Postnatal Effects of Gestational and Lactational Gavage Exposure to Boric Acid in the Developing Sprague Dawley Rat

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ABSTRACT

Human exposure to boron occurs primarily through diet and drinking water sources. Animal studies have found that reduced fetal weight following gestational exposure to boron (as boric acid) is the most sensitive toxicological effect. However, recent studies suggest that newborns in areas with elevated boron in drinking water may receive levels of exposure that exceed the U.S. EPA oral reference dose for B. Currently, there are no data to inform a boron risk assessment accounting for this developmental window. To address this knowledge gap, the National Toxicology Program evaluated developmental toxicity following pre- and postnatal boron exposure. Time-mated female Sprague Dawley (Hsd: Sprague Dawley SD) rats were administered 0–20 mg B/kg/day (as boric acid) via gavage from gestation day 6 to 21; offspring were dosed via gavage at the same respective dose level from postnatal day (PND) 1 to 28. There were no dose-related effects on dam bodyweight, bodyweight gain, or feed consumption. Clinical findings were limited to low incidences of umbilical hernia in the 20 mg B/kg pups which resolved by study completion. Pup plasma boron concentrations increased in dose-proportional manner and were similar between PND 4 and PND 28. Postnatal weight gain was significantly reduced at 20 mg B/kg, with male and female pups weighing 23% less than the controls on PND 28. These findings demonstrate that postnatal growth in the Sprague Dawley rat is sensitive to boron exposure and highlights the importance of evaluating the potential toxicity of agents with known human exposures during early life stages.

Key words: boron; boric acid; prenatal; postnatal; umbilical hernia.

Impact Statement

- This study demonstrates that pre- and postnatal exposure to boron (boric acid) is associated with lower rat pup body weights and low incidence of transient umbilical hernia.
- These findings occurred at 20 mg B/kg/day, an exposure level that is higher than the current point of departure used to calculate the current U.S. EPA oral reference dose.
- This study highlights the importance of evaluating the potential toxicity of agents with known human exposures during early life stages.

Boron is an essential element required for development of plant (Warrington, 1923) and vertebrate species (Fort et al., 2002; Nielsen, 1994; Rowe and Eckhert, 1999). Boron is typically found in the environment in its oxidized form as borates with sodium tetraborate (borax) and boric acid (H_3BO_3) among the most prevalent forms. Boron has been detected at varying concentrations in surface water and groundwater in the United States and is associated with geologic properties of a region (Frey et al., 2004; USGS, 2016). Human exposure to boron occurs predominantly from dietary sources, which provide approximately 1 mg/day in the average adult (Rainey et al., 2002). Borate-containing compounds are also used in industrial, agricultural, and household and consumer products (Moore, 1997; USGS, 2016); however, diet and drinking water are considered the 2 most prevalent sources of exposure in humans.

Given potential human exposure through water and dietary sources, numerous studies have evaluated the potential for boron to induce adverse developmental effects in the fetus. Teratology studies conducted in mouse, rat, and rabbit species identified reduced fetal body weight and rib anomalies as the most common and sensitive outcomes following *in utero* exposure to boric acid in feed. Specifically, lower fetal weights were observed at doses of 13.3 (Price et al., 1996) and 13.6 mg B/kg/day (Heindel et al., 1992) in 2 teratology studies in the Sprague Dawley rat. These data were used to derive a benchmark dose level (BMDL_{05}) of 10.3 mg B/kg/day that was based on 5% reduction in fetal weight as the benchmark response (Allen et al., 1996). Using the BMDL_{05} as the critical effect, the U.S. Environmental Protection Agency (EPA) established an oral reference dose (RfD) of 0.2 mg B/kg/day (U.S. Environmental Protection Agency, 2004), a dose which is assumed to pose no risk of adverse effects during a lifetime of exposure.

Additional studies report evidence of testicular toxicity in the rat and mouse following exposure to boric acid or borax. In CD-1 mice, a 27-week Reproductive Assessment by Continuous Breeding (RACB) feed study by the National Toxicology Program (NTP) found reduced fertility, lower mating indices, and reduced sperm parameters at 4500 ppm (≈ 111 mg B/kg) and infertility at 9000 ppm (≈ 221 mg B/kg) boric acid in the F_0 generation; cross-over mating trials identified males as the sensitive sex. Reproductive parameters in F_0 and F_1 mice at 1000 ppm were unaffected, except for slight, but statistically significant lower F_2 pup weights (Fail et al., 1991). A 14-week feed study in Sprague Dawley rats also identified sterility associated with testicular atrophy in males and decreased ovulation in females exposed to 6690 ppm boron (as boric acid) (≈ 106 mg B/kg/day) (Weir and Fisher, 1972). In addition, a number of subchronic feed studies in adults showed that boron-related testicular toxicity as a spectrum of outcomes from inhibited spermiogenesis at lower doses (≥ 26 mg B/kg/day) to testicular atrophy and sterility at higher doses (> 100 mg B/kg/day) (Fukuda et al., 2000; Ku et al., 1993; Lee et al., 1978; Yoshizaki et al., 1999).

Although exposure to boron in rats has been shown to produce developmental toxicity at ≥ 13 mg B/kg/day and testicular toxicity in adults at ≥ 26 mg B/kg/day, there are no studies available to assess the potential for boron-mediated toxicity during early postnatal development. Human infants and neonates may be exposed through drinking water used to reconstitute infant formula as well as in the powdered formula (López-García et al., 2009). Under this scenario, the potential for higher exposure exists and there are no developmental toxicity data involving direct exposure to postnatal animals available to inform health-based risk assessments.

To address this knowledge gap, NTP evaluated the potential for boron to induce developmental toxicity following prenatal and postnatal gavage exposure in Sprague Dawley (Hsd: Sprague Dawley SD) rats. In the present study, time-mated females were administered doses of 0, 5, 10, or 20 mg B/kg/day (as boric acid) from gestation day (GD) 6 to 21, and following birth, F_1 offspring were dosed from postnatal day (PND) 1 to 28 to the same dose as their respective dam. Dams were not dosed during lactation to avoid potential boron exposure to pups through lactational transfer. Doses were selected to compare potential postnatal effects with the point of departure ($\text{BMDL}_{05} = 10.3$ mg B/kg) based on findings of lower fetal weight from teratology studies. Thus, the primary objectives of the present study were to characterize the postnatal effects of boron exposure at doses above and below the point of departure and to assess the relative sensitivity of the perinatal exposure window compared with gestational exposure alone.

MATERIALS AND METHODS

Materials. The test article, boric acid (CAS no.: 10043-35-3), was purchased from Sigma-Aldrich (lot no.: BCBW0314; St. Louis, Missouri). Boric acid was chosen as the test article based on its use in prior studies to investigate boron-mediated toxicity. The vehicle was deionized water. Characterization of the boric acid used in this study yielded an overall purity estimate of $>98\%$ based upon the results of the elemental analysis, which indicated boron content of 17.0%, compared with a theoretical value of 17.5% and water content of 0.73%. High-performance liquid chromatography coupled to a refractive index detector (HPLC-RI) analysis showed no reportable impurities, and the inductively coupled plasma-mass spectrometry (ICP-MS) trace impurity screen showed no reportable elemental impurities. The dose formulations, at concentrations of 0, 1, 2, and 4 mg B/ml prepared in deionized water, were analyzed using a validated ICP-MS method (10–1000 ng B/ml; percent relative error [%RE] -21.8% – 4.7% ; percent relative standard deviation [%RSD] $\leq 7.7\%$). Formulation samples were diluted 1:10 with deionized water. A 40- μl aliquot of each diluted sample was added to a centrifuge tube containing 100 mg D-mannitol, which was then mixed and combined with 19.54 ml of deionized water, 400 μl of

nitric acid, and 25 μ l of beryllium internal standard (IS). Samples were subsequently analyzed using ICP-MS (Model 7500ce Octapole Reaction System [ORS]-ICP-MS; Agilent, Santa Clara, California) and were within $\pm 6.5\%$ of their respective target concentrations; animal room samples collected after dosing were within $\pm 8.1\%$ of their target values. Prior to study initiation, stability of dosing formulations up to 70 days was established when stored at room temperature with boron recovery $\geq 99.0\%$ (%RSD = 0.3%) compared with day 0. Formulations were stored in polyethylene bottles at room temperature during study conduct.

Background boron concentrations were measured in drinking water (Birmingham, Alabama municipal water), deionized water, and NIH-07 feed (lots 02-01-17, 030217, 03May18, 04-04-18) using the ICP-MS method described above, with the addition of closed vessel acid digestion to recover the boron from the feed. The %RE values were $\leq \pm 10.9\%$ and %RSD was $\leq 4.8\%$ for feed QC standards run with the samples.

Test animals. The study protocol was in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and National Research Council guidelines and was approved by the Southern Research Institutional Animal Care and Use Committee. The study was conducted in accordance with the U.S. Public Health Service animal care policy and with all applicable federal, state, and local regulations and guidelines. In addition, the study was performed in compliance with the U.S. Food and Drug Administration Good Laboratory Practice Regulations.

Thirty-two time-mated female Sprague Dawley rats (Hsd: Sprague Dawley SD) (Envigo, Haslett, Michigan) were received at approximately 12 weeks of age and weighed between 225 and 265 g. Animals were acclimated to their new environment for up to 5 days prior to dosing and maintained in quarantine for 7 days after arrival. Animals were randomly assigned to treatment groups (8 per group) based on gestation day (GD) 3 body weight. Ten non-mated females were used as sentinel animals for disease screening. Dams were housed in solid-bottom polycarbonate cages (Lab Products, Inc.; Seaford, Delaware) individually during gestation and with their pups in lactation in a climate-controlled (target humidity 35%–65%, temperature 69°F–75°F) room on a 12-h light/dark cycle. Cage filters were obtained from Granville Milling Company (Creedmoor, North Carolina); irradiated Sani-Chips hardwood bedding from Envigo (Madison, Wisconsin) and Crink-I' Nest paper (The Andersons, Maumee, Ohio) for environmental enrichment. Irradiated NIH-07 feed (Zeigler Brothers; Gardners, Pennsylvania) and Birmingham, Alabama municipal water were provided *ad libitum*.

Exposure and experimental endpoints. From GD 6 to 21 time-mated females ($n=8$ per group) were administered either vehicle (deionized water), 5, 10, or 20 mg B/kg/day (administered as 28.6, 57.1, or 114 mg boric acid/kg/day; pH 3.1–5.6) via oral gavage at a dose volume of 5 ml/kg. Dams were weighed on GD 3 and daily beginning on GD 6. Dams were assigned to treatment groups randomly based on GD 3 body weight. Dams and offspring were observed twice daily for signs of morbidity and mortality. Feed consumption was measured at 3- to 4-day intervals during gestation (beginning on GD 3) and lactation. Following delivery, litter parameters including litter size, number of live and dead pups, and sex ratio were assessed.

F1 pups were administered the same dose concentration as their respective dam from PND 1 through 28; dams were not

dosed during the lactational period. Pup viability, clinical observations, and body weights were recorded daily. On PND 4, litters were standardized to 8 pups per litter (4 males and 4 females, when possible); unneeded pups were euthanized via decapitation (for collection of trunk blood) or by intraperitoneal injection of sodium pentobarbital and not examined further. Dams were euthanized on PND 28–30 via 100% CO₂ inhalation and were not examined further. On PND 28–30, pups were euthanized via 100% CO₂ inhalation with some pups used for blood collection. In addition, selected pups on PND 30 were evaluated macroscopically for gross lesions to evaluate the persistence of umbilical hernias observed in several pups. Three pups per sex from the control group, and pups that were observed to have umbilical hernias were subjected to a gross macroscopic examination of the thoracic and abdominal viscera.

Sample collection and analysis for plasma boron concentration. Plasma was collected from PND 4 ($n=4$ –5/sex/dose group from different litters) and PND 28 pups ($n=2$ –3 females/dose group, from different litters). Samples were collected approximately 2 h after the last dose. PND 4 pups were euthanized by decapitation and trunk blood was collected. PND 28 pups were anesthetized via CO₂/O₂ inhalation, and blood was collected from the retro-orbital plexus or by cardiac puncture. PND 28 pups were euthanized by CO₂ inhalation following blood collection. Blood samples were collected into K₃EDTA tubes and plasma was isolated within 1 h of collection. Plasma samples were flash frozen and stored at -70°C for subsequent analysis.

To a 50- μ l aliquot of plasma, 0.020 ml of IS (990 ng Be/ml) and 1.930 ml of diluent consisting of 2% (v/v) aqueous nitric acid, 0.5% (w/v) mannitol were added. Samples were mixed and centrifuged for 10 min at $18\,330 \times g$. Supernatants were transferred to individual 15-ml centrifuge tubes for analysis using ICP/MS (Model 7500ce Octapole Reaction System [ORS]-ICP-MS; Agilent Technologies, Santa Clara, California). Standard curves run with each sample batch were linear ($r \geq 0.9996$) and accuracy, based on QC standards at 15.038 and 751.88 ng/ml run with the samples, was acceptable (%RE < 14%; %RSD $\leq 2.4\%$). For collection and analysis of plasma, care was taken to avoid the use of borosilicate glass.

Data collection and statistical analysis. Data was collected using the Provantis hosted system provided by the NTP (Instem Life Sciences Systems, Ltd., Staffordshire, UK). For F₀ body weights and feed consumption endpoints, extreme values were identified by the outlier test of Dixon and Massey (1957). To identify outliers for F₁ pup weights, all observations across dose groups were fit to a linear mixed effects model with a random litter effect, and the residuals were tested by dose group for outliers using Tukey's outer fences method (Tukey, 1977). All flagged outliers were examined by NTP personnel, and implausible values were eliminated from the final analyses.

Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary, North Carolina). F₀ body weights were analyzed using Jonckheere's trend test (1954) and Williams' or Dunnett's (pairwise) test depending on detection of a significant trend at .01 level (Williams, 1971, 1972; Dunnett, 1955). Gestational length, feed consumption, litter size, and survival endpoints were analyzed using the nonparametric multiple comparison methods of Shirley (1977) as modified by Williams (1986) and Dunn (1964). Jonckheere's trend test was used to assess the significance of dose-related trends. For reproductive performance endpoints, statistical analysis was performed by Cochran-Armitage (trend) and Fisher Exact (pairwise)

Table 1. Boron Concentrations and Estimated Exposures From Drinking Water and NIH-07 Feed During Gestation

Sample	Boron Concentration	Estimated Exposure (mg B/kg/day) ^a
Purified water (18 Ω) ^b	≤4.17 ng/ml ^c	N/A
Animal facility water	32.0 ± 10.9 ng/ml	<0.005
NIH-07 rodent feed	6.75 ± 0.68 µg/g	0.51 ± 0.01

Data reported as mean ± SEM.

^aExposure estimates are based on a water intake rate of 11 ml/100 g body weight/day (McGivern *et al.*, 1996) and mean feed consumption from control dams in the present study.

^bDosing vehicle.

^cTwo samples were analyzed. One measured 4.17 ng/ml and the other did not measure above the limit of detection.

2-sided tests (Gart *et al.*, 1979). F₁ pup weights were first adjusted for litter size and then analyzed with mixed effects linear models with a random litter effect, and the Dunnett-Hsu procedure (Hsu, 1992) was used to adjust for multiple comparisons. Adjustment of pup weights by litter size was performed by fitting a linear model to pup weights as a function of dose and litter size. The estimated coefficient of litter size was then used to adjust each pup body weight based on the difference between its litter size and the mean litter size. Pup weights measured from PND 1 to PND 4 (standardization) were adjusted relative to live litter size on PND 1, and post-standardization pup weights were adjusted based on the number of post-standardization live pups. Dosed groups different from control at $p \leq .05$ were considered statistically significant.

RESULTS

Summary data relevant for evaluating toxicological findings are presented below. Additional data including individual dam and pup data are available in the NTP Chemical Effects in Biological Systems (CEBS) database.

Background Boron Concentrations

To determine boron contribution from background sources, boron concentrations were measured in NIH-07 feed and drinking water (Table 1). Drinking water from the animal facilities at the study laboratory contained 32.0 ± 10.9 ng B/ml and did not represent a significant source of exposure (<0.005 mg B/kg/day) when estimated based on daily consumption of 11 ml/100 g body weight/day in pregnant Sprague Dawley rats (McGivern *et al.*, 1996). Boron concentrations of 6.75 ± 0.68 µg B/g were present in NIH-07 feed. Based on measured feed consumption from this study (approximately 20–25 g/day), the NIH-07 diet contributed 0.51 ± 0.01 mg B/kg/day to dams during gestation. Contribution from NIH-07 diet therefore represented approximately 10% of the lowest administered dose of 5 mg B/kg/day. Contribution from these sources was not estimated during the lactational period since dams are co-housed with their litters. Deionized water, which comprised the vehicle, contained ≤ 4.2 ng/l and did not contribute a significant source of boron exposure based on a dosing volume of 5 ml/kg.

Maternal Findings and Reproductive Performance

No mortality or signs of overt maternal toxicity were observed during gestation or lactation in dams exposed to 20 mg B/kg or less. Clinical findings of red vaginal discharge, loose feces, and

Figure 1. Maternal growth curves for pregnant rats during gestation and lactation. Body weights are shown as mean ± SEM for each treatment group (N = 6–7 dams/treatment group). Nonpregnant dams were excluded from statistical analysis of gestational weights. No statistically significant pairwise changes were observed with administration of boric acid.

alopecia were observed in 1 or 2 dams in the 20 mg B/kg group; however, given their minimal severity, short duration, and singular or low incidence, these were not suggestive of a B-related effect. Feed consumption, body weights, and body weight gains during gestation were similar across groups. During lactation, feed consumption for the dams in the 20 mg B/kg group was 10%–25% lower than dams in the control group (CEBS); however, no effects on dam body weights were observed during lactation at any dose group (Figure 1, CEBS).

Gestation length was similar (approximately 22 days) for all pregnant females that delivered a litter (6–7 per dose group) (Table 2). There was a slight, but not statistically significant decrease in number of live pups per litter and number of live males per litter in the 20 mg B/kg group when compared with controls. Two, 4, and 7 pups were found dead, cannibalized, or were euthanized moribund in the 5, 10, and 20 mg B/kg groups on PND 0, respectively. The 7 dead pups (3 males and 4 females) in the high dose group were from 1 dam which had 6 dead pups at delivery and an additional pup was euthanized on PND 0. In addition, mean pup weights per litter on PND 0 were comparable to the controls across all dose groups (males, females, or males and females combined).

Plasma Boron Concentrations

Plasma boron concentrations were measured in pups on PND 4 (male and female) and PND 28 (females only) and are shown in Table 3. Plasma concentrations on PND 4 increased in a dose-proportional manner and ranged from 18.9 to 11 164 ng B/ml for males and 39.9 to 9465 ng B/ml for females, which suggests no apparent sex differences across the dose range assessed in the present study. A similar dose-proportional increase was observed in PND 28 females. The approximately 2.5-fold increase in control plasma boron levels between PND 4 (39.9 ng/ml) and PND 28 (106.2 ng/ml) females likely reflects the contribution of boron from the diet.

Pup Findings

Between PND 1 and PND 3, 3 and 5 pups were found dead in the 5 and 10 mg B/kg groups, respectively, and in the 20 mg B/kg group, 1 pup was found dead and another euthanized in moribund condition on PND 8 (Table 4). Umbilical hernias were observed beginning on PND 7 in 11 pups (3 males and 8 females) from 5 litters in the 20 mg B/kg group. Of those 11 pups affected, 1 female pup displayed signs of dehydration, the absence of a milk band, and was found dead on PND 8. The umbilical hernias

Table 2. Maternal and Litter Parameters for Time-Mated Sprague Dawley Females Administered 0, 5, 10, or 20 mg B/kg/day From GD 6 to 21

	mg B/kg/day			
	0	5	10	20
No. of time-mated females	8	8	8	8
No. of pregnant	7	6	7	6
Gestation length	21.7 ± 0.2	21.7 ± 0.2	22.0 ± 0.0	22.0 ± 0.0
Litter parameters (PND 0)				
No. of viable litters	7	6	7	6
Total no. of pups	94	82	97	73
No. of live pups	94	80	93	66
No. of dead pups (no. of litters)	0	2 (2)	4 (4)	7 (1)
Total no. of pups/litter	13.4 ± 0.6	13.7 ± 0.3	13.9 ± 0.6	12.2 ± 1.5
No. of live pups/litter	13.4 ± 0.6	13.3 ± 0.2	13.3 ± 0.6	11 ± 1.4
No. of dead pups/litter	0	0.3 ± 0.2	0.6 ± 0.2	1.2 ± 1.2
Percent (%) of live males	60.2 ± 3.7	43.6 ± 4.6*	53.3 ± 3.9	47.0 ± 3.0
PND 0 pup weights ^a				
Combined	6.43 ± 0.24	6.11 ± 0.18	6.42 ± 0.09	6.28 ± 0.10
	—	− 4.9%	0.0%	− 2.2%
Male	6.53 ± 0.24	6.23 ± 0.17	6.51 ± 0.10	6.47 ± 0.12
	—	− 4.5%	− 0.3%	− 0.9%
Female	6.29 ± 0.24	6.02 ± 0.18	6.30 ± 0.09	6.14 ± 0.10
	—	− 4.3%	0.2%	− 2.4%

Data reported as total counts or mean ± SEM. Pup weights are reported as mean ± SEM of litter means.

^aPup weights were adjusted for litter size. Statistical analyses were performed using mixed models with dam ID as a random effect and Dunnett-Hsu adjustment for multiple comparisons.

*Significantly different ($p \leq .05$) from vehicle control by Shirley or Dunn pairwise tests.

Table 3. Plasma Boron Concentrations for F₁ Offspring

Timepoint	Sex	mg B/kg/day			
		0	5	10	20
PND 4	Male	18.9 ± 2.5	3426 ± 213	6499 ± 804	11 164 ± 2875
	Female	39.9 ± 14.8	1808 ± 564	7253 ± 300	9465 ± 3006
PND 28	Male	—	—	—	—
	Female	106.2 ± 5.3	2583 ± 59.0	4411 ± 548	8755 ± 581

Data are mean ± SEM; plasma concentrations are in ng/l.

Table 4. Postnatal Mortality and Survival of F₁ Pups Administered 0, 5, 10, or 20 mg B/kg via Gavage From PND 1–28^a

	mg B/kg/day			
	0	5	10	20
Pup mortality ^b				
PND 0	0 (0)	2 (2)	4 (4)	7 (1)
PND 0–1	0 (0)	2 (2)	4 (3)	1 (1)
PND 1–4	0 (0)	1 (1)	1 (1)	0 (0)
PND 4 (post-standardization) –7	0 (0)	0 (0)	0 (0)	0 (0)
PND 7–14	0 (0)	0 (0)	0 (0)	2 (1)
PND 14–28	0 (0)	0 (0)	0 (0)	0 (0)
Survival ratio ^c				
PND 0	1.00 ± 0.00 (7)	0.98 ± 0.01 (6)	0.96 ± 0.01 (7)	0.93 ± 0.07 (6)
PND 1–4	1.00 ± 0.00 (7)	0.96 ± 0.02 (6)	0.95 ± 0.02 (7)	0.99 ± 0.01 (6)
PND 5–28	1.00 ± 0.00 (7)	1.00 ± 0.00 (6)	1.00 ± 0.00 (7)	0.96 ± 0.04 (6)

^aLitters were standardized to 8 pups (4 per sex, when possible) on PND 4. All calculations are based on the last observation of the day.

^bData shown are number of dead pups (number of affected litters).

^cData shown are mean ± SEM (number of litters). For each litter, survival ratio was calculated as the number of pups alive at the beginning of the interval/number of pups per alive at the end of the interval.

Figure 2. Postnatal growth of F1 offspring administered 0, 5, 10, and 20 mg B/kg/day. Mean of litter means for absolute (A) and relative weights (% of control) (B) are shown for males and females combined, males only, and females only. Pup weights were adjusted for litter size and statistical analyses were performed using mixed models with the dam as the random effect and Dunnett-Hsu adjustment for multiple comparisons. Asterisks in (A) denote a significant pairwise difference in mean pup weight per litter in the 20 mg B/kg group when compared with the vehicle control (* $p \leq .05$, ** $p \leq .01$).

Figure 3. Postnatal weights of individual pups with umbilical hernia (20A-K) compared with the control and 20 mg B/kg group means. No outliers were detected among individuals when compared with the control or 20 mg B/kg group means.

observed in the remaining 10 pups resolved by the end of the study based on macroscopic evaluation on PND 28 or 30. Umbilical hernias were not observed in the control or other treatment groups.

Pup weights in 20 mg B/kg offspring were significantly lower than controls, beginning as early as PND 5 (Figure 2, CEBS). On PND 28, male and female pups (combined) weighed 1.7%, 5.9%, and 23.3% less than the controls in the 5, 10 and 20 mg B/kg groups, respectively. At 20 mg B/kg, respective male and female pup weights and weight gains were reduced by a similar magnitude. The body weight decrements at the high dose in males and females (combined) reflect lower overall weight gain (–22.9%) from PND 4 to 28 when compared with the controls, with the greatest effect occurring during the PND 4 to 7 (–37.8%) and PND 18 to 21 (–35.9%) intervals. Pups with umbilical hernias at 20 mg B/kg demonstrated similar body weights when compared with the rest of the 20 mg B/kg dose group (Figure 3).

DISCUSSION

The present study examined the postnatal effects following gestational and postnatal (via gavage) exposure to boron. Here, we provide evidence of postnatal growth restriction in Sprague Dawley pups at 20 mg B/kg/day which occurred in the absence of maternal toxicity. Key findings observed at this dose level included reduced weight gain in pups and the occurrence of transient umbilical hernia during postnatal development. Assessment of internal dose demonstrated a dose-proportional increase in plasma boron concentrations on PND 4 and PND 28, with no apparent sex differences observed in PND 4 pups. In addition, plasma concentrations were similar among dose groups between PND 4 to PND 28 suggesting little to no bioaccumulation, and is consistent with previous studies reporting rapid elimination and a short plasma half-life of approximately 3–4 h for boron when administered to rats via gavage (Usuda *et al.*, 1998; Vaziri *et al.*, 2001).

Of the endpoints assessed, postnatal weight gain displayed the most sensitive response and resulted in PND 28 pup weights that were 2%, 6%, and 23% lower than the controls at 5, 10, and 20 mg B/kg, respectively. Importantly, the lack of an observed effect on PND 0 pup weights at any exposure level indicates that postnatal gavage dosing significantly contributed to the lower weights observed on PND 28. Given the sensitivity of the perinatal developmental window, a 23% reduction in weight gain could significantly and irreversibly impact or delay key developmental milestones. However, relatively few studies have investigated the effects of direct (the present study) or indirect (lactational transfer) postnatal exposure in offspring. An RACB feed study in mice conducted by the NTP found no effects on postnatal weight gain in F₁ pups whose dams were exposed to 1000 ppm boric acid (≈27 mg B/kg/day) during gestation and lactation. F₂ pup weights on PND 0 were slightly lower

(approximately 3%) than the controls in F₂ pups at the same dose level (Fail et al., 1991). Another multigeneration breeding study in Sprague Dawley rats found no effects on offspring weight or survival at weaning in the F₁, F₂, and F₃ generations at dose levels of 170 (≈10 mg B/kg/day) and 310 ppm (≈30 mg B/kg/day) boron (as boric acid) in feed (Weir and Fisher, 1972). It is important to note that these pups likely did not receive direct boron exposure from feed until the onset of feeding, approximately 2 weeks into postnatal development. To the authors' knowledge, our study is the first to demonstrate a significant reduction in postnatal weight gain in rats following gestational exposure and direct exposure to boron during early postnatal development.

The findings of pup mortality and umbilical hernias are more difficult to interpret given the limited number of dams and litters assessed in the present study. The reduced number of live pups and live males per litter on PND 0 was attributed to 1 dam with 7 dead pups; the same dam had 2 additional pup deaths on PND 7 and 8, with only 1 pup death observed from the remaining 5 litters in the 20 mg B/kg group. Additional studies with a larger number of animals per group are needed to confirm if the observed pup mortality is indeed treatment related. The presence of umbilical hernias on PND 7 in 20 mg B/kg pups was an unexpected finding, as umbilical hernias commonly manifest at birth due to a delayed closure of the abdominal wall. In addition, only singular incidences of umbilical hernia have been observed in previous prenatal toxicity studies of boric acid in the Sprague Dawley rat (Heindel et al., 1992; Narotsky et al., 1998). One could speculate that a minor closure defect was present in pups but not visibly apparent at birth, and that postnatal exposure to boric acid exacerbated or prevented the early resolution of this defect. That in conjunction with increased abdominal pressure associated with rapid weight gain in the first postnatal week could provide one explanation for this delayed finding. Interestingly, the umbilical hernias in the present study were resolved by PND 30 (based on gross evaluation at necropsy), which is consistent with human cases where resolution occurs with subsequent postnatal growth (Kelly and Ponsky, 2013). In addition, of the 11 pups with umbilical hernias, only 1 did not survive at the end of study and the remaining 10 did not appear to have dramatic differences in weight when compared with other pups in the 20 mg/kg group. It remains unclear whether this finding represents an effect of prenatal boric acid exposure alone, or a combination of prenatal and postnatal exposure; however, given its resolution by PND 30, it likely does not indicate a deleterious effect that would be expected to impact subsequent juvenile development.

The specific aim for this study was to address the hypothesis that postnatal development is sensitive to boron exposure and to place these findings into context with other studies evaluating boron-mediated developmental toxicity. Therefore, dose levels of 0, 5, 10, and 20 mg B/kg/day were selected to probe the existing point of departure for prenatal toxicity (BMDL₀₅ = 10.3 mg B/kg/day) (Allen et al., 1996). The BMDL₀₅ value predicts the lower bound for a 5% reduction in fetal weight and is based on findings from 2 teratology studies where boric acid was administered via feed in the Sprague Dawley rat. In these 2 studies, fetal weight reductions of 6%–7% occurred at 13.6 mg B/kg (Heindel et al., 1992) and 13.3 mg B/kg (Price et al., 1996). The study by Price et al (1996) included a recovery cohort (ie, no postnatal exposure) to determine if fetal findings, which also included rib malformations at 13.3 mg B/kg, persisted with postnatal development. Interestingly, PND 0 pup weights in the Price et al. (1996) recovery cohort were within 3% of control pup

weights at all dose levels tested and the incidence of rib anomalies persisted only at the top dose of 25 mg B/kg. In the present study, we observed no statistically significant reductions in PND 0 pup weights, which align with findings from the Price et al. (1996) study (skeletal assessments were not performed in the present study). The design of the present study was focused specifically on identifying postnatal effects that occurred following *in utero* exposure with direct exposure to boric acid from PND 1 to PND 28 (around the time of weaning). Additional studies are needed to determine whether gestational and lactational exposure to boron, a known testicular toxicant at ≥26 mg B/kg/day (for review, see Chapin and Ku, 1994), at doses at or below the current point of departure affects male reproductive endpoints or functional outcomes.

Assessing exposure during the pre- and postnatal period highlights important considerations for deriving health-based guidance values for chemical and nonchemical agents in drinking water. That is the prevailing notion of differential susceptibility across life stages, especially during postnatal development when infants and neonates may receive greater exposure than adults. Contributing to these higher exposure levels are the higher water intake rates (volume of water per unit mass per day) that can be 6- to 7-fold higher in infants than adults (U.S. Environmental Protection Agency, 2011). As a result, the relative source contribution of drinking water may be greater when considering the total exposure in a growing infant. When taken into consideration, these factors may influence the promulgation of lower health-based guidance values for short duration exposures during critical developmental windows than those derived for chronic exposures (Goeden, 2018).

For boron specifically, recent epidemiological evidence supports the growing concern for exposure during pre- and postnatal development. Mother-child cohort studies in Argentina and northern Chile, where boron is present at levels ranging from 0.38 to 16.1 mg B/l in drinking water, demonstrate a strong association between infant urinary boron levels and reduced infant body weight, head circumference, and length (Hjelm et al., 2019). Therefore, the present study provides important data to contextualize the uncertainty regarding the sensitivity of postnatal development to boron exposure. Though designed to be limited in its approach and scope, the present study identifies a clear effect on postnatal pup weight gain following direct gavage to 20 mg B/kg with no notable effects observed at 10 mg B/kg. The no-observed-adverse-effect level of 10 mg B/kg/day closely aligns with the point of departure for reduced fetal weight derived from previous prenatal toxicity studies, and suggests that postnatal development, especially during the lactational window, is a sensitive developmental period that should be evaluated to fully characterize the toxicologic potential of environmental agents present in drinking water.

ACKNOWLEDGMENT

The authors are grateful to Drs Chad Blystone and Kembra Howdeshell for their review of this article.

FUNDING

Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences (ZIA ES103316-04); and performed for the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services

(HHSN273201300010C [Southern Research; animal study laboratory], HHSN273201400020C [MRIGlobal; chemistry activities], HHSN273201600011C [Social and Scientific Systems; statistics]).

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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