of room levels by the incubator was not investigated. To contribute new knowledge regarding airborne particle sources, levels, and influencing factors pertinent for understanding neonatal exposure, we undertook a study of aerosol characteristics in the NICU environment. The overall effort resulted in two interrelated studies with distinct outcomes. Elsewhere, we report on the results of a yearlong measurement campaign [17] that enables inferences about the sources of airborne particles in an ordinarily operating NICU baby room (i.e., without researcher control over human occupancy and activity conditions).

In the present paper, we report on the results of a weeklong pilot-scale effort conducted in an empty patient room in an otherwise normally functioning NICU. The circumstances allowed for researcher control over human occupancy and activity conditions. The use of an empty room also enabled sampling to be conducted from within the infant incubator under a range of operational conditions. Consequently, the pilot study not only informed the development of the full-year sampling campaign, but also provided new information and insight about an important, yet data-poor, indoor environment.

Specifically, the objectives of the present work were (a) to characterize particle number concentrations in a private-style NICU room across a range of simulated occupancy conditions and (b) to assess the factors influencing those levels. By measuring particle levels indoors, outdoors, and inside neonatal incubators, we sought to evaluate the relationships among concentrations at the three locations in relation to particle size, human activity, and incubator operation parameters. A brief subsequent monitoring investigation in a second NICU in a different part of the United States was conducted to test whether the findings from the first NICU would be reinforced in another setting.

2. Materials and Methods

2.1. Study site

Experiments were conducted in a private-style NICU located in a large hospital building in the western United States. The site was selected based on convenience and access. Physical features of the NICU included hard tile flooring, no visible green plants or water damage, and no obvious dust accumulation on visible surfaces or any visible signs of air infiltration. The NICU had no operable windows. Smoking and pets were not permitted in the unit. Staff and visitors followed hygiene protocols upon entry that included hand-washing and exchanging clothes for

laundered scrubs. Street shoes were retained and, in some instances, street clothes continued to be worn under the scrubs for warmth. Critically ill patients were housed in rooms that formed a semicircle around the nurse's station so that they could be closely monitored with visual contact. The experimental room for this study was situated at one end of the NICU away from this core. The volume of the room was assessed as 30 m^3 based on physical dimensions (length, width, height). The side of the room adjacent to the hallway had a large door, which was left open to mimic typical use conditions. The doorway had a curtain that was usually open, but was occasionally drawn closed. The experimental room was not cleaned during the weeklong study. The three patient rooms closest to it were not in regular use for infant care and were used, instead, for equipment storage and maintenance.

2.2. Mechanical ventilation

Ventilation air was supplied to the room from a heating, ventilating, and air conditioning (HVAC) system. The HVAC system was equipped with an economizer that varied the percentage of recirculated air in the supply stream based on the outdoor air temperature. Recirculated air was drawn from the NICU and from other parts of the building, mixed with filtered outdoor air, and cooled. Cooling coils were treated with UV germicidal irradiation. The mixed supply air was then heated and humidified according to thermostatic demand, and treated with high-efficiency particulate arrestance (HEPA) filters. The ventilation specifications included a requirement for at least six air changes per hour for the entire NICU, of which at least a third was required to be outdoor air. The actual air-exchange rate of the test room was not measured. The air in the test room was deemed to be well mixed, based on the supply air-flow rate, position of ceiling-mounted air delivery and exhaust devices and the physical dimensions of the room.

2.3. Experimental protocols and instruments

The monitoring campaign was conducted during 17-21 June 2013. Continuous sampling in the test room was initiated on 18 June at 00:00 (Eastern Time Zone) and lasted 3.6 days. Air quality parameters were measured from a central location in the infant room, and from the interior of an operating, but empty infant incubator. Sampling was conducted with one-minute time resolution to capture the dynamic response to rapidly changing conditions. Particle number (PN) concentrations larger than 0.3 μm were measured with particle counters (model HHPC 6+, Beckman Coulter Life Sciences, Palatine, IL, USA) in six size bins according to optical diameter: 0.3-0.5, 0.5-1, 1-2, 2-5, 5-10, and >10 μm.

Supplementary parameters monitored and logged included carbon dioxide (model SBA-5, PP Systems, Amesbury, MA, USA; and model 820, LI-COR Biosciences, Lincoln, NE, USA) and temperature and relative humidity (HOBO U12, Onset Computer Corp., Bourne, MA, USA). A light/movement passive infrared (PIR) sensor (HOBO UX90-006, Onset Computer Corp., Bourne, MA, USA) with a range of 12 m was positioned at the door of the room, facing outward, to record occupant movements near the open doorway. Carbon dioxide data from the room were not acquired during the last sampling day because of instrument malfunction. However, carbon dioxide levels in the incubator closely tracked levels in the room during typical operating conditions (Fig. S1.). Therefore, for this paper, incubator-CO_2 levels are utilized in place of room-CO_2 data during the period when the room-CO_2 monitor failed.

The contributions of simulated human activities to indoor concentrations were quantitatively compared with the estimated levels of particles entering the room via the mechanical ventilation system. Outdoor sampling was conducted during two periods totaling 8 h, on 19 and 20 June. The location was a balcony situated on the same level as the NICU unit. Total

PN concentrations were measured with an optical particle counter (model GT-526, Met One Instruments, Grants Pass, OR, USA). The outdoor particle monitor size bins did not match the size categories delineated by the indoor particle sampling devices. For the purposes of indoor/outdoor comparisons, concentration values from instruments in each location were post-processed to fit the following five size categories: 0.3-0.5, 0.5-1, 1-2, 2-5, >5 μm.

The effects of human activities on particle and carbon dioxide concentrations in the study room were evaluated through 21 scripted experimental activities. Each activity was preceded and followed by a period when the room was vacant. The timing, duration, occupancy and activity conditions of each experiment are summarized in Table 1. One or more of the three individuals who constituted the core field research team conducted these experiments. The research team conformed to the common hygiene practices of the health-care workers and visitors, including handwashing, wearing scrubs, and retaining street shoes.

Table 1
Schedule of human activity experiments, along with 15-min maximum size-specific particle number (PN) concentrations (in particles per liter) and carbon dioxide mixing ratios (in parts per million) measured in the room when the activity was conducted.[1,2]

ID	Start date and time (2013)	Experiment			15-min maximum						
		Dur (min)	Activity	Occ	PNa L^{-1}	PNb L^{-1}	PNc L^{-1}	PNd L^{-1}	PNe L^{-1}	PNf L^{-1}	CO_2 ppm
1	18 June 11:35	2	clothes	1	131	82	43	57	14	25	440
2	18 June 17:05	5	clothes	1	98	63	41	65	20	41	404
3	18 June 14:49	1	walk	1	33	10	5	5	1	2	406
4	18 June 15:18	15	sit	1	36	12	7	9	3	3	412
5	18 June 16:32	2	sink	1	44	15	8	10	2	2	400
6	19 June 13:31	1	quick	1	20	9	6	9	2	0.9	436
7	19 June 13:47	1	quick	1	33	15	8	11	2	2	442
8	19 June 13:56	1	quick	1	38	19	9	10	2	2	442
9	18 June 11:11	5	walk	2	132	90	46	60	14	12	494
10	18 June 16:02	1	walk	2	61	26	13	16	4	6	411
11	20 June 11:31	5	walk	2	201	138	74	93	17	12	474
12	20 June 12:08	5	walk	2	171	119	67	85	16	13	471
13	20 June 13:54	5	walk	2	270	179	93	111	21	17	475
14	20 June 14:46	5	walk	2	139	110	67	102	22	21	470
15	20 June 16:10	5	walk	2	77	62	36	49	12	20	465
16	20 June 16:56	5	walk	2	59	45	28	43	12	17	456
17	18 June 14:01	15	sit	2	61	27	14	20	5	6	452
18	19 June 15:34	15	sit	2	46	29	15	22	5	5	479
19	18 June 12:33	5	fabrics	2	610	499	285	383	84	92	422
20	19 June 14:38	5	fabrics	3	942	727	421	566	124	151	540
21	20 June 12:51	5	walk	3	239	186	102	135	30	36	494
Unoccupied[3] (daytime) N = 20 h; mean					39	12	4	4	0.7	0.6	414
Unoccupied (daytime); standard deviation					30	14	5	5	0.9	0.8	20

[1] The particle size categories a, b, c, d, e, and f correspond, respectively, to 0.3-0.5 μm, 0.5-1 μm, 1-2 μm, 2-5 μm, 5-10 μm, >10 μm.
[2] Researchers were sitting (sit), walking (walk), changing into or out of hospital scrubs (clothes), shaking fabrics (fabrics), washing their hands at the sink (sink), or quickly entering and exiting (quick). The curtain was drawn when clothes were being changed. The duration (dur) of each activity and the number of researchers involved (occ) are shown.
[3] For comparison, "unoccupied" levels are also presented. These are mean and standard deviation of 1-min concentrations from daytime periods (9 AM to 5 PM; N = 20 h) during the full duration of sampling, excluding occupied times. A 15-min lag period following each occupancy event was also excluded.

2.4. Incubator tests

An empty infant incubator (model Giraffe Omnibed), positioned in the center of the test room, was operated in closed "incubator mode" with the temperature set point at 33 °C. The mean temperature measured inside the incubator during the study was 32.6 ± 0.1 °C. When

operated in this mode, the incubator interior was ventilated with filtered air that was introduced via a fan at a nominally constant flow rate. The incubator air-exchange rate was assessed by means of tracer-gas decay. Carbon dioxide was used as the tracer gas and released by introducing dry ice into the incubator for a few minutes. The incubator air-exchange rate was 9.0 ± 0.4 per hour (N = 3 tests; Table S1). The incubator interior volume was 0.2 m^3. Consequently, the volumetric airflow rate through the incubator was determined to be 1.7 m^3 per hour.

The incubator water reservoir was refilled as needed with sterile, distilled water. We tested the effect of humidification on particle levels in the incubator by means of operating the incubator at different humidity settings: no control, 30-40%, 60%, 70%, and 90%. The 30-40% setting was chosen to match the approximate RH level in the room, and thus represents a level at which humidity control was turned on but was not expected to be active. A lower bound estimate of the rate of particle emission from the humidifier into the incubator was inferred based on an integral material-balance approach (File S1).

2.5. Data analysis and quality assurance

Approximate PM_{10} levels (defined as the mass concentration of airborne particles smaller than 10 μm) were estimated from the PN concentration data in two steps. First, size-specific particle number concentrations were converted to mass concentrations, based on the assumptions that particles were spherical, had a density of 1 g/cm^3, and that within each particle size group the mass-weighted size distribution, $dM/d(\log d_a)$, was constant. Second, the PM_{10} mass was evaluated as the sum of the mass concentrations determined for particles in size groups smaller than 10 μm. The optical particle counters did not detect particles smaller than 0.3 μm in optical diameter. These are expected to have contributed little to PM_{10} mass concentration in the NICU owing to the effectiveness of HEPA filtered supply air in limiting entry of particles of outdoor

origin and the absence of evident indoor sources of submicron particulate matter.

Instrument calibrations and performance checks were conducted throughout the period of field monitoring. The intercomparability of data from the optical particle counters was improved by post-processing raw data with adjustment factors based on side-by-side tests of instrument performance (Table S2). Flow rates of all active instruments were checked and corrected if the response was outside the manufacturer-specified normal range.

3. Results and Discussion

3.1. Summary of findings

The temperature and relative humidity in the test room were tightly controlled by the HVAC system thermostat, and varied over only a small range throughout the sampling period. The mean ± standard deviation temperature was 24.1 ± 0.3 °C (range: 23-25 °C), and the corresponding RH values were 36 ± 3% (range: 30-43%). The carbon dioxide levels in the room (407 ± 26 ppm, range: 388-614 ppm) were typical of well-ventilated indoor environments [18]. Particle number concentrations were substantially lower than levels previously reported for common indoor environments, such as commercial and residential buildings. For instance, in a review of PM_{10} levels measured in schools and residences, Morawska et al. [19] reported an observed range in indoor-air concentrations of 10 to 250 $\mu g/m^3$. In contrast, the mean ± interquartile range PM_{10} concentration in the NICU room was 0.1 ± 0.04 $\mu g/m^3$ in the absence of simulated activities, and 1 ± 0.07 $\mu g/m^3$ over the full monitoring period. The maximum number concentrations of particles larger than 0.3 μm (when the room was unoccupied) corresponded approximately to ISO 7 cleanroom standards. Within the infant incubator, particle levels were even lower than in room air, except when the humidifier, which was found to be a source of particles, was in operation (Table 2).

Table 2
Minimum (min), maximum (max), and mean (± standard deviation; N = 77 h) carbon dioxide and size-specific particle number (PN) concentrations, measured at a temporal resolution of 1-min in the test room when it was vacant; maximum and mean values corresponding to the full sampling period (N = 87 h) are also shown for comparison.[1]

Parameter	Units	Vacant			All	
		Min	Max	Mean ± s.d.	Max	Mean ± s.d.
CO_2	ppm	388	455	400 ± 14	614	407 ± 25
PN (0.3-0.5 μm)	L^{-1}	8.8	236	35 ± 19	1636	45 ± 77
PN (0.5-1 μm)	L^{-1}	0	112	8 ± 9	1294	17 ± 62
PN (1-2 μm)	L^{-1}	0	36	2 ± 3	742	8 ± 35
PN (2-5 μm)	L^{-1}	0	35	2 ± 3	990	9 ± 48
PN (5-10 μm)	L^{-1}	0	7	0.3 ± 0.7	214	2 ± 11
PN (>10 μm)	L^{-1}	0	7	0.2 ± 0.5	260	2 ± 13

[1] To remove the influence of occupancy, a period of 15 min following each occupied period was also excluded from the results presented for "vacant" conditions.

The activities conducted in the room by researchers were associated with short-term spikes in particle concentration that were evident across all size groups measured. The activities also resulted in increased CO_2 levels. Fig. 1 illustrates the response to two simulated activities: two occupants entering the room and remaining seated for 15 min before exiting, and a person walking in the room for one minute. As is demonstrated by the sharp decays after occupants exited in each case, the emitted particles and CO_2 were rapidly removed because of the high ventilation rate (and, in the case of large particles, also because of deposition onto room surfaces). As a consequence of the short-term, human-activity-related peaks, the overall means of 1-min PN concentrations were associated with high coefficients of variation (COVs). The COV increased monotonically with particle size, ranging from 1.7 for 0.3-0.5 μm particles, to 5.9 for >10 μm particles. The variability in particle means was lower during unoccupied periods, but remained high, with COVs ranging from 0.5 for 0.3-0.5 μm particles, to 2.3 for >10 μm particles. The cause of the high COV values even when the room was unoccupied was

hypothesized to be occupant-associated emissions outside the room. This potential source of airborne particles in the patient room is discussed below.

In the subsections below we describe characteristics of the incubator, discuss relative contributions from source groups, and compare primary findings with observations from a second NICU.

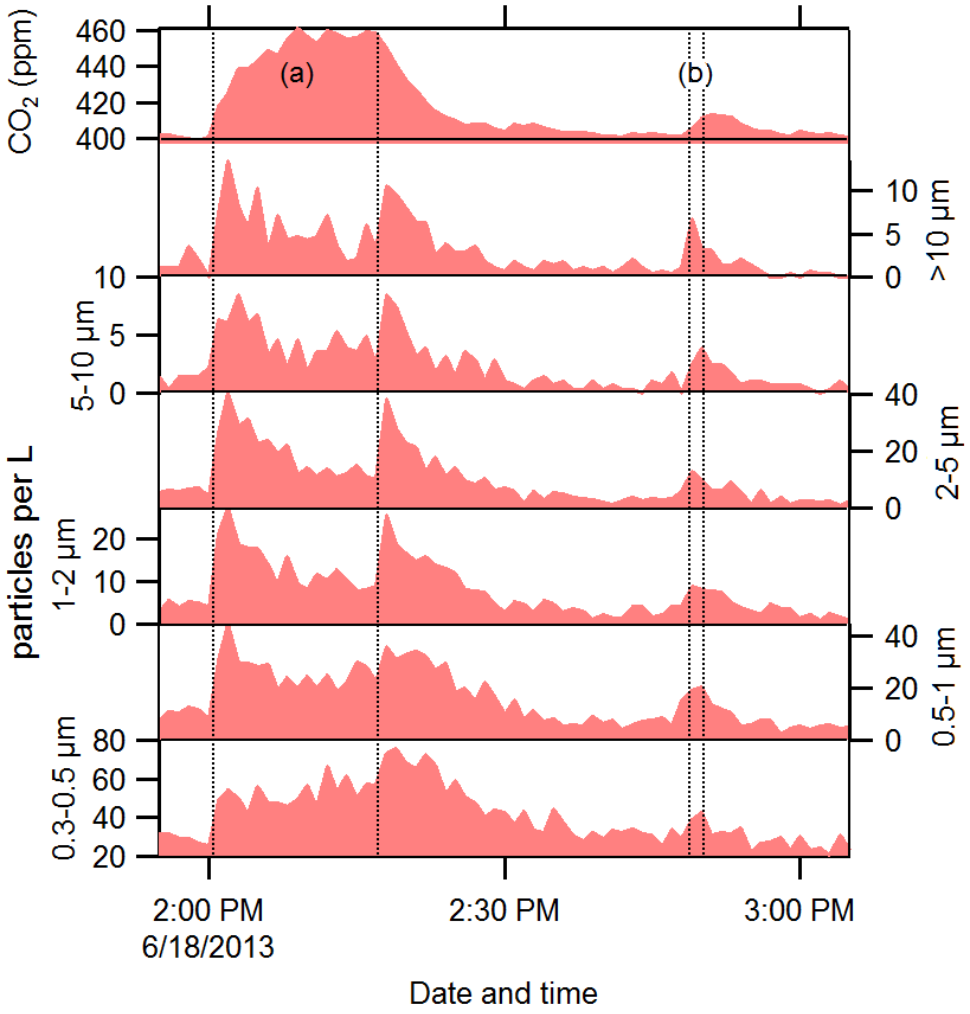

Fig. 1. Time series (at 1-min resolution) of carbon dioxide and size-specific particle number concentration response to selected activities. The dotted lines designate the start and end of (a) a 15-min period when 2 occupants entered the room and were seated quietly before exiting, and (b) a 1-min period when 1 occupant walked around the room. Both activities were associated with distinct carbon dioxide peaks, and particle concentration peaks across all the particle size groups monitored.

3.2. Incubator characterization

As presented in Fig. 2, the mean ratio of incubator to room particle concentration without humidification decreased with increasing particle size, from 0.62 for particles 0.3-0.5 μm in diameter, to 0.17 for particles larger than 10 μm. Mean incubator/room PN concentration ratios, when the humidifier was on at the default setting of RH = 60% (total duration = 2.4 h; measured RH = 61%), are also presented in Fig. 2. The results reveal that: (1) particle concentrations inside an infant incubator were a substantial fraction of those in room air; and (2) humidification significantly increased the incubator/room PN ratio for particles across a broad size range of 0.3 to 5 μm. The humidification process was evidently a source for PM emissions directly into the incubator. The PM mass emission rate was estimated to have a lower bound of 1 μg/h (File S1). This rate did not increase systematically with increasing RH setting.

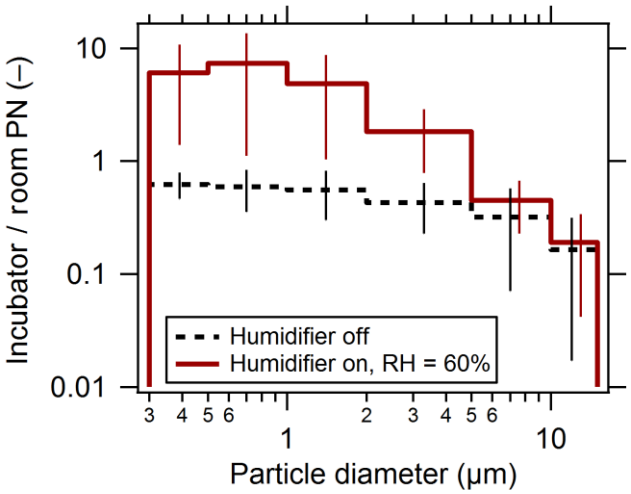

Fig. 2. Incubator/room particle number concentration ratios, by particle size, without humidification, and with humidification at RH = 60%. The means and standard deviations were evaluated based on 5-min mean concentrations (5.6 h for no humidification and 2.4 h for RH = 60%) and as the ratio of means. Periods with very low particle levels, when the average level in the room for any size group was less than one particle per liter, are excluded from the means. The results show that humidification was associated with significant increases in incubator/room particle number concentration ratios for particles across the size range 0.3 to 5 μm in optical diameter. For the largest size channel of the optical particle counter we adopted an upper limit of 15 μm diameter.

According to specifications provided by the manufacturer, incubator humidification is provided through a steam-generation process. While ultrasonic and impeller humidifiers are known to generate particles, steam humidification has not been shown in previous studies to be associated with significant increases in indoor airborne particle mass concentrations [20–22]. Plausible factors for why the NICU incubator steam humidifier led to significant increases in particle concentration include these: (a) background particle levels were very low, and thus levels were sensitive to a measurable perturbation even from a low-emitting source; (b) particles were emitted into the enclosed incubator that provided a small dilution volume compared to the volume of, e.g. a bedroom; (c) droplets formed in the process of creating steam could evaporate to yield residual particles if there were small proportions of nonvolatile matter in the water contained in the humidification reservoir. The low dilution-volume argument is supported by the observation that particle concentrations in the room air did not increase noticeably in response to the emissions inside the incubator.

Additional research would be needed to evaluate the composition of particles generated by the incubator humidifier under typical operating conditions. Prior work has shown incubator humidity chambers can harbor microorganisms [23]. Our research was not designed to probe the chemical composition or biotic status of the observed humidifier-generated particles.

3.3. Relative source contributions to within-room particle concentrations

In sum, we apportion the particle concentrations measured in the room to three underlying sources categories: ventilation supply air (comprising a mix of outdoor and recirculated air); occupant movements outside the room; and occupant activities inside the room. Contributions of particles of outdoor origin to indoor PN concentrations in the NICU room were evaluated, on a size-specific basis, by comparing expected values with those observed. Expected values of the time-averaged indoor proportion of outdoor particles (*IPOP*, or infiltration factor) were modeled, using a material-balance approach, as the rate of particle entry from outdoors divided by the total rate coefficient for particle removal from indoor air [24,25]. For the purpose of this model, the room was treated as a single, well-mixed compartment, ventilated exclusively by the mechanical HVAC system. The contribution of recirculated air (i.e., air making more than one pass through the filtration system) to the supply air particle load was also considered negligible, compared to the much higher contribution from outdoor air. Under these conditions, the *IPOP* for each particle size group *i* can be assessed using Equation (1).

$$IPOP_i = \frac{a_{out} \times (1-\eta_i)}{a+k_i} \quad (1)$$

In Equation (1), a is the total volume-normalized mechanical ventilation rate and a_{out} is the room air-exchange rate with outdoor air (per hour); η_i is the size-specific HVAC filtration efficiency (—); and k_i is the size-specific particle deposition loss coefficient in to room surfaces (per hour). Starting with the smallest particle size tested, we assumed $k_{0.3} << a$. The approximation is valid because the total mechanical ventilation rate, a, specified for the HVAC system (6 air changes per hour) was considerably higher than the expected range of indoor deposition loss rate coefficients for submicron particles [26]. This condition simplifies the right hand side of Equation (1) to $a_{out}(1 - \eta_{0.3})/a$. Using $\eta_{0.3} = 0.9997$ (based on minimum guidelines

for HEPA filters from DOE-STD-3020-97), and an a_{out}/a ratio in the range 0.3-1 (based on specifications that at least a third of the supply air must consist of outdoor air), the *IPOP* for 0.3 μm particles was estimated to be in the range $0.9\text{-}3 \times 10^{-4}$. Using analogous reasoning, the *IPOP*s for particles larger than 0.3 μm were determined to have an upper bound of $\sim 10^{-4}$, as both filtration efficiency and deposition loss rates increase with particle size for particles larger than 0.3 μm [27].

For comparison with the estimated *IPOP* values, Fig. 3a presents the mean measured I/O ratios, by particle size, from two periods when outdoor air was sampled and the test room was unoccupied. In the absence of indoor sources, the I/O ratio is equivalent to the *IPOP* as it represents the fraction of ambient particles that penetrates indoors and remains suspended [25]. The results shown in Fig. 3a support the hypothesis that indoor sources influenced the within-room particle levels even when the room itself was unoccupied. The mean I/O ratio for 0.3-0.5 μm particles, 6×10^{-4}, was 2-7× the predicted $IPOP_{0.3}$. Notably, the I/O ratios increased monotonically and significantly with particle size, instead of decreasing as would be expected if the source were exclusively outdoor air. The indoor source responsible for elevated I/O ratios is thought to be occupant movements that occurred in close proximity to the open doorway. The occupancy data from the PIR sensors facing the hallway and the room opposite the test room support this inference. They showed that occupants were present outside the open doorway, within a 12 m radius, during 30-40% of the time when the I/O ratios for unoccupied conditions were evaluated. We further inferred that the occupant movements emitted particles primarily through the mechanisms of shedding and resuspension. Those processes are known to increase in strength with increasing particle size up to the 5-10 μm range [28–30], and could plausibly account for the size-related I/O trends presented in Fig. 3a.

Fig. 3b displays the estimated relative contributions of near-room occupancy and outdoor air as sources of particles in the room air, under conditions when the room was unoccupied. Upper-bound estimates for concentrations of particles of outdoor origin were evaluated as the product of $I/O_{0.3\text{-}0.5}$ and each size-specific outdoor mean concentration. This approach was based on treating $I/O_{0.3\text{-}0.5}$ as an upper-bound estimate of *IPOP* for particles with a diameter equal to or greater than 0.3 μm. Unoccupied periods on 19-20 June when outdoor sampling was included were applied for the analysis. Lower bounds for the fractions of particles emitted by occupants were then assessed as one minus the maximum fraction from outdoors. The results of this analysis show that occupants were responsible for at least 80% of the concentrations of supermicron particles in the room even when it was nominally unoccupied. The fraction from occupants increased with particle size, and was close to 100% for particles larger than 5 μm. On the other hand, supply air and near-room occupancy both contributed significantly to levels of submicron (i.e., 0.3-1 μm) particles.

To better understand how near-room occupancy might have influenced within-room particle levels, we explored the relationship between the occupancy sensor signal and the particle size group (> 5 μm) whose levels were clearly dominated by indoor sources (Fig. 3b). The analysis focused on periods when the room was unoccupied, and utilized 5-min mean concentrations of particles larger than 5 μm as the outcome variable. The results showed that particle levels were positively linearly correlated ($r^2 = 0.43$) with the sensor signal. This finding suggests that an outward-facing PIR sensor may provide a useful source of data for predicting near-room effects.

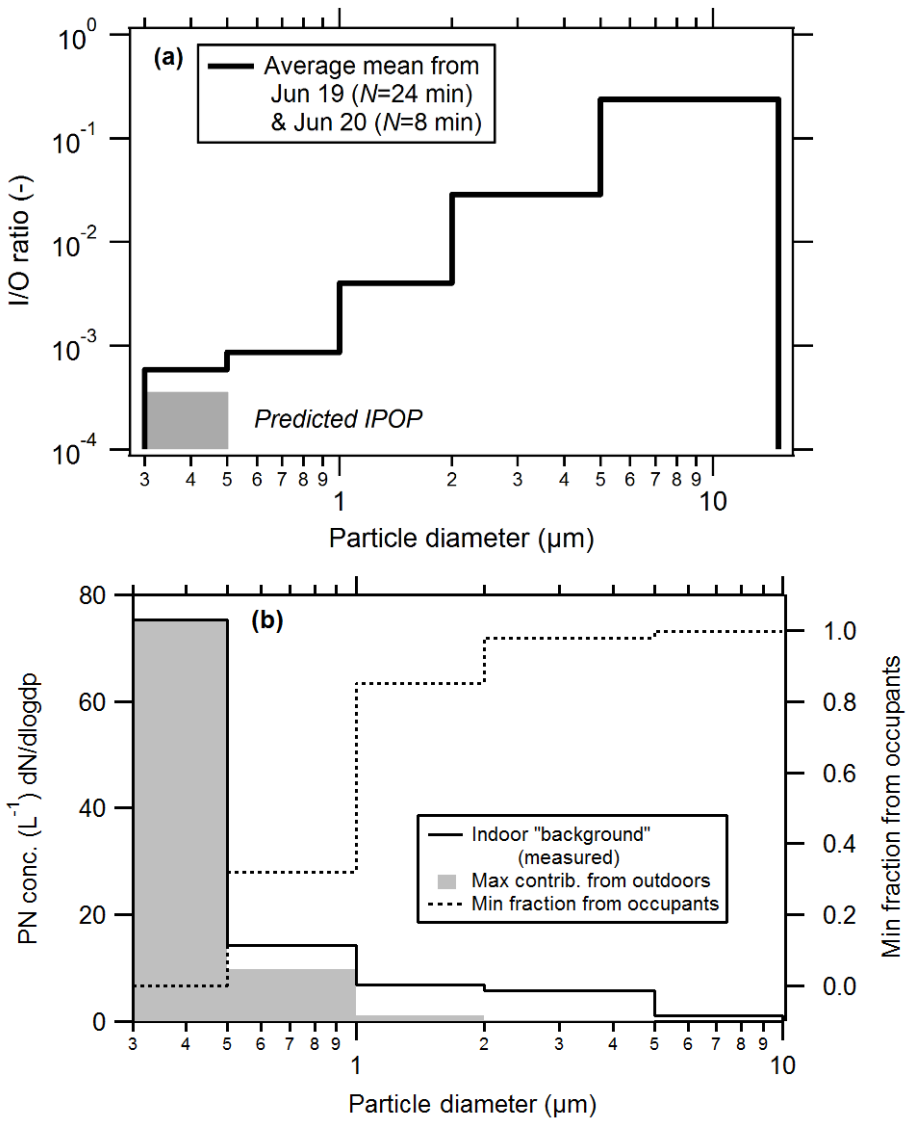

Fig. 3. (a) Indoor/outdoor particle number concentration ratios, and (b) lower bound estimates of the fraction of indoor concentration attributable to occupants. These results are based in part on an analysis of data from two periods (19 June for 24 min; 20 June for 8 min) when simultaneous outdoor sampling was conducted and the room was unoccupied. The indoor concentrations reflected the combined influence of particles of outdoor origin (entering through the ventilation supply air), and particles that entered the test room through its open doorway. The I/O ratios presented in frame (a) were evaluated as the average of the ratio of means from the two periods. For comparison, the modeled indoor proportion of outdoor particles for 0.3 µm particles is shown. Frame (b) presents size distributions of the measured mean indoor concentration, along with the maximum concentration attributable to outdoor air (evaluated as $I/O_{0.3\text{-}0.5} \times PN_{out}$ for each size group). The minimum contribution from occupants is evaluated as one minus the modeled maximum fractional contribution from outdoors. The biggest size group is displayed as the sum of the two largest size channels of the optical particle counter with an adopted overall upper diameter limit of 15 µm.

3.4. Effects of human activities on indoor PN concentrations

We evaluated the effect on indoor PN concentrations of a range of simulated activities conducted in the hospital room. Table 1 summarizes the size-specific, maximum, 15-min PN and CO_2 concentrations in the room during each activity. The "unoccupied" means are from daytime periods (9 AM to 5 PM) when the room was vacant. The results show that while near-room activities had a large effect on particle levels in the room when considered relative to outdoor air as a source, their influence was small compared to most within-room activities, especially for supermicron particles. Against the low mean unoccupied level of 60 particles per liter (for all particles larger than 0.3 μm) and 9 particles per liter (for supermicron particles), even small perturbations – such as from a person briefly entering and exiting the room – were discernible.

As expected, the activity-specific 15-min maximum PN concentrations reported in Table 1 were observed to vary with the duration and vigor of the activity, the number of people involved, and whether fabrics were manipulated. Fig. 4 displays representative, activity-specific concentrations for (a) large particles (>10 μm), and (b) small particles (0.3-0.5 μm). Concentrations corresponding to the smallest and largest size group illustrate the two extremes in the trend observed across particle sizes. Results are also presented for (c) carbon dioxide, to explore the usefulness of this parameter as an indicator of expected particulate matter changes with occupancy. The figure provides visual evidence of the following patterns. (i) Within each activity type, larger numbers of occupants or longer duration activity resulted in higher airborne levels of all outcome measures. (ii) The vigor of the activity was a stronger driver for particles than it was for CO_2. For example, for particle concentrations, sitting in the room for 15 min was approximately equivalent to walking for 1 min, whereas for CO_2, sitting in the room for 15 min

resulted in levels similar to walking for 5 min. (iii) For particles, the highest concentrations were observed when fabrics were handled.

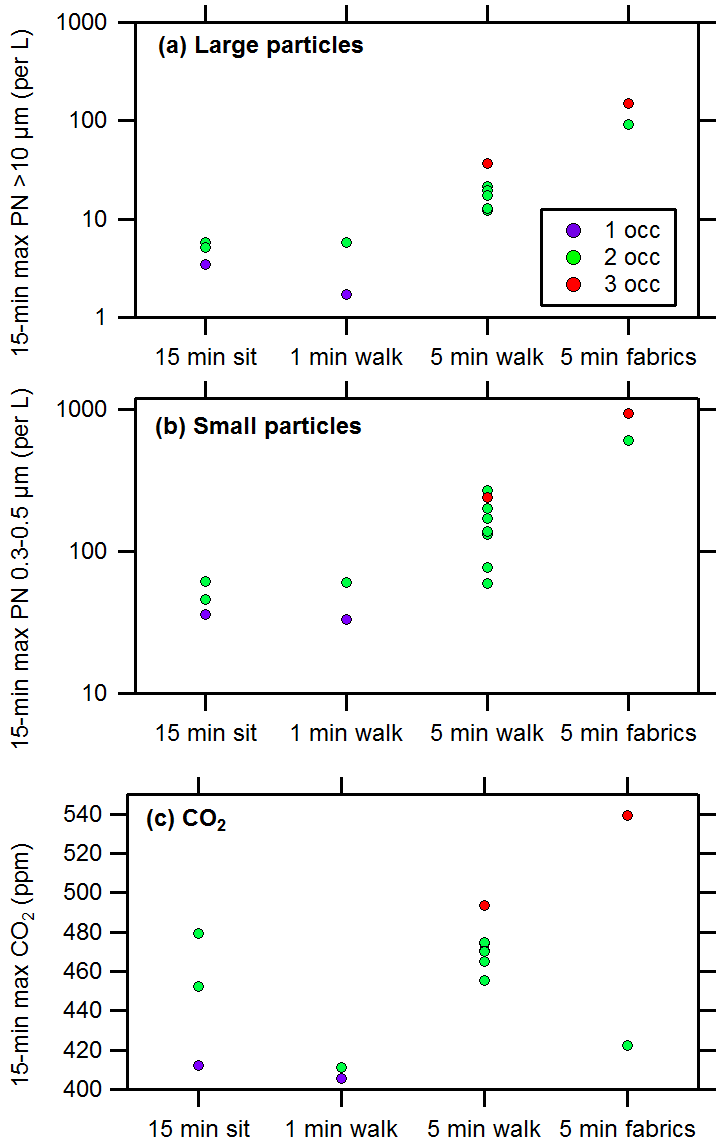

Fig. 4. Activity-specific 15-min maximum particle number concentrations for (a) large particles (>10 µm), (b) small particles (0.3-0.5 µm), and (c) CO_2. The activities are organized by type (sitting, walking, manipulating fabrics), duration (1, 5, or 15 min), and numbers of room occupants (1-3). The results indicate that the type of activity, its duration, and the number of occupants each influenced the particle number concentration.

Fig. 5 presents the net effect of experimental activities, near-room normal occupancy patterns, and changes in supply air concentrations on diurnal profiles of 1-h mean particle and carbon dioxide levels in the test room. Concentrations of large particle were strongly coupled to within-room occupancy. On average, levels were at or near zero when the room was unoccupied, and both the magnitude and variability in concentration increased in relation to the number of people in the room. This outcome measure emerged as the most sensitive indicator of within-room occupancy. Carbon dioxide levels remained elevated during the early evening after the room was vacant. This finding is expected: recirculated air contributes substantially to indoor levels of CO_2 but not of particles. Hence, elevated CO_2 levels in a patient's room above that in outdoor air may reflect both the contributions from local occupancy as well as distributed occupancy throughout the NICU.

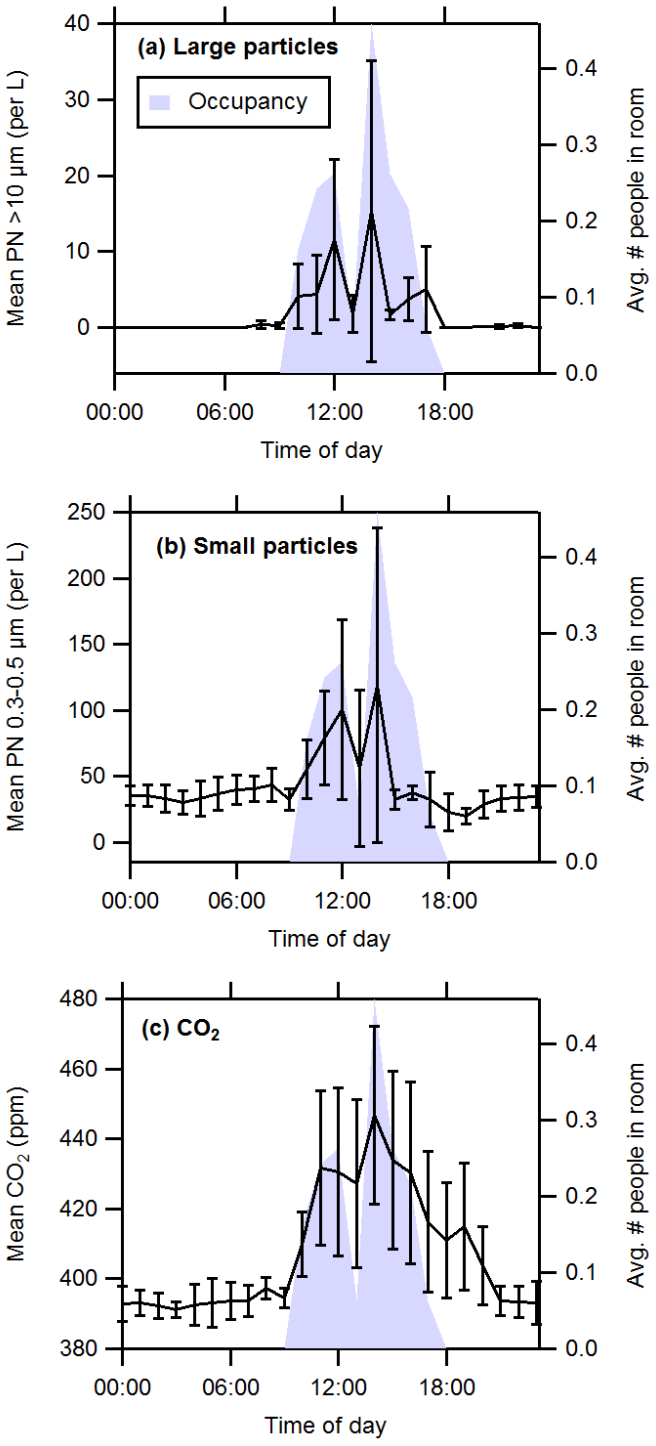

Fig. 5. Diurnal profiles of 1-h mean (± standard deviation) concentrations of (a) large particles (>10 µm), (b) small particles (0.3-0.5 µm), and (c) CO_2 in the test room, over the full sampling period. The patterns in variability across occupied and unoccupied periods, and day and night, are suggestive of the relative role of source groups in influencing each outcome measure.

A substantial body of prior research has linked human movements, especially walking, to particle and bioaerosol emissions via shedding and resuspension [30–32]. Whyte et al. [33] and Hathway et al. [34] showed that microbes originating on the floor or on human surfaces in hospitals are released into the air and redistributed onto room surfaces via walking and caregiver activities. McDonagh and Byrne [35] and Spilak et al. [36] showed that clothing, blankets, and other textiles release large numbers of submicron and supermicron particles when manipulated. Several studies have demonstrated substantial emissions of airborne bacteria from fabrics. Davies and Noble [37] and Doig [38] demonstrated that used bedding and clothing were laden with bacteria that were released during activities such as bedmaking. The bacteria were carried on heterogeneous particles comprising desquamated skin and (less commonly) textile fabrics [37]. Clothing has been demonstrated to facilitate the detachment and release of skin flakes via friction [38]. In some cases, gowns worn over clothing suppressed emissions [39,40], but typical loose cotton gowns were found to be ineffective barriers [41,42].

3.5. Comparison with a second NICU

The potential applicability of our findings to other similar environments was assessed via a second investigation conducted in a NICU located in eastern half of the US. Physical features were similar to the first NICU, including a private-style configuration, HEPA filtration of supply air, no exterior windows, and hard tile flooring. The rooms had incubators of the same make and model. The experimental design was similar in terms of instruments, sampling locations, measurement frequency, and study objectives. However, access to the site was more constrained owing to hospital operation considerations. We were able to conduct fewer experiments; and outdoor air was not sampled. Results from the second NICU are compared with findings from

the primary study site considering several attributes: average indoor concentrations during a 24-h sampling period, characteristics of the incubator, and observed effects of simulated activities.

Average values and variability patterns of sampled parameters were similar between the two NICU sites. During a 24-h sampling period in the second NICU, the temperature and relative humidity were relatively stable with means of 23.3 ± 0.4 °C (range: 23-25 °C) and 42 ± 1% (range: 39-44%), respectively. The range in measured indoor CO_2 levels was 390-640 ppm. At both sites, minimum particle levels were effectively zero for size fractions larger than 1 μm. But at the second site, the mean and maximum levels of particles were somewhat higher than at the first site (Fig. S2).

The incubator air-exchange rate, evaluated through a single test at the second NICU site, was ~ 6 per hour. As shown in Fig. 6, the incubator/room PN concentration ratio was similar at the small end of the particle size range evaluated (i.e., 0.72 for particles 0.3-0.5 μm in diameter). However, the concentration ratio for the second incubator did not vary systematically with particle size. A visual inspection of the incubator revealed numerous open ports that might have facilitated the entry of air bypassing the filters; such bypass would contribute to a weakened size-dependent response for the inside/outside concentration ratio. As with the first incubator, the humidification process was observed to be a strong source of particles smaller than 5 μm, resulting in marked increases in incubator/room PN concentration ratios when it was utilized (Fig. 6).

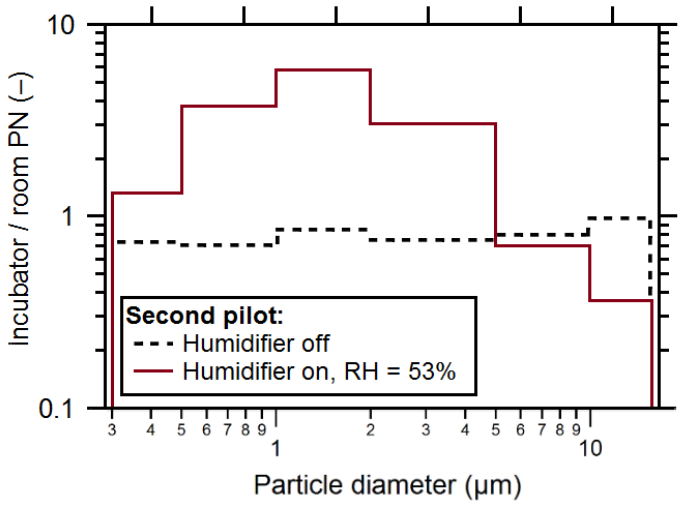

Fig. 6. Size-resolved incubator/room PN concentration ratios evaluated at the second NICU site. When the humidifier was off, incubator/room PN concentration ratios were consistently less than one. At the primary (but not the secondary) site (Fig. 2), the ratio decreased with particle size. At both sites, humidification was associated with a strong increase in the ratio for particles smaller than 5 µm. (At the second site, this effect was muted for particles 0.3-0.5 µm because of the high baseline concentration for this size fraction). For the largest size channel of the optical particle counter we adopted an upper limit of 15 µm diameter.

Within-room activities caused short-term increases in particle and CO_2 levels in the second NICU as they did at the first. Time series of the response (at 1-min resolution) to two activities are presented in Fig S3. These activities were tested: (a) one person entering the room for 5 min to check instruments; and (b) one person shaking a jacket in the room for 20 s before exiting. Results show that deliberately manipulating fabrics (even for a short period) led to strong increases in PN concentrations that were many times higher than levels associated with entering the room for instrument maintenance. Since the baseline levels of the smallest particle size group were higher at the second NICU compared with the first, the particle concentration signal associated with researcher activity was not as clearly discernible in the submicron particle range at the second site.

4. Conclusions

This study highlights several distinctive aspects of the environment inhabited by infants housed in incubators in neonatal intensive care units. The baseline intrusion of outdoor particles in the patient's room at two NICU sites studied was low owing to effective HEPA filtration of mechanical ventilation supply air. Despite supplemental filtration, particle concentrations inside an infant incubator were a substantial fraction of those in room air and ranged from 0.17 for particles larger than 10 μm to 0.62 for particles with diameter 0.3-0.5 μm. Against this low background, the NICU environment was sensitive to the influence of indoor emissions. Two distinct indoor sources were identified and investigated: occupants and the incubator humidifier. When operating, the humidifier significantly increased concentrations of particles smaller than 5 μm inside the incubator. It had no significant influence on within-room concentrations. Occupant movements conducted outside the study room, in close proximity to the open doorway, were inferred to be the cause of elevated baseline I/O ratios in the room, especially for supermicron particles. These near-room activities contributed strongly to within-room levels of supermicron particles relative to outdoor air, but their influence was small compared to most within-room activities that were the strongest driver of short-term increases in particle and CO_2 levels. Activity-specific particle concentrations were shown to be a function of the vigor and duration of the activity, the number of occupants, and whether the disturbance of fabrics was involved. Because of the low baseline contribution from outdoors and near-room activities, perturbations associated with even brief entries and exits were typically discernible. The relative strength of occupancy effects increased with particle size; yet, for most activities the signal was detectable down to the smallest particle size measured, 0.3-0.5 μm optical diameter. Our study findings show that supermicron particle levels, passive infrared occupancy sensors, and CO_2 levels can

each be utilized as distinct indicators of the extent and type of human occupancy and activity strength in NICU rooms. They further contribute to the observations by Ramos et al. [43] on the utility of beam-break counts and "room source CO_2" data for inferring occupancy patterns in hospitals.

Findings from the primary study site were generally corroborated by observations from a second NICU. Two distinctive aspects were observed at the second site: (a) its incubator filtration system proved to be less effective than at the first site, probably because of bypass associated with modifications that created larger openings in the incubator shell; and (b) it displayed significantly higher baseline concentrations of submicron particles in the patient room. The second site was located in a more highly urbanized environment than the primary site, and it had an active construction site nearby. We surmise that higher levels outdoors probably caused the indoor baseline submicron particle concentrations to be higher.

These investigations were conducted in an inactive room to enable deliberate investigations into the particle response to specific activities and to enable us to study characteristics of the infant incubator. Future work will draw on lessons learned from this pilot study, to investigate particle sources and dynamics in NICU rooms under normal operating conditions. The ultimate goal of this line of research is to improve knowledge about the sources, dynamic behavior, and fate of airborne particles in the NICU environment so that management and control practices can be maintained at a high level, benefiting the health and welfare of the premature infants treated there.

Acknowledgements

Thanks are expressed to FG Vasiknanonte for contributing to the data collection effort. We are grateful for the cooperation of hospital staff members who facilitated monitoring at the sites.

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Appendix A. Supplementary data
Pilot Study of Sources and Concentrations of Size-Resolved Airborne Particles in a Neonatal Intensive Care Unit

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Table S1. Air-exchange rate assessments for the incubator at the primary study site.[1]

Date and time (2013)	a (h^{-1})
6/17 15:27	9.5
6/19 16:26	8.6
6/20 11:00	8.8

[1]Estimates are based on the first-order decay of carbon dioxide, which was released by introducing dry ice into the incubator chamber for a few minutes and then removing it from the room. Coefficients of determination for the linear correlation between $\log(dCO_2)$ and time were greater than 0.99 in all instances. dCO_2 was evaluated as the difference between within-incubator and within-room levels.

Table S2. Adjustment factors from OPC side-by-side tests conducted with a reference device (OPC1). [1,2,3]

d_a size range (µm)	OPC2	OPC3
0.3 – 0.5	1.03	1.17
0.5 – 1	1.09	1.10
1 – 2	0.96	0.98
2 – 5	1.16	0.87
5 – 10	0.96	
>5		0.46
>10	0.78	

[1] The slopes are based on single parameter (intercept = 0) linear regression.
[2] Comparisons were conducted using 1-min averaged particle number concentrations (with the exception of one test, when 5-min data were used).
[3] The OPC used for outdoor monitoring (labeled OPC3) was of a different model than OPC1 and OPC2. Consequently, data from OPC1 and OPC3 were post-processed to yield matching bin size limits before being compared.

File S1. Evaluating the incubator humidifier particle emission rate

Rates of particle emissions (in µg/h) from the humidifier into the incubator were inferred from the incubator and room particle number (PN) concentrations, using a model based on the principle of material balance (equation S1). The analysis assumes that the incubator interior was well mixed, that the room air sampled was representative of the air entering the incubator, and that the incubator air-exchange rate and particle penetration efficiencies were steady.

$$\frac{dM_i(t)}{dt} = \frac{E_i(t)}{V} + M_{i,R}(t)aP_i - aM_i(t) \qquad \text{(S1)}$$

In equation S1, $E_i(t)$ is the rate of emissions of particles in the size group *i* from the humidifier into the incubator chamber, at time *t*, in units of mass per time; *V* is the interior volume of the incubator, evaluated from physical dimensions; $M_i(t)$ and $M_{i,R}(t)$ are, respectively, the PN concentrations measured in incubator and room air, at time *t*, in units of mass per volume; *a* is the incubator air-exchange rate in units of inverse time; and *P* is the particle penetration efficiency (dimensionless) associated with the incubator filtration system. The incubator air exchange rate was assessed using a tracer decay method with carbon dioxide as the tracer gas. The incubator particle penetration efficiency, *P*, was estimated as the mean incubator/room PN concentration ratio (based on 5-min average concentrations, and a total sampling duration of 5.6 h), by particle size, when the humidifier was off. Ratios from periods when the average PN level in the room was less than 1 particle per liter were excluded from the mean. We assumed that particle deposition (*k*) within the incubator was negligible compared to air-exchange (i.e., $k << a$ for all particle size groups). This assumption was based on the high incubator air-exchange rate (of about 9 h^{-1}) relative to the range of *k* values presented by Thatcher et al. [S1] for particles smaller than 5 µm (which is the largest size of interest for this analysis). However, since the incubator surface area to volume ratio is higher than observed in a typical room, we expect

values of k in the incubator to be somewhat greater than were estimated by Thatcher et al. Consequently, the emissions results presented here are lower bound estimates.

To evaluate the average rate of particle emissions associated with each humidification setting, we first integrated both sides of equation S1 and solved for the cumulative emissions, per incubator volume, from the start (t_1) to the end (t_2) of the humidification period (equation S2).

$$\int_{t1}^{t2}\left[\frac{E_i(t)}{V}\right]dt = a\int_{t1}^{t2} M_i(t)dt - aP\int_{t1}^{t2} M_{i,R}(t)dt + \Delta M_i \qquad \text{(S2)}$$

In equation S2, ΔM_i represents the change in the concentration within the incubator from the beginning to the end of the integral period. Since the integrals are carried out over the duration of humidification, starting with conditions just before the rise and ending just after the return to near baseline conditions, the value of this term is negligible. The average PM_{10} emission rate associated with each humidification level was evaluated in two steps. First, the size-specific average emission rate was assessed by means of multiplying the cumulative emissions term by volume, and dividing by the duration (t_2 minus t_1). Next, the mass emission rates for particle size groups smaller than 5 µm were summed. Emissions of particles larger than 5 µm were assumed to be zero, based on the observation that there was no significant difference between the incubator/room particle number concentration ratios with and without humidification for these size fractions.

By means of this process, a lower bound estimate for the humidifier PM mass emission rate was 1.9 µg/h. Lower bound within-incubator particle emission rates assessed from control periods when humidification was either off, or at a setpoint that was under or close to the room RH level (i.e., 30-40%), ranged from 0 to 0.1 µg/h. Lower bound emission rates associated with higher RH settings of 70-90% ranged from 1.0 to 1.4 µg/h. Therefore, our observations did not suggest that higher RH settings were necessarily associated with an increased particle emission

rate. In aggregate, these experimental results suggest that the humidifier PM emission rate was quantifiable as being at least 1 μg/h and of comparable magnitude to this value. More careful experiments would be needed to increase the quantification certainty.

Reference

S1. Thatcher TL, Lai ACK, Moreno-Jackson R, Sextro RG, Nazaroff WW. Effects of room furnishings and air speed on particle deposition rates indoors. Atmos. Environ. 2002; 36: 1811-1819.

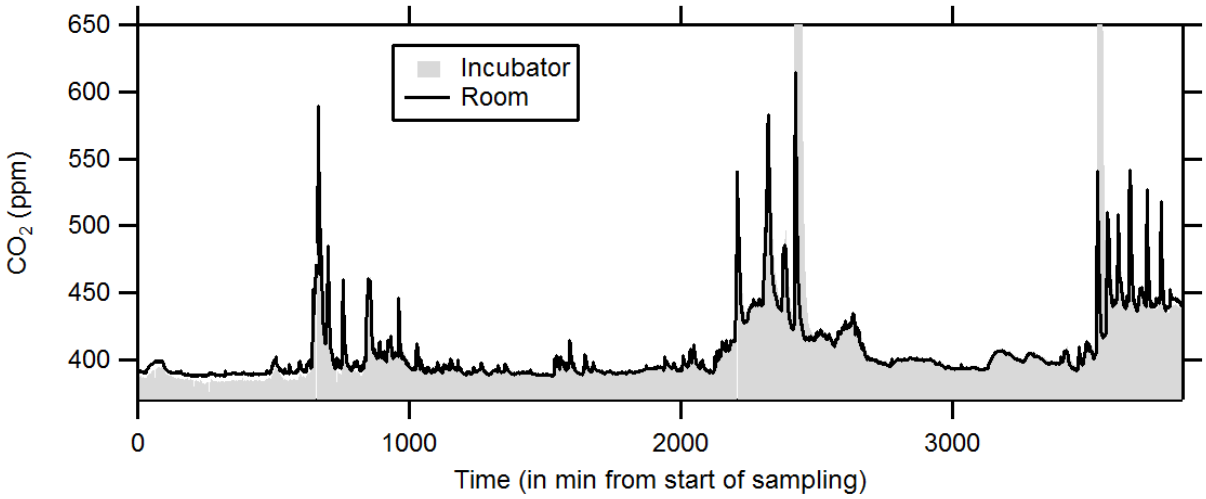

Fig. S1. Time series of CO_2 levels in the room and in the incubator. Data show that mixing ratios at the two locations tracked each other closely during typical operating conditions. Peaks in incubator-CO_2 were associated with the air-exchange rate tests and are truncated on this plot. Mean ± standard deviation CO_2 levels in the incubator and the room, during matched periods that excluded air-exchange rate tests and other incubator perturbations, were both 405 ± 24 ppm.

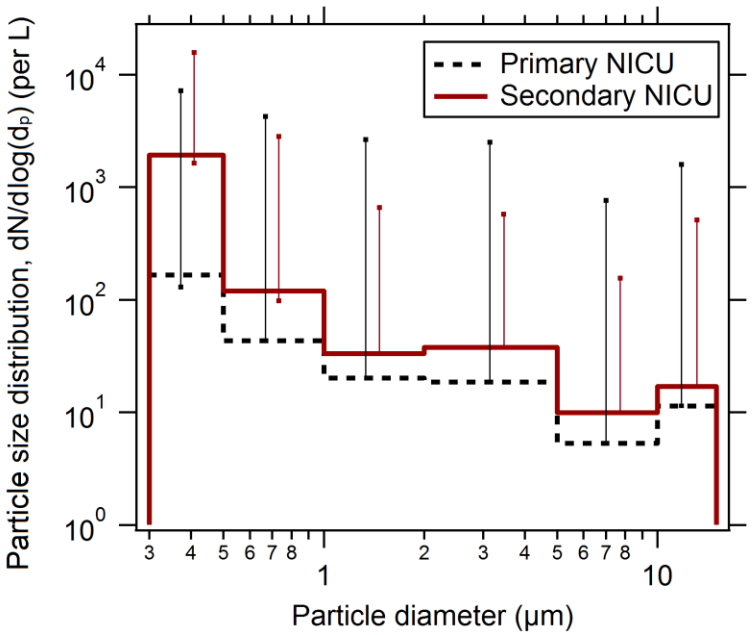

Fig. S2. Size-specific, mean (+ maximum) PN concentrations evaluated at the primary (N = 3.6 d) and secondary (N = 1 d) NICU sites. Minimum concentrations are not shown, as they were effectively zero for supermicron particles at both sites. The ranges of levels measured at the second site were bounded by the ranges at the first site for all particle sizes except 0.3-0.5 µm. For this smallest particle size group, the mean, minimum, and maximum PN concentration were each higher at the second site. For the largest size channel of the optical particle counter we adopted an upper-bound diameter of 15 µm.

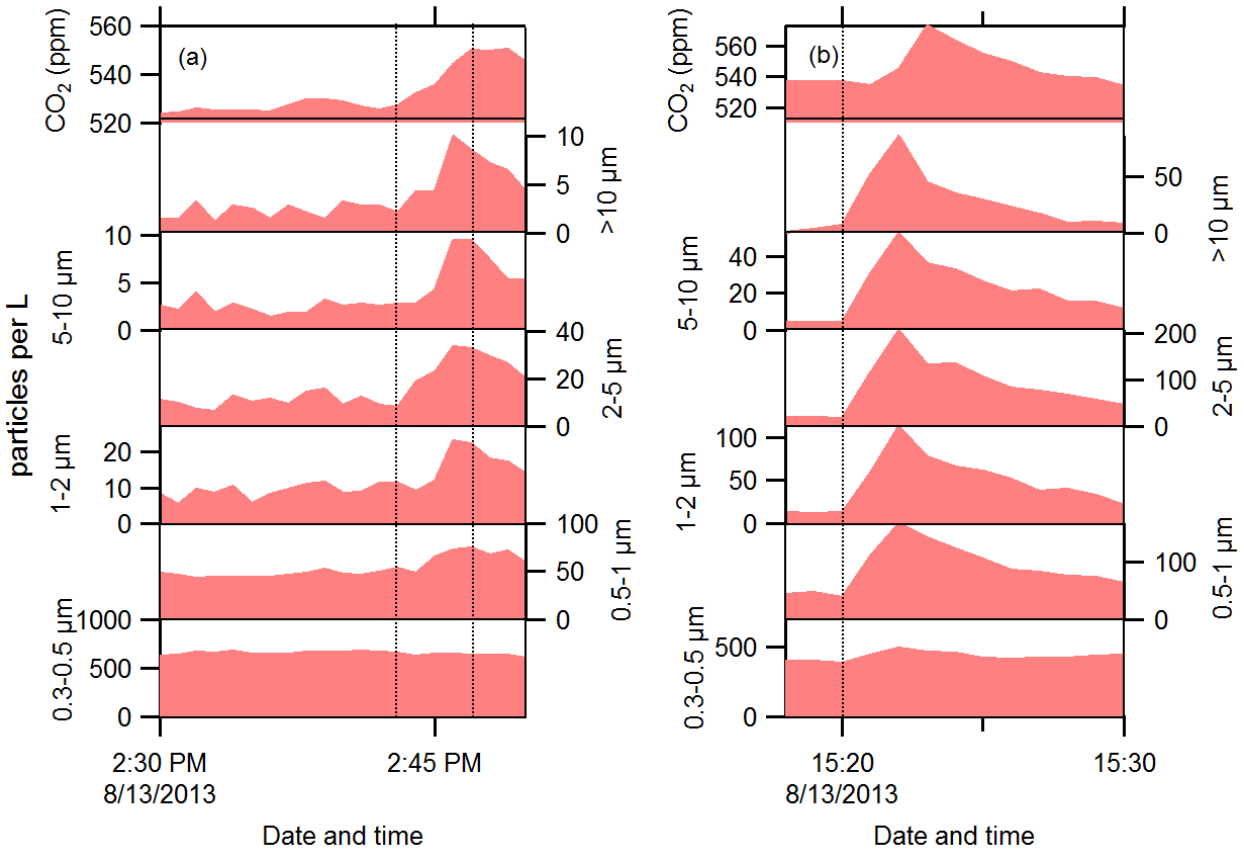

Fig. S3. Time series (at 1-min resolution) of carbon dioxide and size-specific particle number concentration at the second NICU site in response to selected activities. (a) A person checked the instruments in the room for 5 min. (b) A person shook a jacket for 20 s in the room and then exited. The dotted lines designate the start and end of the activities. Both experimental activities were associated with distinct carbon dioxide peaks, and particle concentration peaks for all sizes equal or larger than 0.5-1 µm. Owing to high baseline concentrations of 0.3-0.5 µm particles, the activities did not discernibly influence levels of this size fraction.