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RESEARCH ARTICLE

Repeated dose inhalation developmental toxicity study in rats exposed to cellulose insulation with boric acid additive

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ABSTRACT

Cellulose insulation (CI), a common building material, is a mixture of cellulose fibers and borates. Borates are approximately 20% of the product weight and act as a flame retardant. Given possible exposure to workers and consumers, an inhalation toxicity study was conducted following Organization for Economic Co-operation and Development (OECD) 414 for Prenatal Development Toxicity to evaluate if CI is a developmental toxicant. Pregnant female rats were exposed by nose-only inhalation to CI aerosols containing 20% boric acid for six h/day, from gestational day (GD) 6–19, and fetuses were evaluated for developmental parameters. Respirable CI was produced by grinding to produce respirable particles (MMAD 2.7–2.9 μm, geometric standard deviations (GSD) 1.9–2.6), which were then aerosolized. Target air concentrations were 15, 90, and 270 mg CI/m^3. Controls were exposed to air only. Slight body weight reductions (average decrease <7% vs. control) were observed in male and female GD 20 fetuses in the mid and high dose groups. No embryo/fetal developmental toxicity or alterations in any other measured variable were reported at any dose. The no observed adverse effect level (NOAEL) for developmental outcomes was 270 mg/m^3.

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Boric acid; cellulose insulation; developmental toxicity; rats; particulates; respiratory toxicity

Introduction

Manufactured cellulose insulation (CI) is produced from recycled newsprint and other paper sources with borate compounds or other additives added as a fire retardant at up to 22% of the product weight. Loose CI is installed by blowing into wall and roof cavities in homes and businesses, where the fibers settle to produce trapped pockets of air that impede air infiltration and establish a barrier between temperature differences.

Human exposure to CI fibers is most likely to occur through inhalation by workers who manufacture or install the material. Particles measured during installation generally exceed inhalable size (<15 μm diameter): e.g. during installation, the mean CI particle size was ~260 μm (range 5–3000 μm) with small inhalable fibers (≤5 μm) comprising <2.5% (Colby 2014). The fraction of small inhalable fibers (e.g. ≤5 μm) is what is of concern for risk during human exposure.

In 2006, the National Toxicology Program (NTP) studied the physical and chemical properties of CI particles and assessed the exposure conditions at several workplaces, based in part on a field study conducted by the National Institute of Occupational Safety and Health (NIOSH), to determine if a hazard exists to those who are occupationally exposed to CI. Less than 0.1% by weight of the CI particle samples assessed was respirable. The respirable fractions consisted primarily of fire retardants and smaller quantities of clays and did not contain cellulose material. The results of the studies performed indicated that few respirable particles or fibers are generated during the aerosolization of CI (Morgan 2006). Specifically, concentrations of total dust measured in samples from the personal breathing zone for attic installers had a mean concentration of 74.8 mg/m^3 while respirable dust concentrations had a mean of 1.53 mg/m^3. The highest concentrations were seen in attic installers performing dry applications (Morgan 2006). Others have observed similar total dust concentrations during CI installation. For insulation of attic spaces, Breum et al. (2003) reported mean concentrations of total dust ranging from 40 to 520 mg/m^3 for the installer and from 4.9 to 23.3 mg/m^3 for the helper. Exposure to residents could occur following installation upon entrance into an attic or roof cavity, although the exposure would likely be brief and concentrations low.

Boric acid has been classified as a Category 2 Reproductive Toxicant (suspected human reproductive toxicant) under HAZCOM 2012, following the principles of classification and labeling set by the United Nations' Globally Harmonized Standard (GHS) for Classification and Labelling (UN 2011), on the basis of animal data that suggest potential effects on male reproduction following oral exposure.

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Specifically, boric acid treatment of male rats, mice, and dogs was dose-dependently associated with testicular toxicity, characterized by inhibited spermiation, and reduction of epididymal sperm counts (Fail et al. 1991; Fukuda et al. 2000; Ku et al. 1993; Kudo et al. 2000; Weir & Fisher 1972; Yoshizaki et al. 1999). For example, Weir & Fisher (1972) determined that following 14 weeks of exposure at boric acid dietary concentrations of 1170 ppm, male rats were unable to conceive and were sterile, Fail et al. (1991) found that male reproductive organs were negatively affected at feed concentrations of 4500 ppm after 27 weeks of exposure, and Ku et al. (1993) reported inhibited spermiation at food concentrations of 3000 ppm after nine weeks of exposure. The NOAEL for fertility effects of boric acid in male rats (oral feeding, Sprague-Dawley strain) was determined to be 17.5 mg boron (B)/kg body weight (bw)-d (ECHA 2010, based on the data of Weir & Fisher 1972), equivalent to 100 mg boric acid (BA)/kg bw-d based on the molar fraction of boron in boric acid. A more recent study evaluating semen characteristics in highly exposed Chinese boron workers (Robbins et al. 2010), supplemented by biological monitoring data (Xing et al. 2008) and a review of male reproductive studies (Scialli et al. 2010), did not detect male reproductive effects attributable to boric acid exposures in humans.

No studies reporting developmental effects in humans from exposure to boric acid or boron were identified. However, some studies of oral exposure in rodents suggested developmental effects of borates, including decreased fetal weight and developmental abnormalities. In Sprague–Dawley rats, Heindel et al. (1992) reported decreased fetal weights at 78 mg BA/kg bw-d (13.7 mg B/kg bw-d) and above after exposure to boric acid in feed throughout gestation. In another study, oral dosing of pregnant Sprague–Dawley rats throughout gestation at 55 mg BA/kg bw-d (9.6 mg B/kg bw-d) produced no marked effects, with fetal skeletal effects observed at 76 mg BA/kg bw-d (13.3 mg B/kg bw-d) and above (Price et al. 1996a). Subsequent reviews identified 9.6 mg B/kg bw-d as a NOAEL for developmental effects for boric acid (WHO 1998; ECHA 2010).

Allen et al. (1996) used combined data from Heindel et al. (1992), Price et al. (1994), and Price et al. (1996a) to derive a benchmark dose for developmental effects of boric acid, defined as the 95% lower bound on the dose corresponding to a 5% decrease in mean fetal body weight ($BMDL_{05}$), of 10.3 mg B/kg bw-d. WHO (2009) derived a tolerable daily intake (TDI) for boric acid of 0.2 mg B/kg bw-d, by dividing the $BMDL_{05}$ by a total uncertainty factor of 60 (10 for interspecies variation and 6 for intraspecies variation).

No studies evaluating the reproductive/developmental toxicity of CI delivered to animals or humans via inhalation were identified. However, given that studies of boric acid in animals report developmental toxicity at lower exposure levels than reproductive effects in males, this investigation focused on the potential for CI to cause developmental effects. It is acknowledged that males are likely to be exposed to higher concentrations of CI than pregnant females; however, this study is designed to determine protective concentrations for the most sensitive endpoint and would be protective for other, less sensitive endpoints.

This study was undertaken to address the lack of information on the developmental effects of CI and its borate additives. The target concentrations for this study were chosen based in part on an earlier 14-day inhalation study conducted with the same CI fibers (e.g. 80% recycled paper fibers, 20% boric acid; MMADs 2.22–2.54 µm) in male and female rats at air concentrations up to 30 mg CI/m^3 for six hours per day (see Supplementary Appendix A). The range of target CI concentrations was increased in the current study as no adverse effects were noted in that study (data not presented), and to assess the potentially increased sensitivity of fetal rats. The current study administered borate-treated CI at air concentrations of 0, 15, 90, and 270 mg/m^3.

Materials and methods

Laboratory

Animal testing was conducted by IIT Research Institute (IITRI), located in Chicago, IL. Necropsies were performed by Charles River Laboratories, Pathology Associates. The study was conducted in compliance with the United States Environmental Protection Agency (US EPA) Toxic Substances Control Act (TSCA) 40 CFR Part 160 Good Laboratory Practice Standards (US EPA 2011).

Test article

The test article was borate-treated CI. The parent material was received from CIMA on 14 March 2014, and was stored in its original container in a secured area at room temperature (15–30° C) until used. Analysis of samples of the material by U.S. Borax, Inc. described it as consisting of loose gray CI, with a boric acid content ranging from 20.7–21.9% by weight (Schubert 2014).

Testing guidance for animal inhalation studies recommends that the particle size of inhalable test articles be around 1−3 µm MMAD with a geometric standard deviation (GSD) in the range of 1.5–3.0 (OECD 2017), to ensure respirability and "allow for exposure to all relevant regions of the respiratory tract" (OECD 2017). To generate the test article, the parent material was mechanically ground into fine powder. Briefly, the test article was ground for 90 min in a blender (Oster Push Button Blender (Model BCBG08), Sunbeam Products, Inc., Boca Raton, FL) then passed through a sieve (850 µm US Standard Sieve Series, (Sieve No. 20), Baxter Healthcare Corporation, McGaw Park, IL) (Sullivan 2015). The sieved material was then ground 15 times with a grinder (KitchenAid Pro Line Series Burr Grinder (Model KPCG100OB1), St. Joseph, MI).

Exposure concentrations

Maternal rats were exposed to target concentrations of 15, 90, and 270 mg CI/m3 by nose-only inhalation. The exposure system consisted of a 52-port, flow-past type nose-only inhalation chamber (Lab Products, Inc., Seaford, DE), a jet mill aerosol generation system, a real-time aerosol monitor, and appropriate flow controls (IITRI 2015b). IITRI (Chicago, IL) established and validated the nose-only inhalation exposure system for CI fibers (IITRI 2014).

The test atmosphere mass concentration in each chamber was determined gravimetrically by collecting the test atmosphere on glass-fiber filters placed in closed-face filter holders (one filter taken every two hours of exposure). Samples were collected at a constant flow rate equal to the port flow of the delivery tube, and the total volume of air sampled was measured by a dry gas meter. Test atmosphere mass concentration in the breathing zone of the rats was determined at least three times per day during the six-hour inhalation exposure period.

The particle size distribution of the test atmosphere was measured by a Quartz Crystal Microbalance (QCM) based cascade impactor (California Measurements, Inc.) (IITRI 2015b). The MMAD and GSD were calculated from the mass accumulated on each stage of the QCM. MMAD of the test aerosols was maintained in the range of 1 to 4 microns with geometric standard deviation in the range of 1.5–3.0 µm consistent with the requirements of OECD (2017). Determination of the test atmosphere particle size was conducted at least once each week during the exposure phase of the study. Temperature, relative humidity, and airflow rate within a preselected enclosure were recorded at approximately one-hour intervals during the exposure.

The concentration of boric acid in air was estimated based on the total particulate matter (TPM) air concentration and nominal percent boric acid as follows:

$$Conc\ boric\ acid\ \left(\frac{mg}{m^3}\right) = Particulate\ air\ conc\ \left(\frac{mg\ particulate}{m^3\ air}\right) \times Fraction\ boric\ acid\ \left(\frac{mg\ boric\ acid}{mg\ particulate}\right) \quad (1)$$

Where, Fraction boric acid = 0.20 (mg boric acid/mg particulate), assuming CI particulate has a nominal boric acid concentration of 20% by weight.

Animals

One-hundred and four timed pregnant female rats (CD® IGS) were obtained from Charles River Laboratories, 100 of which were used in the developmental toxicity study following release from quarantine (extending from receipt until the first day of exposure on gestation day (GD) 6) in which animals were observed daily for mortality or evidence of moribundity. Animals were assigned to the exposure group by a computer program using a randomization process based upon body weight to produce similar group mean values (ToxData® version 3.0 (PDS Pathology Data Systems, Inc., Basel, Switzerland)). Twenty five pregnant rats were placed in each group. Rats were approximately 11 weeks old and weighed approximately 200–275 g on GD 0 (IITRI 2015b).

Animals were handled in compliance with all applicable sections of the Animal Welfare Act (AWA; Title 9, *Code of Federal Regulations*), the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (PHS 1986), and the *Guide for the Care and Use of Laboratory Animals* (NRC 2011). The Institutional Animal Care and Use Committee (IACUC) at IITRI reviewed the protocol and deemed its study design appropriate to meet the objectives of the study, while minimizing both pain and distress to the test animals (IITRI IACUC Approval No. 15-004). No anesthetic, analgesic, or tranquilizing/sedating drugs were used during the in-life portion of the study, except for the purposes of euthanizing the animals prior to necropsy.

During non-exposure periods, all animals were single-housed in Lab Products Inc., polycarbonate "shoe-box" cages (10-1/2 × 19" × 8") with absorbent hardwood chip bedding (i.e. Sani Chips or Beta Chips). Fresh bedding was provided weekly. Racks and cages were washed and sanitized biweekly.

Temperature and relative humidity (20–26° C) in the animal room were manually recorded each day. Animal rooms were generally held within temperature and relative humidity ranges as recommended by IITRI's Standard Operating Procedures (SOPs). Animals were maintained on a 12-hour light/dark cycle (maintained with an automatic timer).

The animals were fed Harlan's Certified Global 18% Protein Rodent Meal (2018CM). Each certified lot of diet was analyzed for contaminants to ensure that none were present at concentrations that would be expected to interfere with the conduct or purpose of this study (IITRI 2015b).

Coarse-filtered City of Chicago water was provided *ad libitum* to all rats via an automatic watering system. Supply water was analyzed periodically for bacterial contamination and chemical composition (e.g. electrolytes, metals, etc.). No contaminants expected to interfere with the study were known to be present in the water (IITRI 2015b).

CI exposures to pregnant test subjects

Per the study protocol (IITRI 2015b), GD 4 and 5 pregnant rats were placed in nose-only exposure tubes for two and four hours, respectively, to habituate the subjects to the chambers prior to the start of the experiment on GD 6.

Animals were exposed via nose-only inhalation for six hours per day, with exposure beginning on GD 6 and continuing through GD 19. Test article (CI particulate) was maintained at a nominal concentration of 0, 15, 90 or 270 mg/m^3 for the control, low, medium, and high exposure groups, respectively.

Endpoints for assessment

The OECD 414 Prenatal Developmental Toxicity Study (OECD 2001) and Office of Prevention, Pesticides and

Toxic Substances (OPPTS) 870.3700 Prenatal Developmental Toxicity Study (US EPA 1998) guidelines were followed in the CI developmental toxicity study except for the following few deviations: exposure started on GD 6 verses GD 5 and body weights were taken on GD 4, 6, 9, 12, 15, 18, and 20 verses every day. Both alterations were requested by IITRI in order to allow for an extra day of habituation to the new laboratory and training of the pregnant rats to the chamber. Body weights were taken according to IITRI laboratory procedures.

Necropsy and examinations

All dams were euthanized by CO_2 asphyxiation on GD 20, and a gross necropsy was conducted on all animals from all exposure groups. The lung and main stem bronchi were collected and fixed in 10% neutral buffered formalin. Tissue masses, and suspect lesions if present, were collected, dissected, sliced into appropriately sized sections, and fixed in 10% neutral buffered formalin. Any uteri that appeared non-gravid were further examined (i.e. by ammonium sulfide staining) to confirm the non-pregnant status.

For each dam, the uterus was removed, trimmed of excess adherent fat, and weighed with the ovaries prior to removal of the fetuses from the uterine horns. After weighing, the corpora lutea were counted and recorded for the left and right ovaries.

Gross fetal examination

Each uterine horn was opened and inspected for implantations and the contents were recorded and classified as:

- Early resorption (placenta only or unrecognizable fetal tissue)
- Late resorption (placenta with an autolyzed recognizable fetus)
- Dead fetus (fetus with no signs of autolysis)
- Live fetus (fetus pink in color and responds to touch)

External examination

Fetuses were removed, counted, weighed, and given an external morphological examination (including the palate). All fetal abnormalities were recorded. Observations (e.g. normal, within normal limits) and fetal variations or malformations were documented for each fetus. Sex was determined by ano-genital distance.

Visceral examination

Visceral examinations were performed on approximately one-half of the fetuses from each litter using a modified Staples' technique. The soft tissues (excluding the head) of each fetus were examined using a stereomicroscope. Fetuses were sexed prior to visceral examination and again internally for verification purposes during the visceral examination. Adverse or abnormal findings were classified as variations (a change in the normal structure that does not affect function) or malformations (a primary structural defect that results from a localized error of morphogenesis and interferes with normal function) (Staples 1974).

Cephalic examination

Decapitated fetal heads from approximately one-half of each litter (those selected for visceral examination) were fixed in Bouin's solution for a minimum of one week prior to examination using a modified Wilson's razor blade technique (Wilson 1973). A stereomicroscope was used, as needed, to examine the cephalic structures and organs. All abnormalities were recorded and classified as variations or malformations.

Skeletal examination

Approximately one-half of the fetuses from each litter (those not selected for visceral examination) were processed for skeletal examination. Fetuses were eviscerated, skinned, and fixed in isopropyl alcohol. Tissue was macerated in a potassium hydroxide solution and stained with Alizarin Red-S and cleared in glycerin. A stereomicroscope was used to examine the cartilage and skeletal formations. All skeletal abnormalities were recorded and classified as variations or malformations.

Criteria used for categorizing fetal external, soft tissue, and skeletal alternations were defined in IITRI SOPs.

Statistical analysis

Statistical analysis was performed using data management system/software (e.g. ToxData® (PDS Pathology Data Systems Ltd., Basel, Switzerland, version 3.0), SYSTAT software (Systat Software Inc., Chicago, IL), or SigmaStat® Software, (Systat Software Inc., Chicago, IL)). For all comparisons, a probability value of $p < .05$ was considered significant.

For continuous data (e.g. dam body weight/body weight change, dam food consumption, dam implantation sites, gravid uterine weight, corpora lutea per dam, preimplantation and postimplantation loss, number of live fetuses per litter, fetal body weight), if the data set was normally distributed and of equal variance, statistical comparisons to the control group were conducted using a one-way analysis of variance (ANOVA), with *post hoc* comparisons made (if necessary) using Dunnett's test. If normality and/or equal variance failed for a data set, statistical comparisons were conducted using nonparametric Kruskal-Wallis ANOVA, with *post hoc* comparisons made (if necessary) using Dunn's test or the Mann-Whitney *U* Test.

Incidence data (incidences of malformation and variations) were compared using Chi-square analysis and/or Fisher's exact test, with the fetus and litter as the experimental unit (separate analyses). The total number of litters with malformations and the total number of litters with variations were statistically compared. In addition, the average number of malformed fetuses per litter for each

dose group was compared using Mann-Whitney Rank Sum Test.

Results

Aerosol characterization and inhalation dose

Particle size distribution, expressed as MMAD, was determined for two of the study days and ranged from 2.63–2.90 µm with a GSD of less than 3.0. Mean TPM concentration was measured for three samples in each exposure group on each day of the study. Mean TPM air concentrations ranged from 13.7–16.3 mg/m^3, 86.3–97.3 mg/m^3, and 255.3–279.7 mg/m^3 in the low, mid, and high groups, respectively. The overall mean gravimetrically measured concentrations of the test substance in the test atmosphere were 0, 15, 92, and 265 mg/m^3 for the control, low, mid, and high groups, respectively, corresponding to the respective CI target concentrations of 0, 15, 90, and 270 mg/m^3. The estimated average daily boric acid air concentrations are shown in Table 1.

Maternal results

All twenty-five dams placed in each group survived to GD 20. Each dam was observed for clinical signs prior to exposure on GD 6 through post exposure on GD 19.

Ten clinical signs were monitored during the experiment. The results are presented in Table 2. The only clear dose-response observation was a dose-related increase in signs of test article around the face. A slight increase in vaginal discharge was observed with increasing air concentration. This effect was not noted to be adverse but could be a sign of stress. At the highest air concentration, more dams were observed with porphyrin staining compared to the control group. Regardless of air concentration, all animals exhibited signs of wet inguinal fur, likely due to restraint of all test animals for six hours during each study day (IITRI 2015a).

Body weight for all dams in the control, low, mid, and high exposure groups was recorded on GD 4, 6, 9, 12, 15, 18, and 20 (See Table 3). Mean body weights for all dams were similar across all exposure groups. No decreases in food consumption were observed in the test substance-treated groups compared to the control group.

Organs were grossly evaluated in the dams on GD 20. Key organs evaluated were the lung and liver (see Table 4). As the concentration of test article increased, the percentage of dams showing lung effects increased, with the numbers of dams with pale lungs (pigmentation loss in the lungs) and mottled lungs showing the greatest increase. The number of animals with any gross lesion in the lung or liver increased from 0/25 in the control group to 19/25 in the highest exposure group; the increase was statistically significant in the mid and high exposure groups ($p < .05$). The number of animals with pale lungs was also significantly increased ($p < .05$) in the mid and high exposure groups.

Uterine contents of the dams in each exposure group are summarized in Table 5. All dams in each exposure group had viable litters with no difference between treatment and control groups for mean number of corpora lutea, total number of implants, percent preimplantation loss, total number of live implants, mean number of live implants per litter, percent litters with deaths, number of resorptions, percent litters with resorptions, percent litters with abnormal fetuses, or litter sex ratio. One dead fetus was reported in the high exposure group, and one abnormal fetus was reported in the low exposure group. The sex ratio (male:female) was approximately 50:50 in each exposure group.

Uterine weights are summarized in Table 6. These weights reflect the weights with fetuses at GD 20. Evaluation of the data showed no differences in uterine weights for any of the exposure groups.

Table 1. Estimated boric acid air concentrations for the developmental inhalation toxicology study of CI in pregnant Sprague–Dawley rats.

Exposure Group	Target total particulate matter (CI) air concentration (mg/m^3)	Measured TPM air concentration (mg/m^3)[a]	Mean boric acid air concentration (mg/m^3)[b]
Control	0	0	0
Low	15	13.7–16.3 (15.0)	3
Mid	90	86.3–97.3 (92.0)	18
High	270	255.3–279.7 (265.0)	53

[a]Range of TPM concentration for three samples on each day of study (overall mean).
[b]Based on overall mean TPM concentration, assuming 20% boric acid on CI particulate.

Table 2. Clinical signs in pregnant Sprague–Dawley rats exposed to CI[a,b].

Parameter	Value	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Signs-material around the face (color = gray)	*N* (%)	0 (0%)	1 (4%)	0 (0%)	25 (100%)
Signs-material around the nose (color = gray)	*N* (%)	0 (0%)	25 (100%)	25 (100%)	25 (100%)
Signs-porphyrin staining	*N* (%)	14 (56%)	18 (72%)	16 (64%)	22 (88%)
Signs-redness around nose fur	*N* (%)	24 (96%)	24 (96%)	24 (96%)	19 (76%)
Signs-salivation	*N* (%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)
Signs-vaginal discharge	*N* (%)	9 (36%)	9 (36%)	14 (56%)	16 (64%)
Signs-wet inguinal fur	*N* (%)	25 (100%)	25 (100%)	25 (100%)	25 (100%)

[a]Clinical signs were recorded from GD 6 to GD 19. Each exposure group included 25 animals.
[b]No statistical analysis performed on these data.
N number of animals.

Table 3. Body weights of pregnant Sprague–Dawley rats exposed to CI.

Day	Value	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
GD 4	Mean ± SD (kg) (*N*)	233 ± 16.0 (25)	234 ± 16.4 (25)	235 ± 17.2 (25)	236 ± 16.1 (25)
GD 6	Mean ± SD (kg) (*N*)	243 ± 16.7 (25)	245 ± 18.2 (25)	247 ± 18.1 (25)	246 ± 16.7 (25)
GD 9	Mean ± SD (kg) (*N*)	255 ± 17.9 (25)	259 ± 18.6 (25)	258 ± 20.3 (25)	255 ± 19.1 (25)
GD 12	Mean ± SD (kg) (*N*)	273 ± 19.1 (25)	279 ± 19.2 (25)	276 ± 20.9 (25)	273 ± 19.9 (25)
GD 15	Mean ± SD (kg) (*N*)	290 ± 20.7 (25)	297 ± 20.9 (25)	294 ± 23.8 (25)	293 ± 19.3 (25)
GD 18	Mean ± SD (kg) (*N*)	327 ± 22.1 (25)	334 ± 22.5 (25)	331 ± 26.6 (25)	330 ± 32.2 (25)
GD 20	Mean ± SD (kg) (*N*)	352 ± 24.0 (25)	360 ± 23 (25)	355 ± 28.5 (25)	351 ± 25.0 (25)

GD: gestation day; *N*: number of animals; SD: standard deviation.

Fetal results

Male fetal body weights for the high exposure group were found to be different when compared to controls. The average body weight of male fetuses in the high exposure group was 6% lower ($p < .05$) than the males of the control group (see Table 7). Female fetal body weights for the high and mid exposure groups were also different when compared to controls. The average body weight of female fetuses in the mid and high exposure groups were about 6% and 7% lower, respectively, than the females of the control group ($p < .05$). When female and male fetal body weights were combined, both the mid and high exposure group were different from the controls ($p < .05$). However, as reported in Table 6, no differences in uterine weight per dam, measured on GD 20, were observed.

Table 4. Lung and liver observations in pregnant Sprague–Dawley rats exposed to CI[a].

Parameter	Value	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Any gross lesion in lung or liver	*N* (%)	1 (4%)	1 (4%)	16 (64%)*	19 (76%)*
Lungs pale	*N* (%)	0 (0%)	1 (4%)	10 (40%)*	16 (64%)*
Lungs mottled	*N* (%)	1 (4%)	0 (0%)	9 (36%)*	2 (8%)
Lungs with dark foci	*N* (%)	0 (0%)	0 (0%)	2 (8%)	4 (16%)
Lungs with red foci	*N* (%)	0 (0%)	0 (0%)	0 (0%)	4 (16%)
Lungs with white foci	*N* (%)	0 (0%)	0 (0%)	2 (8%)	1 (4%)
Liver pale	*N* (%)	1 (4%)	0 (0%)	3 (12%)	5 (20%)
Liver mottled	*N* (%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)

[a]Each exposure group included 25 animals; *N*: Number of animals.
*Statistically significant compared to control group as determined by Fisher's exact test ($p \leq .05$).

Table 5. Summary of the examination of uterine contents of pregnant Sprague–Dawley rats exposed to CI.

Parameter[a]	Value	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Initial group size (sperm positive)	*N*	25	25	25	25
Actual group size (gravid)	*N*	25	25	25	25
Viable litters (at least one live implant)	*N*	25	25	25	25
Non-viable litters (no live implant)	*N*	0	0	0	0
Total number of corpora lutea	*N*	356	336	361	349
	Mean ± SD[c]	14 ± 3.1	13 ± 2.4	14 ± 3.2	14 ± 2.1
Total number of implants	*N*	324	313	329	324
	Mean ± SD[c]	13 ± 1.9	13 ± 1.8	13 ± 2.1	13 ± 1.6
% Preimplantation loss[b]	Mean ± SD[c]	8 ± 11.7	6 ± 10.8	8 ± 10.8	7 ± 10.7
% Live implants/Total Implants	%	97	99	98	98
% Non-live implants/Total implants	%	3	1	2	2
Total number of live implants (fetuses)	*N*	314	311	321	318
	Mean ± SD[c]	13 ± 2.2	12 ± 1.8	13 ± 2.2	13 ± 1.7
Number of deaths (implants)	*N*	0	0	0	1
Litters with deaths	*N* (%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
Number of resorptions	*N*	10	2	8	5
	Mean ± SD[c]	0.4 ± 0.6	0.1 ± 0.3	0.3 ± 0.7	0.2 ± 0.4
Litters with resorptions	*N* (%)	8 (32%)	2 (4%)	5 (20%)	5 (20%)
Number abnormal fetuses[d]	*N*	0	1	0	0
Litters with abnormal fetuses	*N* (%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)
% Abnormal/Live implant	%	0	0	0	0
% Non-live and abnormal/Total implants	%	3	1	2	2
Total male fetuses	*N*	160	169	151	156
Male/Litter	Mean ± SD	4.05 ± 0.295	4.16 ± 0.324	3.89 ± 0.304	3.78 ± 0.221
Total female fetuses	*N*	153	142	170	163
Female/Litter	Mean ± SD	3.85 ± 0.332	3.92 ± 0.249	3.64 ± 0.260	3.76 ± 0.270
Sex ratio	Male:Female %	51/49	54/46	47/53	49/51

[a]Reported means and standard deviations utilize the litter (number gravid on Gestation Day 20) or viable litter (if the parameter requires an intact fetus) as the unit of observation.
[b]% Preimplantation loss = [(corpora lutea – total implants)/total corpora lutea] × 100. When the number of implants is greater than the number of corpora lutea, preimplantation loss was considered to be 0%.
[c]Parameter statistically analyzed (ANOVA/Kruskal–Wallis ANOVA followed by *post hoc* Dunnett's/Dunn's).
[d]Gross external, visceral and cephalic abnormalities are included.
N: number of animals; SD: standard deviation.

Table 6. Uterine organ weights in pregnant Sprague–Dawley rats exposed to CI[a].

Parameter	Value	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Uterine weight (g)	Mean ± SD (*N*)	76.7 ± 11.36 (25)	77.5 ± 9.60 (25)	76.4 ± 11.83 (25)	74.3 ± 8.76 (24)

[a]Measured on gestation day 20.
N: number of animals; SD: standard deviation.

Table 7. Mean fetal body weights of offspring of Sprague-Dawley rat dams exposed to CI.

Parameter	Gender	Value[a]	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Body weight (g)	Male	Mean ± SD (*N*)	4.04 ± 0.299 (25)	4.16 ± 0.316 (25)	3.90 ± 0.310 (25)	3.79 ± 0.219* (25)
Body weight (g)	Female	Mean ± SD (*N*)	3.86 ± 0.338 (25)	3.91 ± 0.247 (25)	3.63 ± 0.259* (25)	3.60 ± 0.237* (25)
Body weight (g)	Male + Female	Mean ± SD (*N*)	3.95 ± 0.308 (25)	4.04 ± 0.281 (25)	3.75 ± 0.271* (25)	3.69 ± 0.224* (25)

[a]Reported mean and standard deviation based on average fetal weight/litter.
*Statistically significant difference from control; $p < .05$ (ANOVA/Kruskal–Wallis ANOVA followed by *post hoc* Dunnett's/Dunn's).
N: number of animals; SD: standard deviation.

Table 8. Summary of gross fetal external abnormalities by litter and fetuses from Sprague–Dawley rat dams exposed to CI.

Parameter	Value[a]	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Minor observations[b]					
Left foot cut off at necropsy	Number of fetuses (Number of litters)	0 (0)	1 (1)	0 (0)	0 (0)
Tail cut off at necropsy	Number of fetuses (Number of litters)	0 (0)	0 (0)	0 (0)	1 (1)
Gross abnormalities					
Tail bent/kinked[c]	Number of fetuses (Number of litters)	0 (0)	1 (1)	0 (0)	0 (0)

[a]Number of fetuses examined was 314, 311, 321, and 319 for control, low, mid, and high groups, respectively. Number of litters examined was 25 per group.
[b]Caused by handling of the fetus and are considered by the test administrators to be artifacts.
[c]Classified as a malformation by test administrators.

No exposure related external alterations (i.e. physical deformities) in the fetuses were noted (see Table 8). One gross abnormality (tail bent/kinked) was observed in one fetus in the low exposure group. No other gross abnormalities were reported. Other minor observations were assumed to be the result of handling of the fetus by the test administrators and were considered artifacts.

No exposure related visceral abnormalities were noted for any of the exposure groups (see Table 9).

No exposure related cephalic alterations were noted for any of the exposure groups (see Table 10). In the high exposure group, a statistically significant ($p < .05$) increase in the number of fetuses with alterations in the interparietal and strenebrae bones (reported as reduced ossification of the interparietal bone in the skull and unossified sternebrae (5th and/or 6th)) compared to the control was noted (Table 11). However, no exposure-related skeletal malformations were observed using the litter as the unit of comparison, consistent with US EPA (1998) and OECD (2001) developmental toxicity study guidelines.

Discussion

Because the particle size of the test article (MMAD 2.63–2.9 µm with a GSD of less than 3.0) is considerably smaller than mean particle sizes during CI installation (mean ~260 µm, range 5–3000 µm, with small inhalable fibers (≤5 µm) comprising <2.5%; Colby 2014), the inhaled particulate dose delivered to the test animals provides a conservative estimate of inhaled dose during human exposure.

The statistically significant increase in pale and mottled lungs observed in the mid (90 mg/m^3) and high exposure (270 mg/m^3) groups was considered by the study pathologist to be due to deposition of CI fibers in the lung. Other studies in animals have reported CI deposition in the lungs, including several studies of short-term exposures (<4 weeks) of rats to CI or other forms of cellulose (mean boron or boric acid concentrations in CI, where reported, up to 20%) via the respiratory tract (inhalation or intratracheal instillation (II)) that reported increased inflammatory responses in the lungs likely confounded by impairment of clearance (Cullen et al. 2000; Hadley et al. 1992; Morgan 2006).

Deposition of cellulose fibers in the lung is expected to be stressful to the pregnant dam. However, other than an increase in vaginal discharge assumed to be related to stress, no other signs of maternal stress were reported, including statistically significant differences in maternal body weight between exposure groups. Maternal stress is a potential confounder in studies of reproductive and developmental toxicity, with reported effects including increased fetal anomalies (Michel & Fritz-Niggli 1978) and effects on fetal endocrine function (Mairesse et al. 2007) or offspring behavior (Baker et al. 2009; Velíšek 2011; Weinstock 2001).

No indication of maternal toxicity was evident based on examination of the uterine contents. All dams in each exposure group had viable litters with no difference between treatment and control groups in mean number of corpora lutea, total number of implants, percent preimplantation loss, total number of live implants, mean number of live implants per litter, percent litters with deaths, number of resorptions, percent litters with resorptions, percent litters with abnormal fetuses, or litter sex ratio.

Table 9. Summary of fetal visceral abnormalities by number of fetuses from Sprague–Dawley rat dams exposed to CI.

Parameter	Value[a]	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Total affected	Number of fetuses	7	9	7	0
Lung, parenchyma, pigmentation, mottled[b]	Number of fetuses	1	3	3	0
Lung, parenchyma, pigmentation, pale/white[b]	Number of fetuses	2	1	1	0
Lung, parenchyma, pigmentation, red[b]	Number of fetuses	2	3	2	0
Lung, right apical lobe, pigmentation, mottled[b]	Number of fetuses	1	1	0	0
Lung, right apical lobe, pigmentation, red[b]	Number of fetuses	2	2	0	0
Lung, left lobe, pigmentation, red[b]	Number of fetuses	2	1	2	0
Lung, left lobe, pigmentation, mottled[b]	Number of fetuses	1	1	1	0
Lung, left cardiac lobe, pigmentation, mottled[b]	Number of fetuses	0	1	0	0
Lung, right cardiac lobe, pigmentation, red[b]	Number of fetuses	0	1	0	0
Lung, right cardiac lobe, pigmentation, mottled[b]	Number of fetuses	0	1	0	0

[a]Number of fetuses examined was 154, 157, 160, and 160 for control, low, mid, and high groups, respectively. Number of litters examined was 25 per group.
[b]Classified as a variation by test administrators.

Table 10. Summary of fetal cephalic abnormalities by number of fetuses from Sprague–Dawley rat dams exposed to CI.

Parameter	Value[a]	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Total affected	Number of fetuses	8	7	3	4
Cleft palate (partial)[b]	Number of fetuses	0	1	0	0
Third ventricle- distended[b]	Number of fetuses	6	5	3	4
Lateral ventricle- distended[b]	Number of fetuses	0	1	0	0
Olfactory lobes-asymmetrical[c]	Number of fetuses	2	0	0	0

[a]Number of fetuses examined was 154, 157,160, and 160 for control, low, mid, and high groups, respectively. Number of litters examined was 25 per group.
[b]Classified as a malformation by the test administrators.
[c]Classified as a variation by test administrators.

No biologically significant fetal effects were noted. A slight body weight reduction was observed in the male and female GD 20 fetuses (combined and separate) for the high exposure group (average body weight decreased 6–7% compared to the control) and for the mid exposure group for the female and combined male and female fetuses (average body weight decreased 5–6% compared to the control). However, given that no statistically significant increase in alterations was observed in any of the other variables when using the litter as the experimental unit (including external, visceral, cephalic, and skeletal alterations), fetal toxicity is not evident. The decreased fetal body weights were considered to be due to maternal stress related to the deposition of CI fibers in the lung.

The literature reports that slight changes in fetal body weight at GD 20 following exposure to boric acid may be transient. For example, Price et al. (1996a) reported reductions in fetal body weight (males and females) at GD 20 for groups of pregnant Sprague-Dawley rats fed boric acid at doses of 0.1% in feed (approximately 76 mg BA/kg bw-d or 13 mg B/kg bw-d) or 0.2% in feed (approximately 143 mg BA/kg bw-d or 25 mg B/kg bw-d) from GD 0 to GD 20. At GD 20, mean body weights of male fetuses decreased 6.2% and 12.9% in the 0.1% and 0.2% dose groups, respectively, compared to control, and in female fetuses, mean body weights decreased 7.1% and 13.6% in the 0.1% and 0.2% dose groups, respectively, compared to control. However, no differences in body weight were observed in offspring in any of the dose groups when measured at either birth [Postnatal Day (PND) 0] or at PND 21.

While some skeletal variations in individual fetuses were seen at the 270 mg/m^3 dose level, consisting mainly of signs of delayed ossification as evidenced primarily by unossified 5th and/or 6th strenebrae and reduced ossification of the interparietal bones in the skull, the differences in incidence of skeletal variations were not significant when using litter as the experimental unit for statistical analysis per US EPA (1998) and OECD (2001) developmental toxicity study guidelines. Using the fetus as the experimental unit biases the analysis, as it places disproportionate weight on litters with more fetuses and neglects the potential for correlation among observations within a litter (Holson et al. 2008; Wainwright 1998), when the objective is to determine if the chemical agent is causing effects to more litters.

These skeletal variations are commonly seen effects usually indicative of slight developmental delays that accompany depressions in fetal body weight secondary to maternal stress (Kavlock et al. 1985; Khera 1984, 1985; Kimmel & Wilson 1973). Thus, these skeletal variations are not considered indicative of an adverse treatment-related effect.

Because boric acid is a key ingredient in CI, its potential toxicological effects are of interest. The estimated average boric acid concentrations for the three CI exposure groups are 3, 18, and 53 mg/m^3. To facilitate qualitative comparisons of delivered inhalation doses to studies examining effects of boric acid delivered via other routes (e.g. oral), the daily delivered dose of boric acid to the rats was estimated based on an assumed average inhaled minute volume for the rat, as follows (adapted from US EPA 1989):

Table 11. Summary of fetal and litter skeletal alterations by litter and fetuses from Sprague–Dawley rat dams exposed to CI.

Parameter	Value[a]	Control	Low (15 mg/m^3)	Mid (90 mg/m^3)	High (270 mg/m^3)
Skull					
Parietal – any alteration[b]	Number of litters affected (%)	2 (8%)	6 (24%)	4 (16%)	5 (20%)
	Number of fetuses affected (%)	2 (1.3%)	7 (4.5%)	7 (4.4%)	7 (4.4%)
	Average number of malformed fetuses per litter	0.08	0.28	0.28	0.28
Interparietal – any alteration[b]	Number of litters affected (%)	5 (20%)	4 (16%)	4 (16%)	8 (32%)
	Number of fetuses affected (%)	5 (3.1%)	6 (3.9%)	9 (5.7%)	13 (8.2%)*
	Average number of malformed fetuses per litter	0.2	0.2	0.4	0.5
Frontals – any alteration	Number of litters affected (%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)
	Number of fetuses affected (%)	0 (0%)	0 (0%)	1 (0.6%)	0 (0%)
	Average number of malformed fetuses per litter	0.0	0.0	0.04	0.0
Supraoccipital – any alteration	Number of litters affected (%)	6 (24%)	6 (24%)	7 (28%)	7 (28%)
	Number of fetuses affected (%)	13 (8.1%)	9 (5.8%)	13 (8.2%)	13 (8.2%)
	Average number of malformed fetuses per litter	0.5	0.4	0.5	0.5
Squamosal – any alteration	Number of litters affected (%)	0 (0%)	1 (4%)	1 (4%)	0 (0%)
	Number of fetuses affected (%)	0 (0%)	1 (0.6%)	1 (0.6%)	0 (0%)
	Average number of malformed fetuses per litter	0.0	0.04	0.04	0.0
Hyoid – any alteration[b]	Number of litters affected (%)	3 (12%)	2 (8%)	6 (24%)	4 (16%)
	Number of fetuses affected (%)	7 (4.4%)	8 (5.2%)	15 (9.4%)	10 (6.3%)
	Average number of malformed fetuses per litter	0.28	0.32	0.60	0.40
Mandible – any alteration	Number of litters affected (%)	1 (4%)	0 (0%)	0 (0%)	1 (4%)
	Number of fetuses affected (%)	1 (0.6%)	0 (0%)	0 (0%)	1 (0.6%)
	Average number of malformed fetuses per litter	0.04	0.0	0.0	0.04
Vertebral Column					
Thoracic centra – any alteration[b]	Number of litters affected (%)	8 (32%)	12 (48%)	13 (52%)	7 (28%)
	Number of fetuses affected (%)	19 (11.9%)	17 (11%)	22 (13.8%)	10 (6.3%)
	Average number of malformed fetuses per litter	0.8	0.7	0.9	0.4
Lumbar centra – any alteration	Number of litters affected (%)	1 (4.0%)	0 (0%)	1 (4%)	0 (0%)
	Number of fetuses affected (%)	2 (1.3%)	0 (0%)	1 (0.6%)	0 (0%)
	Average number of malformed fetuses per litter	0.1	0.0	0.04	0.0
Sternebrae and Ribs					
Sternebrae – any alteration[b]	Number of litters affected (%)	21 (84%)	19 (76%)	24 (96%)	24 (96%)
	Number of fetuses affected (%)	76 (47.5%)	51 (33.1%)	83 (52.2%)	105 (66.0%)*
	Average number of malformed fetuses per litter	3.0	2.0	3.3	4.2
All rib alterations – any alteration[b]	Number of litters affected (%)	2 (8%)	4 (16%)	4 (16%)	6 (24%)
	Number of fetuses affected (%)	4 (2.5%)	5 (3.2%)	4 (2.5%)	8 (5.0%)
	Average number of malformed fetuses per litter	0.2	0.2	0.2	0.3

[a]Number of litters examined was 25 for each group. Number of fetuses examined was 160, 154,159, and 159 for control, low, mid, and high groups, respectively.
[b]Parameter statistically analyzed.
*Statistically significant compared to control as determined by Chi-square analysis ($p \leq .05$; statistically significant increases only). However, the litter is the appropriate experimental unit for assessing differences in skeletal variations.

$$Dose = \frac{Conc\ (mg/m^3) \times MV\ (\mathrm{L}/min) \times ET\ (h/d) \times 0.001\ \mathrm{m}^3/\mathrm{L} \times 60\ min/h}{BW\ (kg)} \quad (2)$$

Where, Dose = Estimated average delivered dose of boric acid to rats via inhalation (mg/kg bw-d); Conc = Average concentration of boric acid in air (mg/m^3); MV = Rat daily minute volume (0.170 L/min) (average, non-anesthetized animals; Bide et al., 2000); ET = Exposure time of rats to CI particulate in air (6 h/d); BW = Rat body weight (0.284 kg, approximate average body weight of dams during the study)

Delivered doses of boron were estimated by multiplying the estimated delivered doses of boric acid by the molar fraction of boron in boric acid (17.5%).

For the maternal rats, average delivered doses of boric acid for the low, mid, and high exposure groups were 0.65, 4.0, and 11 mg BA/kg bw-d. Considering boron mass only, the estimated average delivered boron doses for the low, mid, and high exposure groups were 0.11, 0.69, and 2.0 mg B/kg bw-d, respectively. Based on the gross necropsy observations (pale lungs) in dams, the NOAEL for maternal toxicity was 15 mg/m^3 CI (estimated delivered doses: 0.65 mg BA/kg bw-d or 0.11 mg B/kg bw-d), and the LOAEL for minimal maternal toxicity was 92 mg/m^3 CI (estimated delivered doses: 4.0 mg BA/kg bw-d or 0.69 mg B/kg bw-d). The NOAEL for developmental toxicity was the highest concentration tested, 265 mg/m^3 CI (estimated delivered doses: 11 mg BA/kg bw-d or 2.0 mg B/kg bw-d). No LOAEL for developmental toxicity was found in this study as no adverse effects were reported at the highest dose tested.

Several *in vivo* developmental toxicity studies report effects from oral exposure to boric acid. Price et al. (1996a) fed pregnant Sprague Dawley rats boric acid in feed at 0.025, 0.05, 0.075, 0.1, and 0.2% (approximately 19, 36, 55, 76, or 143 mg BA/kg bw-d or 3.3, 6.3, 9.6, 13, or 25 mg B/kg bw-d) from GD 0 to 20. The NOAEL for maternal toxicity was 76 mg BA/kg bw-d (13 mg B/kg bw-d) based on increased relative kidney weight at 143 mg BA/kg bw-d (9.6 mg B/kg bw-d). Price et al. report a NOAEL of 55 mg BA/kg bw-d (9.6 mg B/kg bw-d), and a LOAEL of 76 mg BA/kg bw-d (13 mg B/kg bw-d) for developmental toxicity based on decreased fetal weight and increased incidence of wavy rib (classified as a variation) and short rib XIII (classified as a malformation) (Price et al. 1996a). They note that the fetal body weight and wavy rib reversed on PND 21, but the short rib did not.

In another study, Price et al. (1996b) administered boric acid to pregnant New Zealand white rabbits in feed at doses of 62.5, 125, or 250 mg BA/kg bw-d (approximately 11, 22, or 44 mg B/kg bw-d) on GD 6–19. They report a NOAEL of 125 mg BA/kg bw-d (22 mg B/kg bw-d) for both maternal and developmental toxicity, based on decreased maternal body weight gain during treatment, decreased maternal relative kidney weight, decreased gravid uterine weight, decreased number of ovarian corpora lutea, increased prenatal mortality, and reduced live litter size at 250 mg BA/kg bw-d (44 mg B/kg bw-d). An increase in the percentage of malformed fetuses per litter was also reported at 250 mg BA/kg bw-d.

Heindel et al. (1992) conducted a developmental toxicity study of boric acid in rats and mice. Rats were fed boric acid at 78, 163, and 330 mg BA/kg bw-d (approximately 14, 28, and 58 mg B/kg bw-d) on GD 0 to 20, and mice were fed boric acid at 248, 452, and 1003 mg BA/kg bw-d (approximately 43.4, 79.1, and 175.5 mg B/kg bw-d) on GD 0 to 17. The rat study was originally published as NTP (1990), with an additional dose of 539 mg BA/kg bw-d (approximately 94 mg B/kg bw-d) administered only during the period of major organogenesis (GD 6 to 15) in order to limit early embryolethality. In rats, the maternal NOAEL was 78 mg BA/kg bw-d (14 mg B/kg bw-d) and the LOAEL was 163 mg BA/kg bw-d (28 mg B/kg bw-d) based on increased liver and kidney weights. Decreased fetal weight was observed at all doses, and fetal malformations were observed at 163 mg BA/kg bw-d (28 mg B/kg bw-d) and above. At the 539 mg BA/kg bw-d dose, the same maternal and fetal effects were observed, as was decreased food and water intake and an increase in prenatal mortality. In mice, no maternal NOAEL was identified and the LOAEL was 248 mg BA/kg bw-d (43.4 mg B/kg bw-d) based on increases in kidney lesions. Reduced fetal body weight was observed with a NOAEL of 248 mg BA/kg bw-d (43.4 mg B/kg bw-d) and a LOAEL of 452 mg BA/kg bw-d (79.1 mg B/kg bw-d).

Estimated delivered doses of boric acid associated with inhalation of CI assessed in this study are below the most conservative LOAELs found in the literature for boric acid maternal toxicity or reproductive or developmental toxicity when delivered via the oral route. However, given that a potential effect from inhalation exposure of CI or other particulate is local respiratory tract effects and that route-specific differences in absorption and metabolism (e.g. first-pass effects in the respiratory tract or liver) may impact internal dose as well as observed effects (US EPA 1994), critical effects in developmental studies of inhalation exposure to CI could differ from those observed in studies of oral exposures to boric acid.

No studies evaluating the reproductive or developmental toxicity of CI in rodents following inhalation were identified. Several studies evaluated other endpoints following short-term exposures of rats to CI or other forms of cellulose via the respiratory tract (via either inhalation or II) (Cullen et al. 2000; Hadley et al. 1992; Morgan 2006). In all four studies, increased inflammatory responses in the lungs were observed. For example, diffuse macrophage infiltration, microgranuloma formation, alveolitis (acute inflammation of the pulmonary alveoli), and epithelial hyperplasia (increase in number or proliferation of cells) were seen in rats administered Thermolite® CI particles (38–47% rat-respirable particles) by nose-only inhalation for six hours per day, five days per week, for 21 exposures at concentrations of 100, 500, or 2000 mg/m^3 (Hadley et al. 1992). The results of the study reported here also suggest that the lung is a target organ for CI toxicity based on the observed changes in the dam's lungs at the mid and high dose.

Workers engaged in CI manufacturing or installation are expected to be the most highly exposed group to respirable CI particles. In the field study of CI insulation applicators conducted by NIOSH (Morgan 2006), eight hour time-weighted average (TWA) total dust concentrations were measured in personal breathing zone samples at ten contractor sites during CI installation. Total dust concentrations measured at or around locations where employees dumped bags of CI into a hopper or installed CI into attic spaces or wall/ceiling were as high as 64.8 mg/m^3 (range 0.10–64.8 mg/m^3). Respirable dust concentrations in area samples were as high as 2.2 mg/m^3 (range non detect to 2.20 mg/m^3) (eight hour TWA) (Morgan 2006). Given that workers engaged in CI installation or manufacturing would be those with the highest exposures, based on the results herein, no developmental effects from CI exposure are expected.

Disclosure statement

This manuscript was not provided to CIMA or any of its members until it was finally accepted for publication. Dr. Pleus, Ms. Bruce, and Dr. Keenan have provided independent human health consulting for the Cellulose Insulation Manufacturers Association (CIMA) and companies that are members of CIMA.

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