ABSTRACT

TITLE OF THESIS: ALCOHOL EXPOSURE IN PRETERM INFANTS IN NEONATAL ISOLETTES

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Preterm infants admitted to the NICU may spend up to 12 weeks in isolettes (incubators with controlled air temperature and humidity). Infants receive frequent contact with health-care professionals who use alcohol-based hygiene products. Ethanol is a known developmental neurotoxicant, and inhalation may have long-term effects on infant neurodevelopment. This study assessed alcohol concentration in isolette air after inserting hands cleaned with hand sanitizer, and effects of longer hand rubbing before insertion into the isolette. Each exposure consisted of two squirts (1.5 ± 0.1mL) of hand sanitizer, and hands rubbed for 10 or 20 seconds before insertion into isolettes. Air samples were collected by photoionization detector and breathalyzer. Average ethanol peaks were 387.04ppm (10s) and 104.36ppm (20s). Ethanol levels peaked within 1min, dissipated within 5min, and returned to background within 15 – 20min. Alcohol exposure from ethanol based hand sanitizer may be decreased significantly with longer duration of hand rubbing.

ALCOHOL EXPOSURE IN PRETERM INFANTS IN NEONATAL ISOLETTES

by

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Introduction

A neonatal intensive care unit (NICU) is a facility or unit within hospitals that is designed to treat premature and ill newborn babies. Preterm babies spend up to 12 weeks in neonatal isolettes, which are enclosed spaces made of significant amounts of plastics. While spending time in these isolettes, infants come into frequent contact with health-care professionals who utilize alcohol-based hygiene products before coming into contact with the infants. Ethanol-based hygiene products are increasingly used in NICUs to prevent infections. The isolettes have minimal air exchange, and it is possible that alcohol vapors from hand sanitizer build up in isolette with frequent entry into the isolettes. Ethanol is a known developmental neurotoxicant and may have long-term consequences on the neurodevelopment of these babies.

The objective of this study is to determine the alcohol concentration and persistence levels in a NICU isolette air after introduction of hands cleaned with hand sanitizer. Prior to addressing the study design, a discussion is provided of several major issues affecting the preterm infant's vulnerabilities to environmental exposures, followed by a discussion of the NICU environment that these infants are first exposed to upon their birth.

Health Risks of Neonate Alcohol Exposure

Ethanol exposure during gestation has been linked with number of harmful health effects to the neonate, including fetal alcohol syndrome (FAS) (Costa, 2004). Symptoms

of FAS include facial dysmorphologies, growth retardation, and central nervous system (CNS) abnormalities (e.g., mental retardation, microencephaly, and brain malformations) (Costa, 2004). Of particular concern are the CNS effects, as they are believed to be irreversible (Streissguth, 1991).

Research indicates that the timing of ethanol exposure during fetal brain development affects the types of effects that manifest in the neonate (Costa, 2004). Animal studies and human observations have shown that exposure to ethanol during the brain growth spurt in the third trimester of pregnancy in humans (correlating to the first two postnatal weeks in the rat) is associated with microencephaly (Samson, 1986). This effect is present in more than 80% of children with FAS (Samson, 1986). One animal study investigated the relationship between dose, blood alcohol content (BAC), and microencephaly in rats. The study found that doses of 7.4 g/kg/day and above administered during the time period of brain growth spurt in neonatal rats resulted in microencephaly. Interestingly, the researchers found that BAC varied considerably among individual animals at each dose tested, and the amount of brain growth reduction was more dependent on BAC than dose (Pierce, 1986).

One suspected mechanism of this damage is the result of alcohol metabolism. Alcohol metabolism is mediated through a number of important enzymes, including alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and cytochrome P450 (CYP2E1). Alcohol metabolism results in the generation of acetaldehyde, a highly reactive and toxic byproduct that may contribute to tissue damage. Additionally, harmful effects associated with CYP2E1-mediated ethanol metabolism are primarily related to the production of reactive oxidative species (ROS) (i.e., superoxide and hydroxyl radicals).

This ROS production contributes to alcohol-induced damage to a variety of tissues not only by causing oxidative stress but also by enhancing apoptosis triggered by various stimuli (Zakhari, 2006).

This effect is supported through animal studies in which the apoptotic response to ethanol was investigated for its role in loss of brain mass of neonatal rats, in particular during a specific developmental stage (or "window") of brain growth known as synaptogenesis (Ikonomidou, 2000). This time frame correlates to the last three months of gestation. In this study, saline or ethanol (2.5 g/kg at 0 and 2 hours; total dose 5 g/kg) was administered to infant rats. The researchers found that the brain weights of ethanol-treated rats were significantly lower than those of the saline-treated rats. This supports the hypothesis that the immature brain is vulnerable during this important developmental window (Ikonomidou, 2000).

Health effects of exposure through inhalation, however, are not well known, and safe level of exposures have not been defined for infants. For adults, the OSHA limit to ethanol in ambient air is 1,000 ppm (1884 mg/m^3) for an eight-hour period (OSHA, 2012). Limits for children have not been determined, but would likely be much less than that of adults.

Issues Affecting the Neonate

One factor affecting the susceptibility of preterm infants to environmental exposures is their underdeveloped organ systems. Of particular relevance to this study are the neonatal excretory, nervous, and respiratory systems. In regards to the excretory system, studies examining the excretion capabilities of the preterm infant are limited; however, it is believed that the body systems that detoxify and excrete chemicals are not fully

developed in infants (ATSDR, 2012). In a study of premature infants conducted in 2011, the CNS of infants born at 22–26 weeks gestation were found to be at high risk for hypoxic/ischemic brain injury and intraventricular hemorrhage (Boat, 2011). Furthermore, this study found that immature respiratory systems in extremely premature neonates results in significant long-term morbidity in survivors (Boat, 2011).

The same systems that are underdeveloped at the time of birth of preterm are rapidly developing after birth and become better able to handle environmental exposures (ATSDR, 2012). Additionally, there appears to be "windows" in which neonates are more susceptible to environmental insult (Costa, 2004). While studies examining the excretion capabilities of the preterm infant are limited, previous work investigating excretion capabilities of full term and preterm infants indicates that hemodynamic changes occur around the time of birth, which cause a significant (50 to 100%) increase in glomerular filtration rate during the first week of life (Aperia, 1983a; Aperia, 1983b). Specifically, in a study assessing alcohol elimination rates in newborns, Burd *et al* found that the ability of kidneys to excrete ethanol increases after birth. Of note, a newborn's renal excretion is more effective than renal excretion by the fetus, in part, because there is no amniotic fluid reservoir to trap and recycle the ethanol back into the newborn. As the glomerular filtration rates increase, a greater amount of ethanol can be removed from circulation (Burd, 2012). The authors acknowledge that although the changes underlying the increase in elimination capacity are not fully understood, they suspect that there are a number of physiological and environmental changes around the time of birth that contribute to the increased alcohol elimination capability.

Importantly, the intake rates of chemicals by infants are greater proportionally than that of adults. This results in children receiving an increased body burden of toxicants they are exposed to. In particular, children have increased breathing rates (per body mass), resulting in a greater intake volume of air than adults (EPA, 2011). Their skin is also more permeable, permitting more dermal exposures. Additionally, the reduced weight of infants also contributes to the potential for increased body burden of environmental exposures (ATSDR, 2012). All of these factors are compounded further by the degree of infant prematurity.

While the metabolic capability of the newborn is not fully understood, there are a number of known metabolic differences in infants. Several studies suggest that a number of metabolic enzymes undergo postnatal development (LeBel, 1988; Burd, 2012). This is important to consider when studying environmental exposures in preterm infants. As previously mentioned, ethanol metabolism is mediated through the enzymes ADH, ALDH, and CYP2E1. Metabolism of ethanol with ADH produces acetaldehyde, a highly reactive and toxic byproduct (Zakhari, 2006). Levels of these enzymes appear to differ between adults and preterm infants. One study found that mean liver concentrations of class I ADH (there are five classes) was significantly lower in perinatal infants than adults (Tran, 2007). The study also indicated that only one ADH isoform was present in the liver of perinatal infants, while several variations in were present in the liver of adults, indicating the rapidly developing metabolism of neonates. These results are consistent with previous finding reported in enzyme activity between fetuses aged two to six months in gestation, infants aged one week to seven months of birth, and children and adults aged 2–50 years (Pikkarainen, 1967).

Another study also supports this idea, finding that ethanol metabolism enzymes may be less developed for preterm infants than term newborns. LeBel et al found that reduced percentages of benzyl alcohol metabolites were present in the urine of preterm babies than newborns, indicating deficient production of these metabolites. Still, enzyme activity appears to increase upon birth, regardless of gestational age at the time of birth, suggesting infants are better able to handle environmental exposures after birth. Burd et al discussed a study (Grow, 2001) in which levels of CYP2E1 was found to increase significantly at birth. This increase in metabolic activity persisted regardless of gestational age with notable increase on the first post natal day.

NICU Environment

Infants are admitted to the NICU under varying conditions, many of which are associated with preterm birth. These conditions may be low birth weights (less than 2,500 grams [5.5 pounds]) or very low (less than 1,500 grams, or [3.25 pounds]), underdeveloped organ systems, and congenital abnormalities (*In the NICU,* 2009). These factors predispose infants to environmental exposures via the previously mentioned means (underdeveloped organ systems, increased intake rates, and reduced metabolic activity).

When the infants are brought into the NICU, they are placed in isolettes until they reach an acceptable level of health. These isolettes are plastic incubators with controlled air temperature and humidity, allowing the baby to remain at a constant temperature. It is important that the baby stay within this controlled environment as it grows and develops. The isolettes often have ports for necessary tubing such as ventilation support,

intravenous feeding, and other monitoring equipment. These ports are designed to minimize the amount of ambient air that enters the isolette and have minimal air exchange. Figure 1, below, is an image of a Giraffe Omnibed isolette, a common type of isolette used in the NICUs.

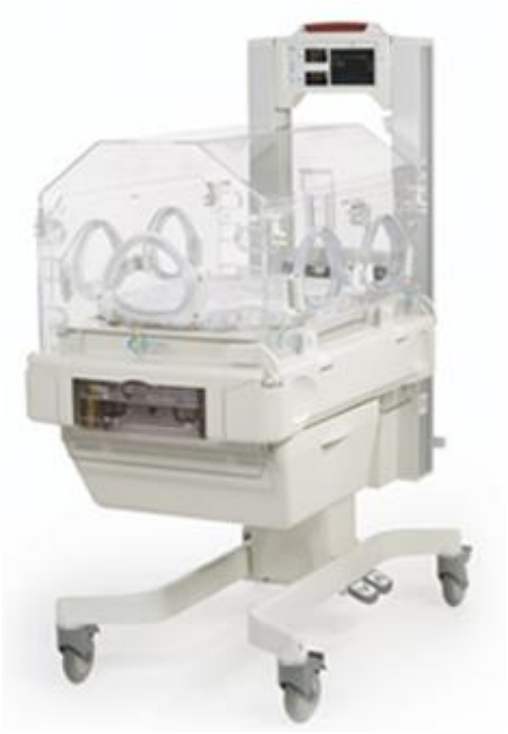

Figure 1. Image of Giraffe Omnibed Isolette.

The average infant in the NICU receive care from healthcare workers every three hours (eight times/day), with each instance of care requiring two to four hand insertions into the isolette, for approximately 24 hand insertions per day. Very sick babies may receive more care (and therefore, more hand introductions) over the course of a day. Additionally, infants remain in the isolettes for varying lengths of time, again depending on their health and prematurity at the time of their birth. More stable babies may stay in isolette for up to 10-12 weeks, while a 25 week baby may remain in the isolette up to 32-

33 weeks. Very sick and/or premature infants may be on ventilation support, so they may not be breathing the isolette air.

Air quality in isolettes is not typically monitored. Because of this, and because infants are more susceptible to environmental exposures than adults, the current study is important piece of work for this topic. One relevant study was identified that studied air quality in isolettes, in particular, that quantified volatile organic chemicals (VOC) in NICU isolettes (Prazad, 2008). In this study, two compounds, 2-heptanone and n-butyl acetate, were found at elevated concentrations inside the incubators compared with ambient room air samples. These VOCs were not found to be toxic in animal models (Lynch, 1981; David, 2001); however, some degeneration of the olfactory epithelium was found. This degeneration effect was associated with the formation of *n*-butanol and acetic acid (David, 2001). Prazad et al suggested that possible sources of VOCs is the isolettes include the plastic materials that comprise much of the internal surface area of typical incubators, or from the incubator's bedding materials.

In addition to the potential VOCs present in the NICU, another environmental exposure of concern is ethanol from alcohol-based hand sanitizers. Alcohol-based, waterless hand sanitizers are used frequently in hospital settings, due to their effectiveness in eliminating disease-causing microbes (Boyce, 2002). Ethanol is relatively volatile compound and evaporates readily into the air. Typical alcohol-based hand sanitizers are over 50% ethanol and since ethanol is volatile, hand sanitizer use increases ethanol concentrations in hospital air. Policy dictates that workers apply hand sanitizer prior to entering occupied isolettes, and as such, babies may receive significant exposure to ethanol. The typical guidance is for health care workers to apply hand

sanitizer, rub hands for 20 to 30 seconds (WHO, 2009) or until hands are dry (CDC, 2013), before proceeding with providing care. It is thought that longer hand rubbing allows for the evaporation of ethanol vapors, prior to hand insertion into the isolette. If healthcare workers rub their hands for less time (or their hands are still wet with sanitizer) in the attempt to provide rapid care, this may be a source of ethanol exposure to neonates.

One study investigating ethanol concentration in hospital air as a result of using hand sanitizer found that during application on hands, ethanol vapors peaked at 20-30 seconds and reach peak concentrations of 14.3 ± 1.4 and 13.2 ± 0.7 mg/mL in the nose (Bessonneau, 2012), corresponding to 7,590 and 7,010 ppm at room temperature and pressure. Because of this direct correlation with hand sanitizer use and ethanol concentration in ambient air, combined with the frequency with which alcohol is used in health care settings, high levels of ethanol may be expected in NICU isolettes.

The effects of dermal exposure to alcohol-based hand sanitizers have also been investigated. A study investigating risk of systemic effects caused by the use of alcohol-based hand sanitizers in adults found minimal amounts of propanol getting absorbed through skin during hand rubs and that risk of chronic systemic toxic effects associated with alcohol hand rubs appeared to be minimal. This study, however, may have implications for preterm infant dermal exposure alcohol-based products from health care providers. The study also did not evaluate the effects of long-term daily and frequent use of hygienic hand rubs, which are typical of health care settings (Below, 2012).

Additionally, there are noted age and racial disparities in NICU admission, including advanced maternal age (AMA) and race. According to one study, AMA women

more likely than teenaged mothers to have a pregnancy result in a NICU admission (de Jongh, 2012). This is supported by a 2011 National Vital Statistics Report, which found that a number of NICU admission disparities were noted, particularly between AMA and race. According to the report, nearly 7 percent of newborns (66.7 per 1,000) in the 27-state reporting area were admitted to a NICU in 2008. Furthermore, in women over aged 40, nearly 10% of babies born were admitted to the NICU, compared to the national average of 7%. Black infants in the same period were 40 percent more likely than white and approximately 60 percent more likely than Hispanic infants to be admitted to a NICU (Osterman, 2011). Further work has shown that Black/Non-Hispanic infants in hyper segregated areas are more likely to be preterm than in non-hyper segregated areas (Osypuk, 2008) and that Black/Non-Hispanic mothers with private insurance had increased odds for NICU admission; lower odds of NICU admission seen with Hispanic and White/Non-Hispanic pregnancies with private insurance (de Jongh, 2012).

In summary, exposure to ethanol, a known developmental neurotoxicant, in NICU is a significant concern to public health, particularly given the extreme vulnerability of these preterm and ill babies. Understanding inhalation exposure at these NICUs are important to inform successful exposure mitigation strategies. The results of our study may be able to inform policies regarding the amount of necessary for hand rubbing during sanitizer application prior to inserting hands into to isolettes. It is hoped that the results of this study could aid in quantifying the effects of NICU admission disparities.

Materials and Methods

This study investigated alcohol levels in unoccupied isolette units over three (3) days. In particular, data was collected on the amount of alcohol present in the isolette environment after insertion of handing over a 30 minute as well as the duration and persistence of alcohol in the isolette environment after insertion of hands over the course of each trial.

In this study, alcohol level within isolettes were determined using unoccupied Giraffe Isolettes (Giraffe Omnibeds) located within the NICU at Mercy Hospital in Baltimore, Maryland. These isolettes were set to maintain an air temperature of 36.5°C, and contained a bed wrapped in baby blankets, and medical equipment monitor leads. To mimic the process of nurse/attending physician's use of hand sanitizer, each exposure consisted of two squirts (1.5mL + 0.1mL) of hand sanitizer (EcoLab Quik-Care Foam Waterless Hand Sanitizer) applied into the palms of the hands. Following the application, hands were rubbed for either 10 or 20 seconds, and then placed into the isolette through ports designed for healthcare worker use. Hands were placed inside the isolette for 5 min to mimic performing various tasks and all port doors remained closed while hands were not placed in the isolette. Exposures occurs at 30-minute intervals, and background levels were assessed before initiation of hand insertion on all days.

For the three (3) days of this study, each day consisted of 16 trials. Each trial consisted of applying equal amounts of hand sanitizer, inserting hands for 5 minutes and collecting data over the subsequent 25 minutes for a total exposure time of 30 minutes. Air samples were collected through the apertures designed for leads on medical devices. Air samples were collected prior to the insertion of hands and throughout the 30 min after

the hands were placed into the isolettes (specific sampling frequency is described in detail, below).

Alcohol Levels

Real time alcohol data (average in 5-second intervals) was collected inside the isolette using a photoionization (PID) detector (MiniRAE3000, 10.6 eV) that is sensitive to volatile organic compounds (VOCs). Air was sampled through Tygon tubing attached to the PID pump. The tube was supported by a moveable arm inside the isolette and placed so that the tube inlet was ~16.5 cm above the infant bed, at the head-end of the bed. The PID sampled isolette air on all three days of trials.

To validate the results from the PID, a supplementary method was used to determine ethanol concentrations in the isolettes. Air from the isolette (~1.5 L) was drawn out from the isolette using a 3-liter syringe and pumped into a breathalyzer (Drager Alcotest 6510) calibrated for 0.5-liter minimum detection volume and 30-second response time. Air was drawn at the time of hand insertion, every 2 minutes for the first 10 minutes post insertion, and every 4 minutes for the subsequent 20 minutes for each exposure. This procedure was performed on Days 2 and 3 of the study, for the first 10 trials of each day. The unit's detection range for ethanol is 3-300 ppm.

Passive alcohol monitoring badges (Vapor-Trak Alcohol Monitors [KEM Medical Products]) were placed in the isolette for eight-hour periods. In the isolette, each monitor was clipped to a moveable arm, and placed so that the monitor center was ~8.5 cm above the infant bed, at the head-end of the bed. After an eight-hour exposure, each monitor was placed in the device's foil bag, as directed by the manufacturer, and mailed to the manufacturer for laboratory analysis. Laboratory analysis came from KEM Medical

Products, who report a detection range of 0.02 to 1000 ppm for exposures ranging from 15 minutes to 8 hours. This data was to be used to determine a time weighted average of ethanol present in the isolette and to serve as another means to validate the PID and breathalyzer data.

Statistical Analysis

PID response is not specific to alcohol; rather it reports data in isobutylene equivalents. Thus, the application of a correction factor of 12 is applied to the data to generate ethanol levels as per manufacturer's recommendation (RaeSystems, 2010). Table 1 illustrates the steps to convert the PID results from isobutylene equivalents to ethanol levels in ppm.

Table 1. Methods to convert PID reading to ppm of ethanol in air.

Step	Conversion
1. PID reading in ppb of isobutylene equivalents → ppm of isobutylene equivalents	PID reading / 1000
2. ppm of isobutylene equivalents → multiplied by the compound of interest's correction factor	Result from step 1 x 12

Prior to analyzing any data, it was noted that the PID read a number of extremely high readings (more than 5 orders of magnitude of the highest peak) over short periods of time (between 30 seconds and 1 minute). These readings were deemed to be equipment errors and were excluded and declared "missing" in the data set prior to statistical analysis.

Similar to the PID, breathalyzer readings also required the application of correction factors. Breathalyzer readings were in BAC%; as such, the data required correction to parts per million (ppm) of ethanol in air. Table 2 shows the conversion that

was used to convert BAC% to ppm of ethanol in air. This method was validated by the breathalyzer manufacturer.

Table 2. Methods to convert breathalyzer reading from BAC% to ppm of ethanol in air.

Step	Conversion
1. Breathalyzer reading → grams of ethanol (EtOH) per mL of air	Breathalyzer reading / 10^2/ Blood:air ratio (2100, obtained from breathalyzer manufacturer)
2. Grams of EtOH per mL of air → moles EtOH per L air	Result from step 1 / molar mass of EtOH (46.07 g/mol)
3. Moles EtOH per L air → moles EtOH per mole air	Result from step 2 x Molar volume of air at 1 atm (L) (24.496004)

PID and breathalyzer data were analyzed using Microsoft (MS) Excel for descriptive statistics (minimum peak, maximum peak, standard deviation of peaks, and average peaks). Results were also tested to ensure they were statistically different from zero. Data was plotted over time to show any conspicuous patterns. Potential patterns expected include, consistent peaks among all exposures for 10- and 20-second hand rubs, approximate peak heights, duration of peaks, and whether alcohol concentrations return to background levels prior to subsequent exposures.

Ethanol levels from the PID and breathalyzer were analyzed using STATA 11 (StataCorp LP) to determine statistical differences between observed alcohol levels for background, 10-second hand rubs, and 20-second hand rubs. To determine the appropriate statistical tests (i.e., parametric vs. nonparametric), the data must be examined for normality. To examine the data for normality, histograms were generated to generate initial thoughts on the normality (or lack of normality) of the data. Subsequently, the data were tested for normality using the Shapiro-Wilk test. This test hypothesizes that the data is normally distributed. If the data are normally distributed,

analysis of variance (ANOVA) would be used to determine if PID data are statistically different between background, 10-second, and 20-second hand rub groups.

If the data fail the Shapiro-Wilk test for normality, nonparametric tests would be used to determine statistical differences between observed alcohol levels for background, 10-second hand rubs, and 20-second hand rubs. Two nonparametric tests identified for potential utility are the Kruskal-Wallis and Wilcoxon ran-sum tests. The Kruskal-Wallis test is a rank-based, nonparametric test for comparing two or more independent samples. The Kruskal-Wallis test is generally used when there is one independent variable with two or more levels. An additional test that may be employed is the Wilcoxon rank-sum test, which is a non-pairwise comparison test.

Risk Assessment

The last step is to generate useful information on the potential dose of ethanol received by infants in the isolette. To do this, average daily dose can be calculated. Average daily dose is the average dose over a pathway-specific period of exposure, expressed on a per-unit-body-weight basis using the following equation:

$$ADD = (C \times IR)/BW$$

Where ADD = average daily dose; C = concentration; IR = intake r(of air/breathing rate); BW = body weight

The information needed for this calculation is concentration of the alcohol. Daily time-weighted averages (TWA) of alcohol concentrations in the isolette were calculated using the breathalyzer data. Any measurements of 0.00 were replaced with half the level of detection (LOD) of the breathalyzer (LOD = 3 ppm, therefore ½ LOD = 1.5 ppm).

TWAs were calculated for both 10 and 20 second hand rubs, and for different numbers of hand insertions per day using the following equation:

$$TWA = \frac{C1T1 + C2T2 + \cdots + CnTn}{1440\ minutes}$$

Additional factors for the calculation of average daily dose include intake rate and body weight. Intake rate was taken from the Exposure Factors Handbook, Chapter 6, which indicated infants from birth to one month have a mean breathing rate of 3.6 m^3/day (95^{th} percentile 7.1 m^3/day). The definition of low birth weight (less than 2,500 grams [5.5 pounds]) or very low birth weight (less than 1,500 grams, or [3.25 pounds]) for body weight was also used in average daily dose calculations.

Infants in the NICU receive varying amounts of care, based on their health and prematurity at the time of their birth, making estimations of exposure more complicated. To help take into account the variables associated with the infant's intake rate of isolette air, Oracle Crystal Ball, Release 11.1.2.2 software was used to define parameters around the components of our average daily dose equation (concentration, intake rate, and body weight) and to forecast average daily dose for both average and most susceptible infants in the NICU. These forecasts were generated using Monte Carlo simulation, run for 1,000 simulations. Average daily dose was evaluated for 50^{th}, 95^{th}, and 99^{th} percentiles, in addition to maximum values. Sensitivity analysis charts were generated to determine which factors (concentration, intake rate, number of hand insertions, etc.) contribute most to the average daily dose of ethanol an infant receives in the isolette.

Results

Analysis of Ethanol Concentration in Isolettes

Passive Alcohol Monitoring Badges

All passive alcohol monitoring badges yielded no detection of alcohol over all days sampled and as such are not included in any of the subsequent results, figures, and tables.

Photoionization Detector

Figure 2 shows a representative peak from each hand rub group over all trials. Criteria for selecting the peaks included, their occurrence on a day where breathalyzer monitoring was conducted, contained a high number of breathalyzer readings, lack of outliers, and average peak height for the length of hand rubbing. Alcohol concentration peaked within 1 minute of insertion of hands and quickly decreased to base line around 20 minutes.

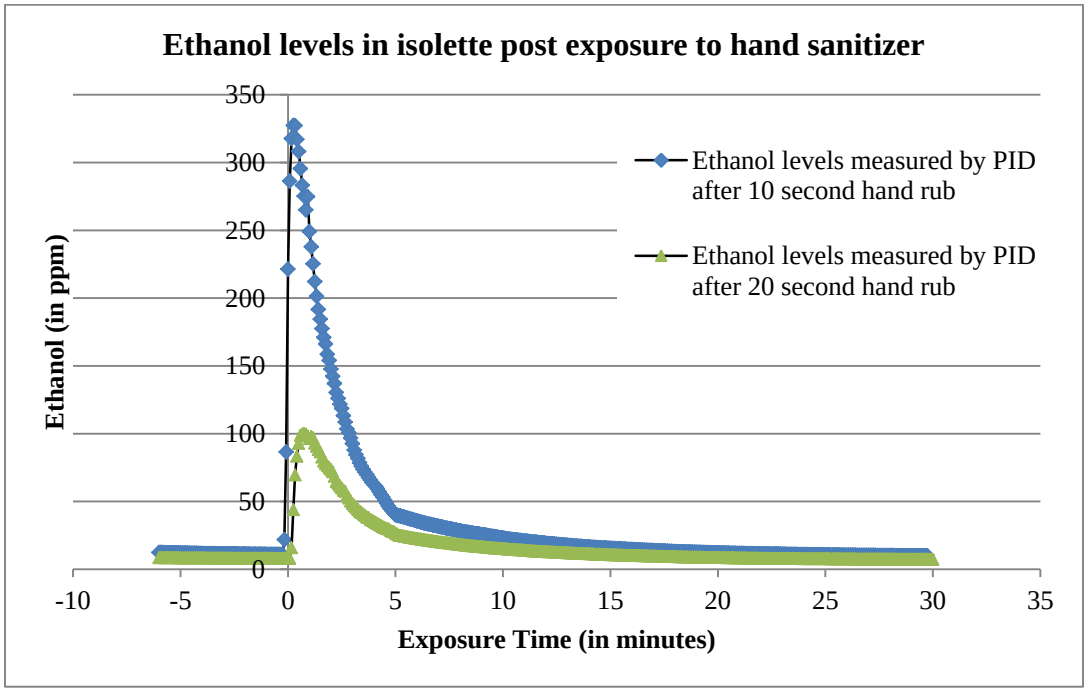

Figure 2. Example peaks of 10 and 20 second hand rubs as measured by the PID.

Over all trials, ethanol concentration peaked at 387.04 ± 191.50 ppm (range, 139.56 - 902.01 ppm) after 10 seconds of hand rubbing and 104.36 ± 50.35 ppm (range, 45.49 - 269.41 ppm) after 20 seconds of hand rubbing. All results were shown to be statistically different from zero. These results are shown below, in Table 3.

Table 3. Peak EtOH concentrations for background trials, 10 second hand rubs, and 20 second hand rubs.

	No of Trials*	Average Peak (ppm)	Range of Peaks (ppm)	Std. Deviation (ppm)	*p*-value
Background	3	6.5	2.1 – 11.2	4.6	$p<0.001$
10s	32	387.0	139.6 – 902.0	191.5	$p<0.001$
20s	16	104.4	45.5 – 269.4	50.4	$p<0.001$

*Background at the beginning of each trial day (2 days with 10 second hand rubs, 1 day with 20 second hand rubs, = 3 total days)

From these results, it appears that there are significant differences between the 0, 10, and 20 second hand rub groups. To validate this, STATA 11 was used to determine statistical differences between observed alcohol levels for background, 10-second hand rubs, and 20-second hand rubs. First, the data were examined for normality, by generating a histogram for PID results for all trials, as well as cumulatively. The histograms for each trial were similar to the shape and pattern of the histogram for all PID values. As such, this histogram of all PID values is shown in Figure 3. The histogram supports the previous observation that the data are not normally distributed, but may have a log normal distribution. A histogram of all log-transformed PID values is shown in Figure 4.

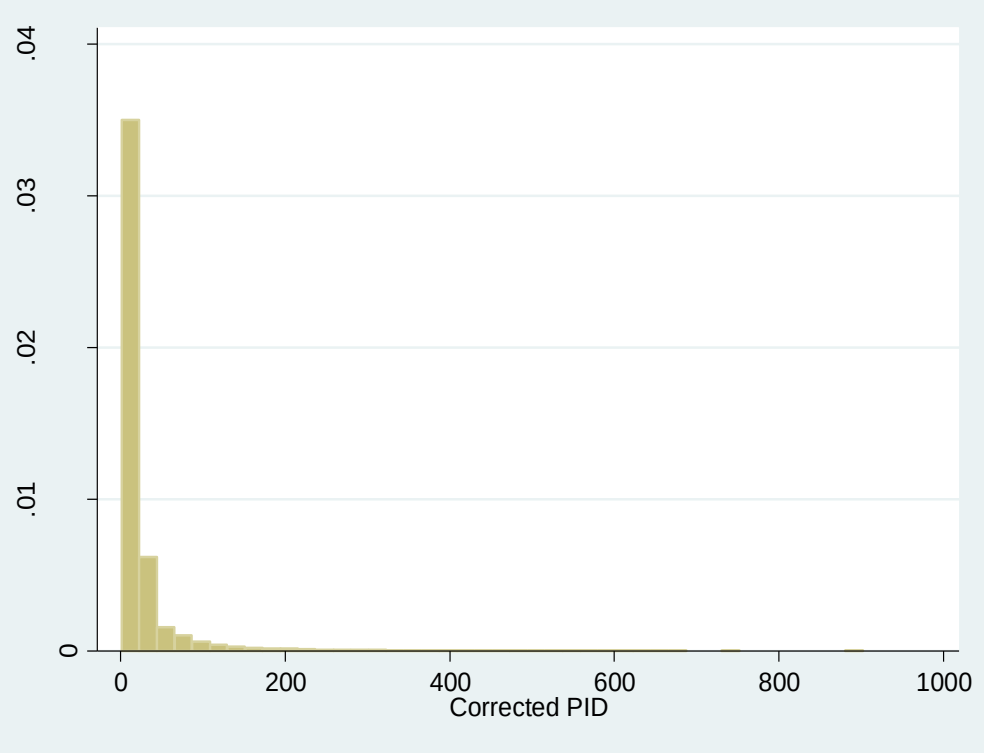

Figure 3. Histogram of values of ethanol, as measured by PID.

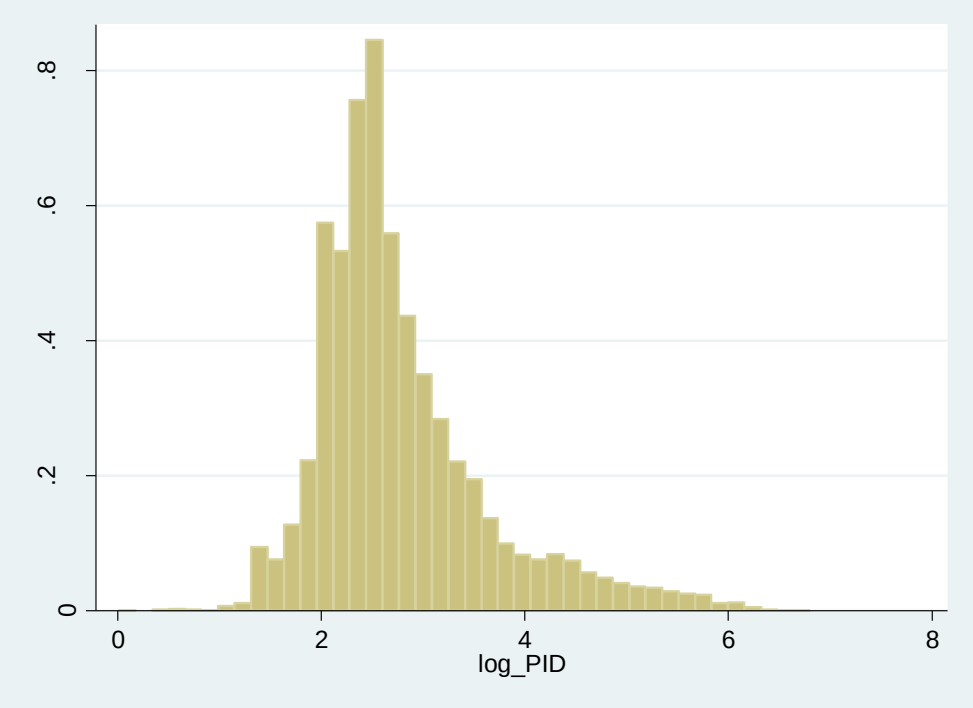

Figure 4. Histogram of log transformed values of ethanol, as measured by PID.

Subsequent analysis confirmed the lack of normality. The Shapiro-Wilk test for normality assumes that data are normally distributed. After running the Shapiro-Wilk test, the results for each trial, 10 and 20 secong groups, and all PID values (shown in Table 4) indicate that we can reject the null that the PID data are normally distributed.

Table 4. Shapiro Wilk test for normality of PID.

Exposure No.	Obs	W	V	z	Prob>z
10 second hand rub trials					
1	315	0.48222	115.192	11.168	0.00001
2	360	0.39975	150.395	11.87	0.00001
3	360	0.39893	150.601	11.874	0.00001
4	346	0.50266	120.281	11.32	0.00001
5	292	0.46089	112.138	11.064	0.00001
6	360	0.3889	153.115	11.913	0.00001
7	348	0.45854	131.624	11.536	0.00001
8	364	0.45691	137.422	11.663	0.00001
9	324	0.42416	131.355	11.492	0.00001
10	360	0.4295	142.94	11.75	0.00001
11	325	0.48726	117.284	11.227	0.00001
12	364	0.41003	149.284	11.859	0.00001
13	359	0.4817	129.54	11.515	0.00001
14	358	0.46927	132.316	11.564	0.00001
15	345	0.47569	126.477	11.437	0.00001
16	340	0.48435	122.78	11.359	0.00001
17	360	0.47584	131.33	11.549	0.00001
18	360	0.50992	122.792	11.39	0.00001
19	360	0.47927	130.47	11.534	0.00001
20	360	0.4639	134.322	11.603	0.00001
21	323	0.48222	117.788	11.234	0.00001
22	360	0.48482	129.081	11.508	0.00001
23	360	0.50373	124.343	11.42	0.00001
24	340	0.45057	130.823	11.509	0.00001
25	358	0.50994	122.178	11.375	0.00001
26	360	0.53602	116.251	11.261	0.00001
27	360	0.43784	140.85	11.715	0.00001
28	306	0.54613	98.41	10.783	0.00001
29	360	0.52096	120.025	11.336	0.00001
30	360	0.60963	97.808	10.852	0.00001
31	360	0.51495	121.531	11.366	0.00001
32	313	0.54691	100.231	10.837	0.00001
20 second hand rub trials					
1	360	0.44571	138.879	11.682	0.00001
2	360	11.747	142.739	11.747	0.00001
3	360	0.44688	138.586	11.677	0.00001
4	336	0.61266	91.262	10.653	0.00001
5	360	0.57523	106.427	11.052	0.00001
6	360	0.58767	103.312	10.981	0.00001
7	335	0.56375	102.514	10.926	0.00001
8	360	0.55321	111.945	11.171	0.00001

9	360	0.55413	111.715	11.166	0.00001
10	360	0.59603	101.216	10.933	0.00001
11	360	0.57996	105.242	11.025	0.00001
12	360	0.56484	109.03	11.109	0.00001
13	355	0.60215	98.447	10.86	0.00001
15	360	0.57141	107.385	11.703	0.00001
15	360	0.5579	111.298	11.157	0.00001
16	445	0.55319	135.346	11.735	0.00001
10 sec	11120	0.43526	3080.854	21.565	0.00001
20 sec	5971	0.56201	1352.091	19.002	0.00001
All Trials	18215	0.40191	4950.387	23.119	0.00001

Subsequent to this assessment of normality, a box plot of the PID data was generated to determine if the groups support the observation that the groups are statistically significant. Because the data appear to have a log normal distribution, a box plot was generated of log-transformed PID values for all trials, as well as for all PID readings. The box plot for all readings is shown in Figure 5. This box plot suggests that there may be statistical differences between the background, 10, and 20 second hand rub groups.

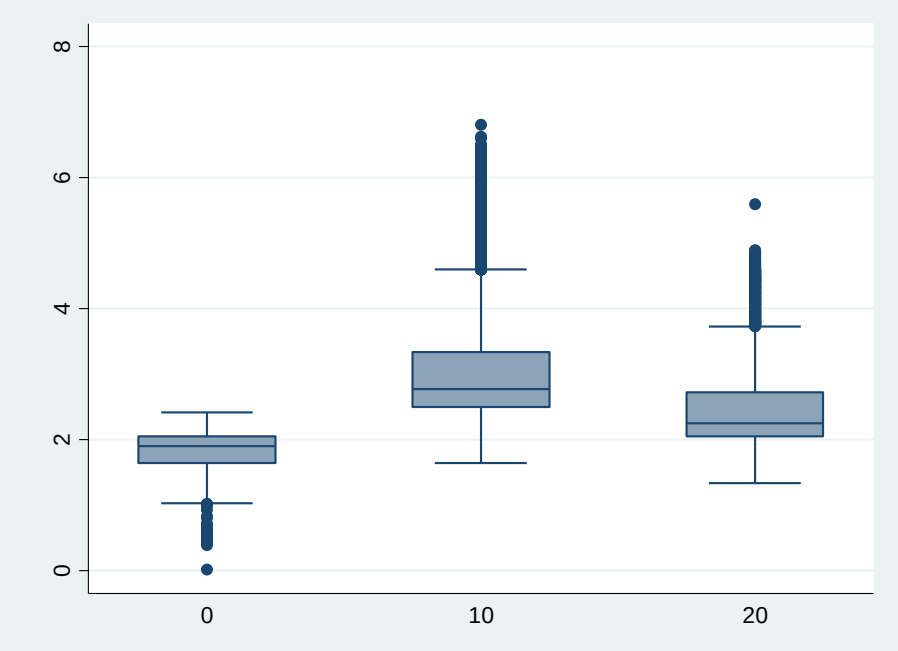

Figure 5. Box plot of log transformed values of ethanol, as measured by PID, by length of hand rub.

As such, subsequent analysis was conducted with nonparametric tests. The Kruskal-Wallis is a nonparametric alternative to ANOVA. The Kruskal-Wallis test assesses if the distributions of multiple groups (which are not normally distributed) are equal. The results of the Kruskal-Wallis equality-of-populations rank test on the PID data indicate that the 0, 10, and 20 second hand rub groups do not have the same distribution ($p<0.0001$) and are thus statistically different.

The Wilcoxon rank-sum test was then employed to assesses whether the rank for each condition (in this case, 10 and 20 second hand rubs) indicates a systematic difference between the two groups. If so, most of the high ranks belong to one condition and most of the low ranks belong to the other. For this study, the results of the Wilcoxon test indicate that the PID results were significantly affected by the length of time hands were rubbed after applying hand sanitizer ($p<0.00001$).

Breathalyzer

As previously mentioned, the breathalyzer was used to validate the readings from the PID. Figure 6 shows a representative peak from each hand rub group over all trials. For ease of comparison, they are the same peaks selected in Figure 2, above. Alcohol concentration peaked within 2 minutes of insertion of hands and decreased to base line by 15 minutes.

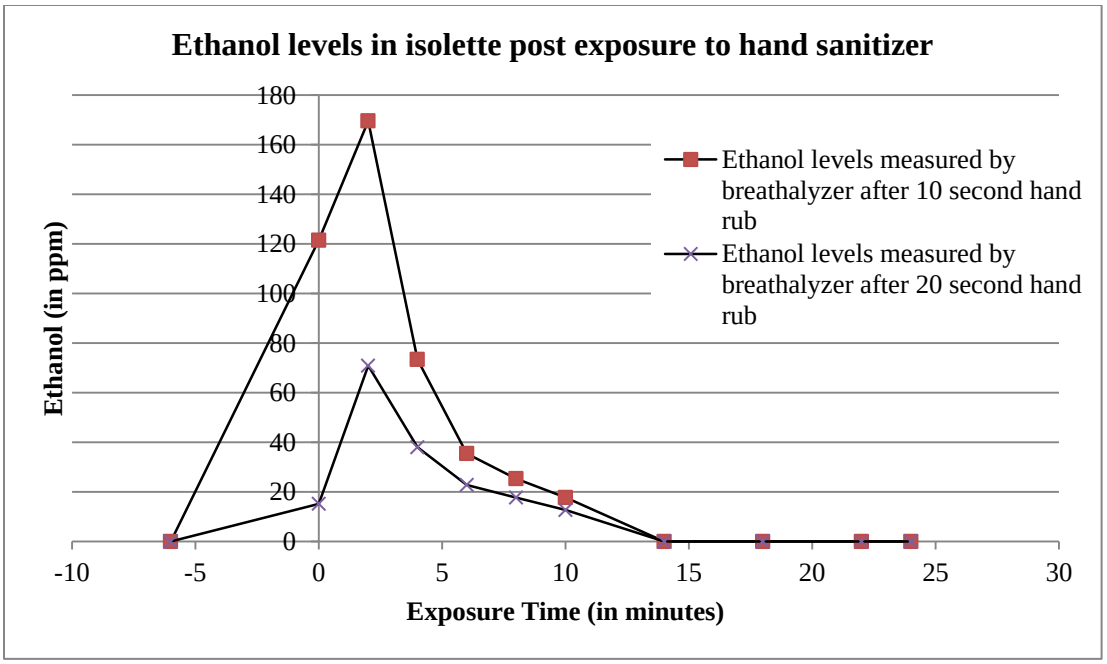

Figure 6. Example peaks of 10 and 20 second hand rubs as measured by the breathalyzer.

The breathalyzer has a much lower time-resolved sampling rate and as such did not provide as many data points and was less effective at characterizing peak ethanol levels within the isolette. Still, the data were assesses in the same manner as the PID data.

As shown in Table 5, ethanol concentration peaked at 141.8 ± 50.7 ppm (range, 78.5 – 250.7 ppm) after 10-second hand rub and 49.9 ± 16.2 ppm (range, 27.9 – 76.0 ppm) after 20-second rub. Alcohol concentration peaked within 1 minute of insertion of hands and quickly decreased to base line by 15 minutes. Breathalyzer samples of the background period (prior to the initiation of daily trials) were not obtained for any of the days.

Table 5. Peak EtOH concentrations for background trials, 10 second hand rubs, and 20 second hand rubs.

	No of Trials*	Average Peak (ppm)	Range of Peaks (ppm)	Std. Deviation (ppm)	*p*-value
Background	N/A	N/A	N/A	N/A	N/A
10s	10	141.8	78.5 – 250.7	50.7	$p<0.001$
20s	10	49.9	27.9 – 76.0	16.2	$p<0.001$

*Background at the beginning of each trial day (2 days with 10 second hand rubs, 1 day with 20 second hand rubs, = 3 total days)

From these results, it appears that there are significant differences between the 0, 10, and 20 second hand rub groups. To validate this, STATA 11 was used to determine statistical differences between observed alcohol levels for 10-second hand rubs, and 20-second hand rubs (recall that no breathalyzer readings were taken during the background sampling time period).

First, the data were examined for normality, by generating a histogram for breathalyzer results for each trial, as well as cumulatively. The histograms for each trial were similar to the shape and pattern of the histogram for all breathalyzer values. As such, this histogram of all breathalyzer values is shown in Figure 7. Similar to the histogram for PID data, the histogram of breathalyzer data supports the previous observation that the data are not normally distributed, but may have a log normal distribution. A histogram of all log-transformed breathalyzer values is shown in Figure 8.

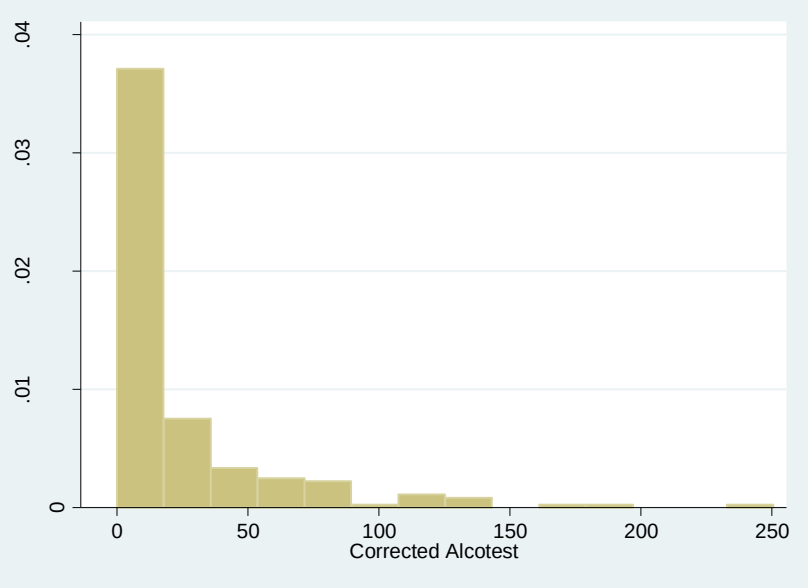

Figure 7. Histogram of values of ethanol, as measured by breathalyzer.

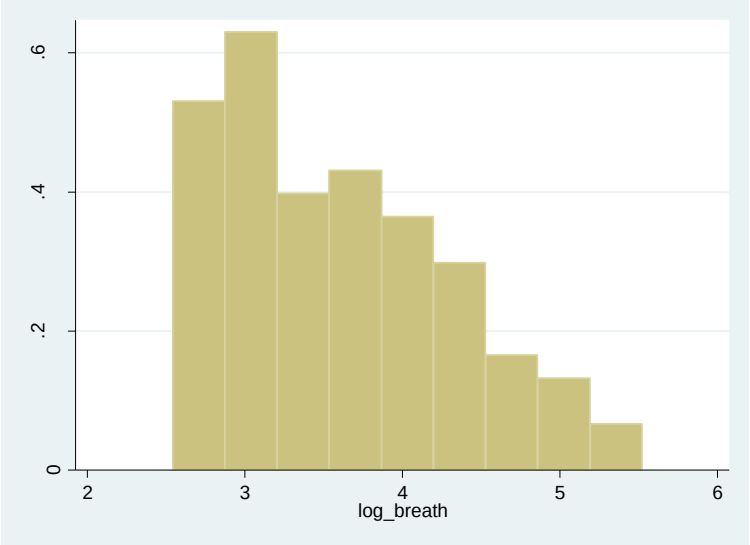

Figure 8. Histogram of values of ethanol, as measured by breathalyzer.

Subsequent analysis using the Shapiro-Wilk test for normality indicate that we can reject the null hypothesis that the breathalyzer data are normally distributed for each hand rub group. The results for each trial, 10 and 20 second groups, and all breathalyzer readings are shown in Table 6, below.

Table 6. Shapiro Wilk test for normality of Breathalyzer.

Exposure No.	Obs	W	V	z	Prob>z
10 second hand rub trials					
1	10	0.81405	2.866	2.024	0.02147
2	10	0.73065	4.151	2.867	0.00208
3	10	0.78982	3.239	2.294	0.01090
4	10	0.74832	3.879	2.706	0.00340
5	10	0.81192	2.898	2.049	0.02023
6	10	0.7897	3.241	2.295	0.01087
7	10	0.83404	2.558	1.781	0.03742
8	10	0.77492	3.469	2.448	0.00718
9	10	0.81661	2.826	1.994	0.02306
10	10	0.77898	3.406	2.407	0.00804
20 second hand rub trials					
11	10	0.75003	3.852	2.69	0.00357
12	10	0.89205	1.664	0.92	0.17882
13	10	0.75192	3.823	2.672	0.00377
14	10	0.75782	3.732	2.616	0.00444
15	10	0.6674	5.126	3.385	0.00036
16	10	0.72264	4.274	2.937	0.00166
17	10	0.77209	3.512	2.477	0.00663
18	10	0.77672	3.441	2.43	0.00755
19	10	0.78927	3.248	2.3	0.01074
20	10	0.7558	3.763	2.636	0.00420
10 sec	100	0.85095	12.306	5.568	0.00001
20 sec	100	0.80698	15.937	6.142	0.00001
All Trials	200	0.77313	33.846	8.103	0.00001

Subsequent to this assessment of normality, a box plot of the log-transformed breathalyzer data was generated to support the observation that the groups are statistically significant for each trial, as well as for all breathalyzer trials. The box plot for all readings is shown in Figure 9. (Again, recall that no breathalyzer measurements were taken during the “background” period used in the analysis).

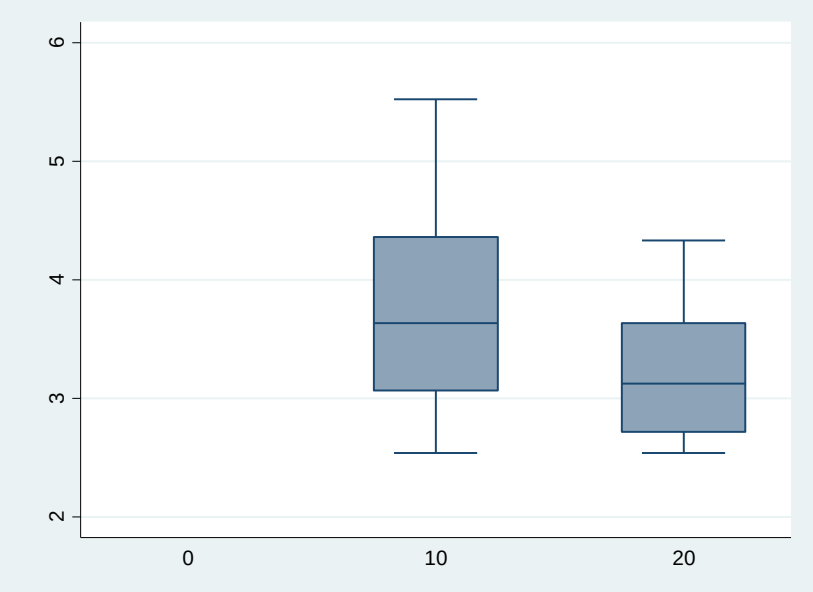

Figure 9. Box plot of values of ethanol, as measured by breathalyzer, by length of hand rub.

Similar to that of the PID, the breathalyzer data were not normally distributed and as such, subsequent analysis was conducted using nonparametric tests. The Kruskal-Wallis was used again as the nonparametric alternative to ANOVA. The results of the Kruskal-Wallis equality-of-populations rank test on the breathalyzer data for the 10 and 20 second hand rub groups indicated that that each hand rub group has different distributions ($p<0.0005$).

The Wilcoxon rank-sum test, was then used to compare the 10 and 20 second hand rub groups. Again, instead of assessing whether the means of two groups are equal, this test assesses whether there is a difference between the medians of the groups. For the breathalyzer data, the Wilcoxon test showed that ethanol levels were significantly affected by the length of time hands were rubbed after applying hand sanitizer ($p<0.0001$).

Side-by-side Comparison of PID and Breathalyzer Results

Figure 10 illustrates the PID and breathalyzer results from Days 1, 2, and 3. From this figure, the breathalyzer results appear highly correlated with the PID results over all trials, with the PID providing significantly greater time resolution of results because of its more frequent sampling rate. As such, the PID appears to give a greater resolution of the decay curve of the ethanol and a more accurate characterization of peak ethanol levels overall trials.

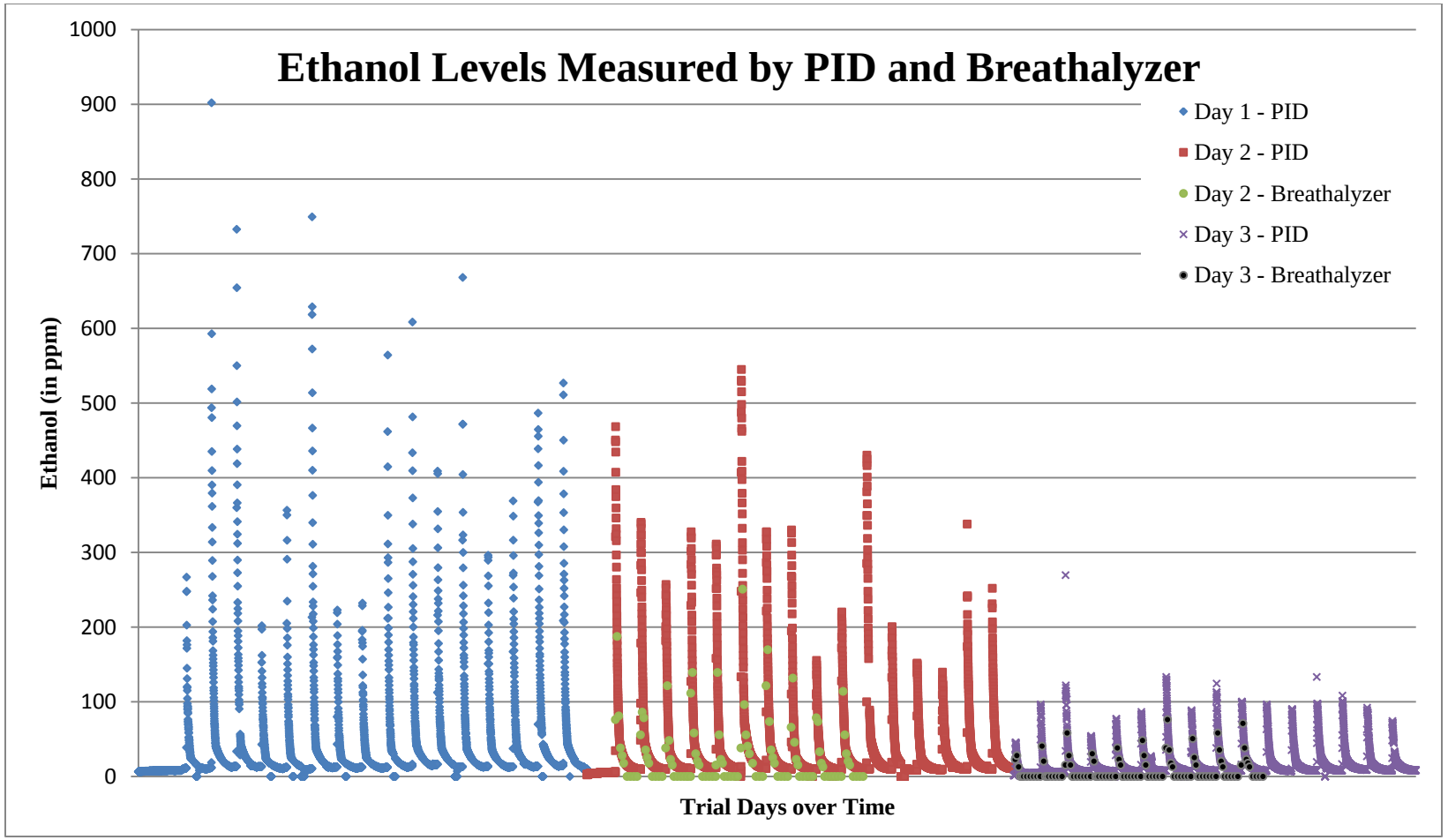

Figure 10. PID data from Day 1, 2, and 3.

To more closely analyze the peaks shown in the figure above, Figure 11 illustrates an overlay of a representative peak from a trial with 10 seconds of hand rubbing and 20 seconds of hand rubbing. Criteria for selecting the peaks included, their occurrence on a day where breathalyzer monitoring was conducted, contained a high number of breathalyzer readings, lack of outliers, and average peak height for the length of hand rubbing. Alcohol concentration peaked within 1 minute of insertion of hands and decreased to base line around 20 minutes.

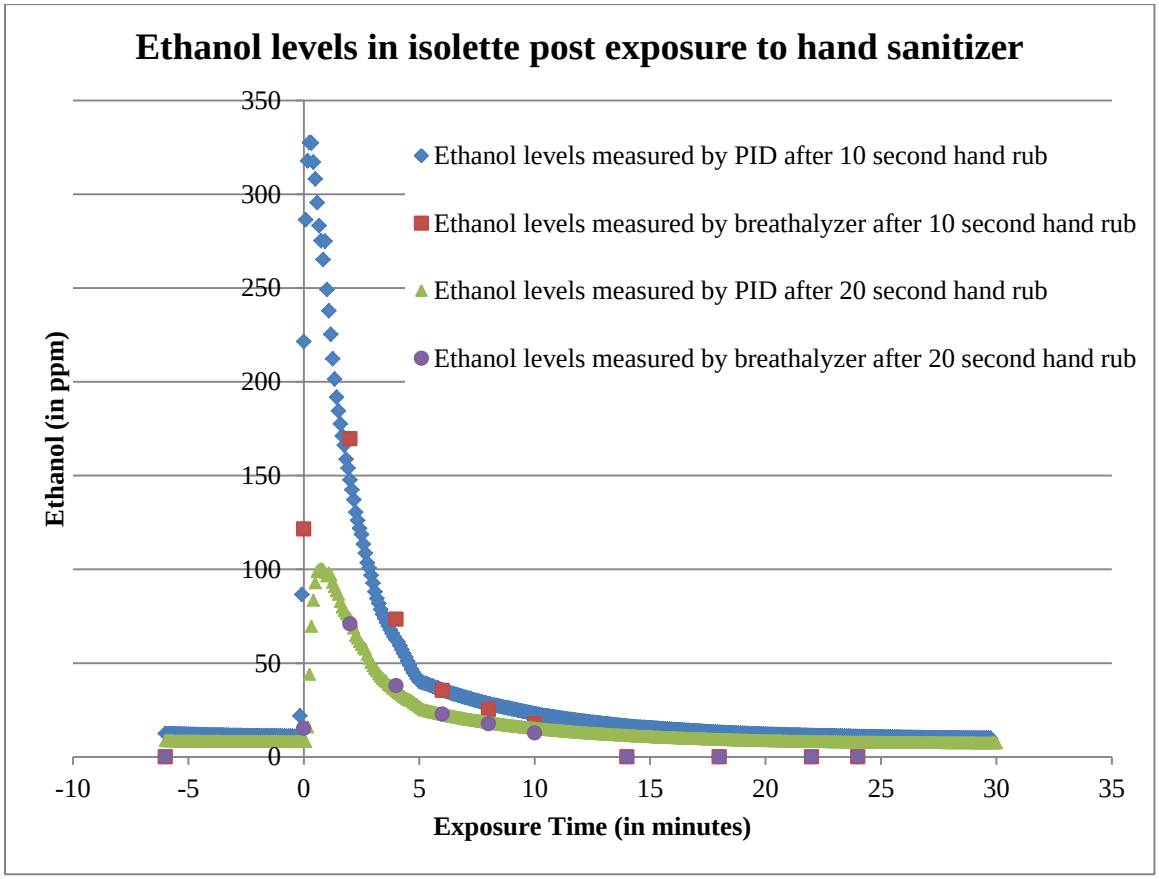

Figure 11. Example peaks of 10 and 20 second hand rubs.

Risk Assessment

The Oracle Crystal Ball was used to run Monte Carlo simulations to generate average daily dose. The software works by using a number of parameters defined around independent variables to generate a forecast of the dependent variable you are looking for. For this study, recall that average daily dose is dependent on concentration of alcohol in the isolette, intake rate of air by the infants, and infant body weight as shown in the equation below:

$$ADD = (C \times IR)/BW$$

ADD = average daily dose; C = concentration; IR = intake rate (of air/breathing rate); BW = body weight

This section describes the parameters used to calculate average daily dose.

10 second hand rub

For the value of concentration, time weighted averages were calculated from breathalyzer data for 10 second hand rubs using the methodology described above (in the Methods section). The TWA for each trial was calculated to be 6.0 ppm (11.2 mg/m^3) with a standard deviation of 14.4 ppm (27.1 mg/m^3). The intake rate was taken from the Exposure Factors Handbook, Chapter 6, which indicated infants from birth to one month have a mean breathing rate of 3.6 m^3/day (95th percentile 7.1 m^3/day). For body weight, the definition of low birth weight (less than 2,500 grams [5.5 pounds]) for body with a standard deviation of 500 grams in average daily dose calculations was used.

To calculate average daily dose, concentration in terms of TWA were input into the Crystal Ball software. Because concentration follows a lognormal distribution, parameters were defined in the model using the TWA, as well as the average and

standard deviation for concentration. In addition, concentration of ethanol in isolette air, is a dependent variable based on both the length of hand rubbing as well as the number of hand insertions into the isolette. A forecast was generated for concentration to take into account the number of hand insertions in a typical day. Using information provided by NICU subject matter experts, a typical infant in the NICU may receive care every three hours, with approximately 2 to 4 hand insertions per instance of care. As such, we assumed an average of 15 hand insertions per day, with a standard deviation of approximately 7 insertions per day. Table 7 below shows the inputs used in the average daily dose modeling.

Table 7. Crystal Ball parameter and forecast inputs, for the 10 second hand rubs.

Inputs for Crystal Ball		
Concentration		
• **Average**	11.2 mg/m^3	Standard Deviation: 27.1 mg/m^3
• **No of Hand Insertions**	15	Standard Deviation: 7
Intake Rate of Air	3.6 m^3/day	95th percentile 7.1 m^3/day
Body weight	2500 grams	Standard Deviation: 500 g

Figure 12 shows the frequency distribution of average daily dose for the 10 second hand rub. This shows a simple histogram of the frequencies of average daily dose in the model. From this, we see that individuals in the highest percentiles (95-99%) receive significantly more exposure to ethanol than those in lower percentiles.

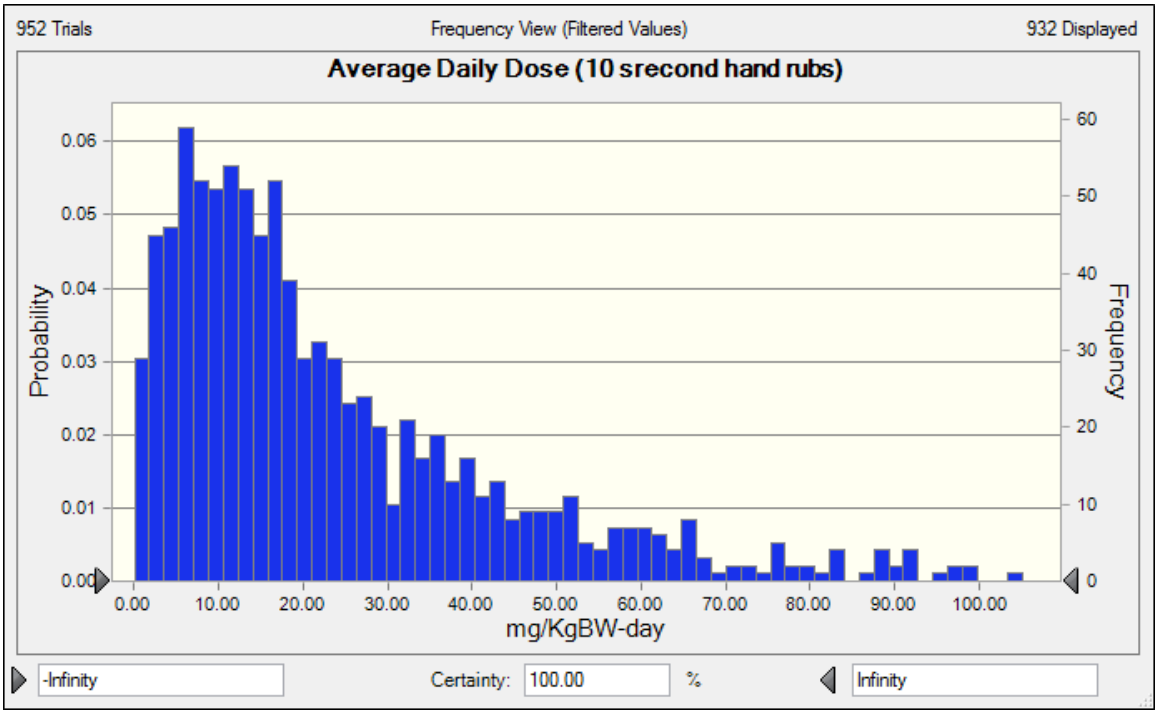

Figure 12. Frequency distribution of average daily dose for the 10 second hand rubs.

Table 8 shows several key percentiles of average daily dose, exported directly from Crystal Ball. From this, we see that the average daily dose of the 50th percentile is 17.3 mg/Kg-BW per day, with the maximum being 400.8 mg/Kg-BW per day. From the 95th percentile to the 99th percentile, the average daily dose appears to double, while the maximum value is nearly triple the 99th percentile.

Table 8. Average daily dose values for key percentiles in the 10 second hand rubs.

Percentile	mg/Kg-BW per day
50%	17.3
95%	76.1
99%	140.7
Maximum	400.8

To see what is driving the average daily dose, we can again create a sensitivity analysis, similar to that done for the concentration forecast. The sensitivity analysis

output is shown in Figure 13. This allows us to see that the intake rate of the infant is the most significant factor (accounting for 40.8% of the average daily dose) .The next greatest contributor to an infant's exposure to ethanol in the isolette is the concentration of ethanol in the isolette (accounting for 31.0% of the average daily dose), while the number of hand insertions accounts for 22.0% of the average daily dose. Conversely, bodyweight appears to be marginally protective, accounting for -6.2% of the average daily dose.

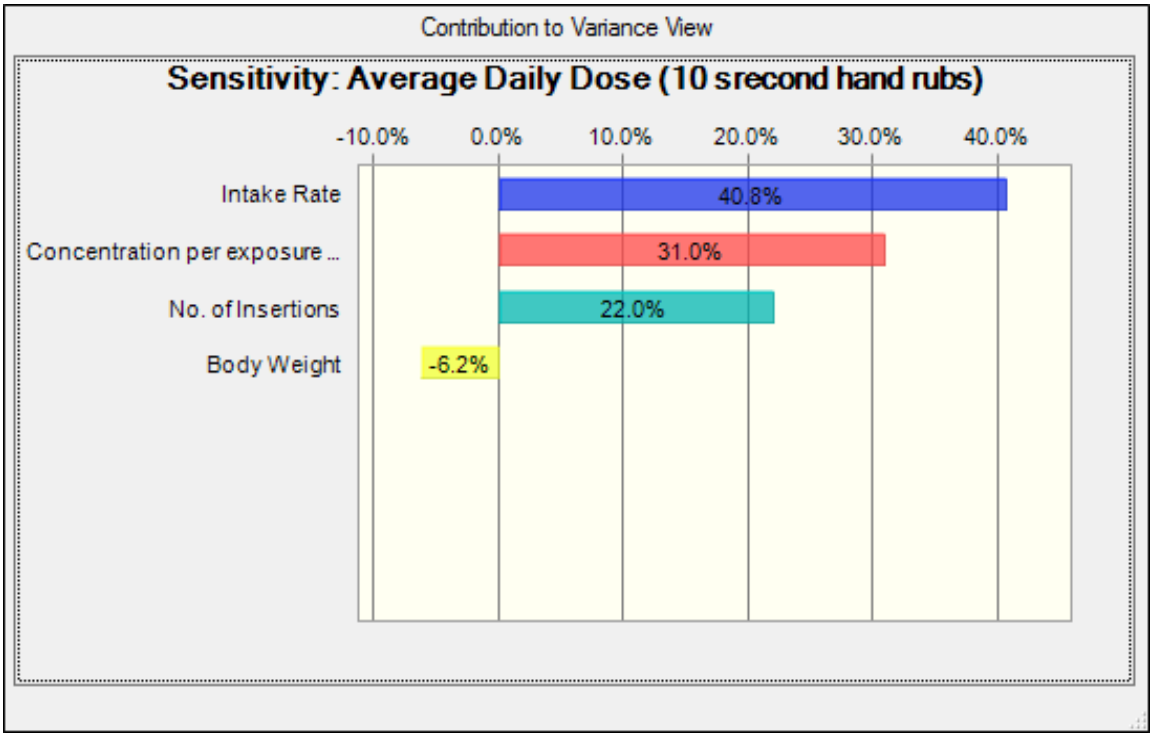

Figure 13. Sensitivity analysis of average daily dose for the 10 second hand rubs.

The results of the 10 second hand rub average daily dose analysis will be compared to the of the 20 second hand rub analysis in the next section.

20 second hand rub

For the value of concentration, time weighted averages were calculated from breathalyzer data for 20 second hand rubs using the methodology described above (in the Methods section). The TWA for each trial was calculated to be 2.8 ppm (5.2 mg/m^3) with a standard deviation of 5.5 ppm (10.3 mg/m^3). Intake rate was taken from the Exposure Factors Handbook, Chapter 6, which indicated infants from birth to one month have a mean breathing rate of 3.6 m^3/day (95^{th} percentile, 7.1 m^3/day). For body weight, the definition of low birth weight (less than 2,500 grams [5.5 pounds]) for body with a standard deviation of 500 grams in average daily dose calculations was used.

To calculate average daily dose, concentration in terms of TWA were input into the Crystal Ball software. Again, we defined parameters around concentration, including the lognormal distribution of the data, the average TWA and standard deviation for concentration. Similar to that of the 10 second hand rub, a forecast was generated for concentration to take into account the number of hand insertions in a typical day. Again, using information provided by NICU subject matter experts, a more stable infant in the NICU may receive care every three hours, with approximately 2 to 4 hand insertions per instance of care. As such, we assumed an average of 15 hand insertions per day, with a standard deviation of approximately 7 insertions per day. Table 9 below shows the inputs used in the average daily dose modeling.

Table 9. Crystal Ball parameters and forecast inputs, for the 20 second hand rubs.

	Inputs for Crystal Ball	
Concentration		
• **Average**	5.2 mg/m^3	Standard Deviation: 10.3 mg/m^3
• **No of Hand Insertions**	15	Standard Deviation: 7
Intake Rate of Air	3.6 m^3/day	95^{th} percentile 7.1 m^3/day
Body weight	2500 grams	Standard Deviation: 500 g

Crystal Ball generated forecast charts of average daily dose. Figure 14 shows the frequency distribution of average daily dose for the 20 second hand rub. This shows a simple histogram of the frequencies of average daily dose in the model. From this, we see that individuals in the highest percentiles (95-99%) receive significantly more exposure to ethanol than those in lower percentiles.

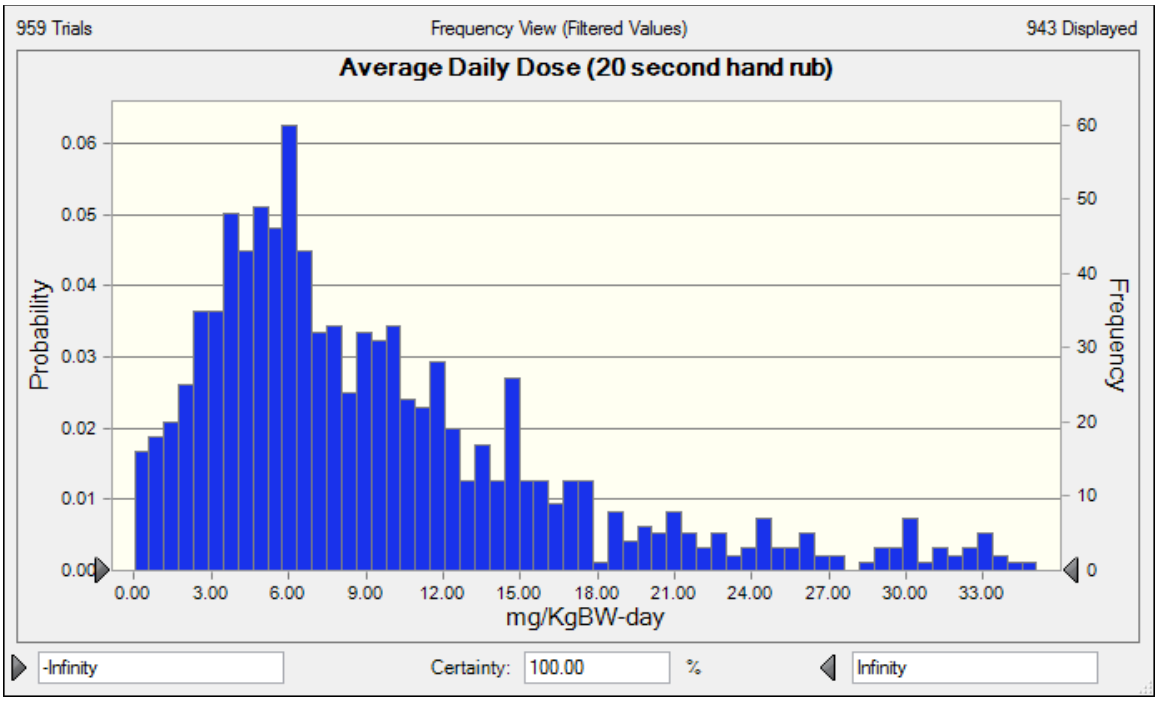

Figure 14. Frequency distribution of average daily dose for the 20 second hand rubs.

Table 10 shows several key percentiles of average daily dose, exported directly from Crystal Ball. From this, we see that the average daily dose of the 50th percentile is 7.7 mg/Kg-BW per day, with the maximum being 107.6 mg/Kg-BW per day. From the 95th percentile to the 99th percentile, the average daily dose almost doubles, while the maximum value is more than double the 99th percentile.

Table 10. Average daily dose values for key percentiles in the 20 second hand rubs.

Percentile	mg/Kg-BW per day
50%	7.7
95%	27.3
99%	40.4
Maximum	107.6

To see what is driving the average daily dose for the 20 second hand rub, we can again create a sensitivity analysis. This analysis is shown in Figure 15. This allows us to see that the intake rate of the infant is again the most significant factor (accounting for 61.4% of the average daily dose) .The next greatest contributor to an infant's exposure to ethanol in the isolette is the concentration of ethanol in the isolette (accounting for 25.0% of the average daily dose), while the number of hand insertions accounts for 7.4% of the average daily dose. Conversely, bodyweight appears to be marginally protective, accounting for -6.2% of the average daily dose.

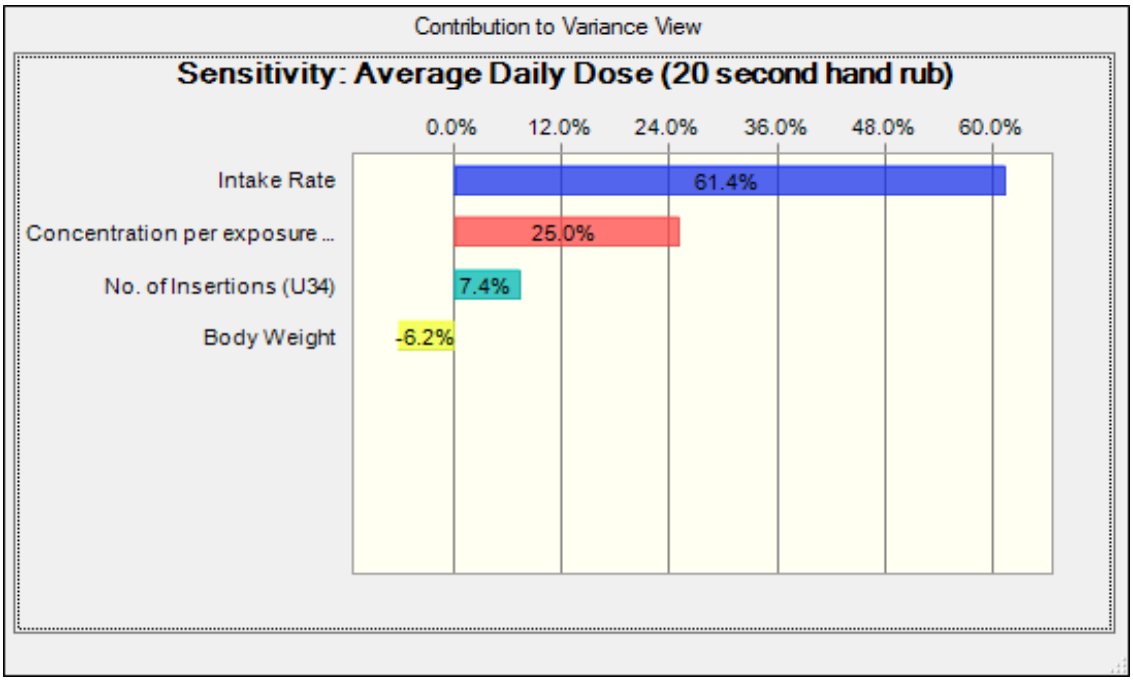

Figure 15. Sensitivity analysis of average daily dose for the 20 second hand rubs.

Table 11 below, shows a comparison of all the generated values from Crystal Ball for the 10 and 20 second hand rubs. From this table, we can see that for the average TWA per insertion, daily concentration, and average daily dose for the 10 second hand rubs appear to be more than double that of the 20 second hand rub.

Table 11. Comparison of Crystal Ball analysis of 10 and 20 second hand rubs.

	10 second hand rub	20 second hand rub
Concentration (TWA)	11.2 mg/m^3 (SD: 27.1 mg/m^3)	5.2 mg/m^3 (SD: 10.3 mg/m^3)
Average Daily Dose		
50%	17.3 mg/Kg-BW-day	7.7 mg/Kg-BW-day
95%	76.1 mg/Kg-BW-day	27.3 mg/Kg-BW-day
99%	140.7 mg/Kg-BW-day	40.4 mg/Kg-BW-day
Maximum	400.8 mg/Kg-BW-day	107.6 mg/Kg-BW-day
• **Intake rate sensitivity**	40.8%	61.4%
• **Concentration sensitivity**	31.0%	25.0%
• **Number of hand insertions**	22.0%	7.4%
• **Body weight sensitivity**	-6.2%	-6.2%

Interestingly, from the sensitivity analysis, it appears that infant intake rate is the most important factor for determining the average daily dose of ethanol. This is followed by the concentration of ethanol in isolette air (as measured by TWA and corresponds to duration of hand rubbing). Together, these two factors account for more than two-thirds of infant ethanol daily dose in isolettes. The number of hand insertions into the isolette appears to be more significant for 10 second hand rubbing than for 20 second hand rubbing. This could be due to the effects of increased ethanol levels per hand insertion (i.e., the greater the concentration per hand insertion, the more influential each insertion is on daily dose). In both instances, body weight marginally protective, with the percentages being the same between that of the 10 and 20 second hand rubs groups.

Discussion

These results suggest that use of alcohol based hand sanitizers within the NICU may result in unintended short term, elevated levels of ethanol exposure among preterm infants. Ethanol levels peaked very quickly in all trials (within one minute) and then dissipated, returning to background levels in approximately 15 to 20 minutes. While this indicates that ethanol levels do not building up over time with each singular exposure despite the minimal air exchanges of isolettes, further tests should be done to determine if ethanol levels accumulate with multiple insertions within a short period of time.

These results also indicated that ethanol levels in isolettes appear to vary based on the amount of time healthcare workers rub their hands after applying hand sanitizer. Alcohol peaks were approximately three times higher for 10 second hand rubs than for 20 seconds. The average peak for 10 second hand rubs was 387.0 ppm (729.3 mg/m^3), while the average peak for 20 second hand rubs was 104.4 ppm (196.6 mg/m^3). These resulted in average daily dose in 10 second hand rub groups of more than double than that for the 20 second hand rub group. This is important to note because the longer a healthcare worker rubs their hands, the longer the ethanol has time to off-gas in the hospital room, rather than off-gassing in the isolette and exposing the neonate to ethanol.

In addition, the severe peaks, and their rapid decline from each exposure to hand sanitizer, contribute to the overall daily TWA for ethanol in isolette air. Even though individual peaks appear to be nearly three times greater for 10 and 20 second hand rubs, daily concentration for 10 second hand rubs appear to be double that of 20 second hand rubs, when using the same amount of hand sanitizer, same number of exposures, and same length of exposure duration.

This study exhibits a number of strengths. This study is one of the first studies assessing air quality of NICU isolettes in the literature and ethanol concentrations, in particular. The earliest study noted in the literature addressing air quality in NICU isolettes was conducted by Prazad in 2008, which investigated VOCs in the NICU. This is an important future direction because isolettes are comprised primarily of plastics. This study looks at ethanol concentrations in NICU isolettes, another important area of investigation for future work for a number of reasons. For example, some babies receive medications to enhance their breathing rate, but if there are elevated levels of ethanol in isolette air, babies may receive an increased daily dose.

This study also demonstrates that ethanol is a significant exposure on a daily basis to infants in the NICU. This is an important area of future investigation because preterm infants are a very susceptible to environmental exposures due to their underdeveloped organ systems, relatively high intake rates for their body weight, and their low body weights, and because ethanol is a known toxicant to the developing systems of infants.

Another strength of this study is the comparison of different ethanol detection and measurement devices. While the passive alcohol monitoring badges yield non-detects for all trials, the PID and breathalyzer showed good correlation with their results, validating their use in detecting ethanol in this study. The PID is a versatile device that measures in near real-time. The PID is typically used in industrial hygiene, as well as leak and hazardous material detection. PIDs use ultraviolet light to break down detected VOCs in the air into ions. The PID then detects this change to determine the concentration of the VOCs in the air. PIDs are especially good at various chemicals in an environment because they measure concentration in isobutylene equivalents. A conversion factor can

be applied to convert the concentration of isobutylene equivalents to the concentration of the agent of interest. Interestingly, the Alcotest breathalyzer utilizes an electrochemical sensor, which is a micro-reactor that produces a small current when reactive gases (i.e., ethanol) are present (Drager, 2011). These complementary methods were shown to correlate very well in the current study. Despite the limitations of the breathalyzer (poorer time resolution, potentially more error) the breathalyzer is cheaper, easier to use, and requires less software and analysis than the PID, and it may be a step in the right direction towards policy compliance or passive monitoring of isolette air for ethanol.

There are number of limitations of this study as well. In this study, hands were inserted into the isolette for 5 minutes every 30 minutes. This may not reflect the true interactions of babies with health care workers in the isolette. For example, each time an infant receives care from a health care worker, there may be multiple hand insertions required, with each requiring application of hand sanitizer. This may not allow the alcohol to fully dissipate before subsequent insertions. Perhaps, this may affect how long it takes ethanol to clear the isolette before returning to background levels and thus the concentration and duration of ethanol exposure in the isolette. There may also be implications for differences in ethanol concentrations at different temperatures; however, isolettes tend to be kept at 36.5°C degrees for keeping babies warm.

An additional limitation is that errors may be introduced into the measurements by drawing out air to conduct the sampling for ethanol. For example, to conduct the breathalyzer testing, 1.5 L of air was drawn out and used to perform the breathalyzer test. It is suspected that the effect of this drawing of air is negligible, as there were no observed dips in PID results during the times in which air was sampled for use in the

breathalyzer tests; however, it would be advisable to investigate, empirically and in the literature, how this may impact the results of the PID and breathalyzer. To address these limitations, a longer study time with more trials should be employed with both the PID and breathalyzer. This would allow for more data to be collected to confirm the positive correlation between the PID and breathalyzer results. These tests should include collect breathalyzer data during background times, as well as collecting breathalyzer data at more frequent intervals.

One additional limitation of this study is that only one type of hand sanitizer was used. This particular hand sanitizer (EcoLab Quik-Care Foam Waterless Hand Sanitizer) is dispensed as a white foam. Vigorous rubbing was needed to completely rub the foam away when rubbing hands for 10 seconds and hands would often feel damp or have visible foam in between fingers when placing them in the isolette. In comparison, when hands were rubbed for 20 seconds, foam was completely gone and hands felt relatively drier before placing them into the isolette. Because the foam was visible, it was clear when the hand sanitizer was not completely rubbed into hands. Thus, the vigor with which hands were rubbed may have been biased by the visibility of the foam. It would be interesting to conduct similar tests with a clear hand sanitizer (e.g., a gel) to see if similar results are obtained over different hand rubs.

Similarly, it was hard to distinguish if the amount of hand sanitizer applied was the same for every pump squirted into hands. The pumping device on sanitizer dispensers may not be accurate or consistent. Further investigation using finite amounts of hand sanitizer per trial would be useful, as well as testing effects on ethanol levels of isolette air using varying amounts of hand sanitizer.

It would also be interesting to see the effects of rubbing hands for longer (e.g., 30 seconds) on ethanol peaks and daily TWAs. This would be beneficial to help inform policy decisions regarding the length of time to rub hands before inserting into the isolette. It is important to note that in health care settings, providing care to infants in the NICU may require quick response times. As such, longer hand rubbing times may result in delays in care, particularly in emergency situations.

Conclusion

Ethanol based hand sanitizer is ubiquitous in healthcare settings, and in NICUs in particular. Premature infants breathing isolette air may receive significant exposure to ethanol vapors for short durations as a result of healthcare workers using hand sanitizer prior to entering the isolettes. Premature infants routinely require multiple instances of care in short time frames (three to four insertions per care, every two to three hours), and may thus be at risk of significant alcohol exposure. Repeated exposure to ethanol may have long-term consequences for developing organ systems, in particular the rapidly developing CNS of preterm infants.

Peak alcohol concentrations and TWA of ethanol per day are significantly decreased if hands are rubbed for more time before placing them in the isolette. It seems that exposure to alcohol may be decreased significantly if hands are rubbed with hand sanitizer for at least 20 seconds.

The OSHA permissible short-term exposure limit for alcohol is 1000 ppm (1884 mg/m^3) for healthy adults, but the acceptable limit for developing preterm babies is unknown. A reasonable permissible exposure limit is expected to be much less for

preterm and developing infants because the effects of ethanol exposure may have long-term development consequences for developing brain. Determining an appropriate level of ethanol for isolette air would be an interesting area of future effort.

The antimicrobial properties of ethanol-based hand sanitizer have been a health intervention strategy that has saved countless; however, this intervention may have unintended consequences on some of the most vulnerable members of the population. It may be necessary to implement policy measures to reduce ethanol exposure in NICU isolettes through a three-pronged approach:

- Mechanical intervention – determine mechanical means to increase ventilation to reduce ethanol levels during periods of hand insertion;
- Training and education of healthcare workers and parents entering isolette – provide training on the appropriate amount of hand sanitizer to use, length of hand rubs, and implications to ethanol air if these measures are not followed; and
- Policy compliance – determine acceptable levels of ethanol in isolette air and identify devices and methods to monitor ethanol levels in the isolette.

In summary, exposure to ethanol, a developmental neurotoxicant, in the NICU is a significant concern to public health given the extreme vulnerability of preterm and ill infants. Understanding inhalation exposure in the NICU is important to inform successful exposure mitigation strategies. As such, alcohol exposure from ethanol based hand sanitizer may be significantly reduced with longer durations of hand rubbing or until hands are completely dry.

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