Tissue Manganese Concentrations in Lactating Rats and Their Offspring Following Combined in Utero and Lactation Exposure to Inhaled Manganese Sulfate

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There is little information regarding the tissue distribution of manganese in neonates following inhalation. This study determined tissue manganese concentrations in lactating CD rats and their offspring following manganese sulfate (MnSO₄) aerosol inhalation. Except for the period of parturition, dams and their offspring were exposed to air or MnSO₄ (0.05, 0.5, or 1 mg Mn/m³) for 6 h/day, 7 days/week starting 28 days prior to breeding through postnatal day (PND) 18. Despite increased manganese concentrations in several maternal tissues, MnSO₄ inhalation exposure did not affect body weight gain, terminal (PND 18) body weight, or organ weights in the dams. Exposure to MnSO₄ at 1 mg Mn/m³ resulted in decreased pup body weights on PND 19 and decreased brain weights in some PND 14 to PND 45 pups. Exposure to MnSO₄ at ≥0.05 mg Mn/m³ was associated with increased stomach content, blood, liver, and skull cap manganese concentrations in PND 1 pups, increased brain, lung, and femur manganese concentrations in PND 14 pups, and elevated olfactory bulb, cerebellum, and striatum manganese concentrations in PND 19 pups. When compared to controls, MnSO₄ exposure to ≥0.5 mg Mn/m³ increased liver and blood manganese concentrations in PND 14 pups and increased liver, pancreas, and femur manganese concentrations in PND 19 pups. Manganese concentrations returned to control values in all offspring tissues by PND 45 ± 1. Our data demonstrate that neonatal tissue manganese concentrations observed following MnSO₄ inhalation are dependent on the MnSO₄ exposure concentration and the age of the animal.

Key Words: manganese; inhalation; rat, pharmacokinetics; lactation.

The essential trace mineral, manganese, is found in several metalloenzymes including manganese-superoxide dismutase, glutamine synthetase, arginase, and pyruvate carboxylase (Takeda, 2003). These enzymes play critical roles in antioxidant defense, glutamine synthesis, the urea cycle, and glycogen metabolism in the brain and other organs. Manganese deficiency can impair growth and skeletal development and may be a risk factor for epilepsy (Carl et al., 1993). Under high-exposure conditions, manganese accumulates within the human striatum and globus pallidus and induces injury to dopaminergic neurons within these sites (Normandin and Hazell, 2002; Olanow, 2004). Manganese neurotoxicity is associated with gait dysfunction, postural instability, micrographia, dystonia, rigidity, bradykinesia, and other extrapyramidal signs (Olanow, 2004) and most commonly occurs as the result of chronic inhalation of high concentrations (>1 mg/m³) of respirable airborne manganese (Pal et al., 1999).

Atmospheric sources of manganese include wind erosion of dusts and soils, ferroalloy production, iron and steel foundries, and power plant and coke oven combustion emissions (ATSDR, 2000). Manganese is also found in methylcyclopentadienyl manganese tricarbonyl (MMT), a fuel additive used in some unleaded gasoline. Automobiles equipped with catalytic converters and using MMT-containing gasoline emit manganese primarily in the phosphate and sulfate forms, although smaller amounts of manganese oxides may also be discharged (Molders et al., 2001; Ressler et al., 1999, 2000; Zayed et al., 1999). Our laboratory has shown that increases in adult rat brain, testes, and liver manganese concentrations are higher following inhalation of the more soluble manganese sulfate (MnSO₄) form than either the phosphate or tetroxide forms of manganese (Dorman et al., 2001; Vitarella et al., 2000).

Andersen and coworkers (1999) have identified critical data gaps for developing a risk assessment for inhaled manganese. One need is an improved understanding of manganese exposure conditions that lead to increased brain manganese concentrations in normal and susceptible individuals. Neonates represent one susceptible subpopulation of concern for manganese neurotoxicity. Neonatal animals, when compared to adults, are at an increased risk for manganese-induced neurotoxicity. Neonates achieve higher brain manganese levels than do adults following identical oral exposures (Dorman et al., 2000). When compared with young adults, the neonatal human (Haycock et al., 2003) and rat (Nomura et al., 1976) striatum contains lower amounts of dopamine. Moreover, neonatal rats given high oral doses of manganese developed significantly increased striatal...
steadystate concentrations of dopamine and its metabolite, DOPAC that were not seen in their adult counterparts (Dorman et al., 2000; Gianoutsos and Murray, 1982; Shukla et al., 1980; Subhash and Padmashree, 1991). However, this effect has not been consistently observed in manganeseexposed neonatal rats (Kontur and Fechter, 1985, 1988; Pappas et al., 1997). Neonates also develop more pronounced brain pathology than do adults following similar manganese exposures (Chandra and Shukla, 1978; Kristensson et al., 1986; Pappas et al., 1997).

The pharmacokinetics and neurotoxicity of manganese in neonates have been most thoroughly examined using the orally administered manganese chloride (MnCl2). To our knowledge, pharmacokinetic data on inhaled manganese in neonatal animals are not available. The objective of this study was to determine manganese tissue concentrations in CD rats following MnSO4 inhalation during pregnancy and lactation.

MATERIALS AND METHODS

Experimental design. Figure 1 provides a schematic representation of our study design. Adult (F0 male and female rats) CD rats (10 rats/gender/exposure concentration) were exposed to either air or MnSO4 (0.05, 0.5, or 1 mg Mn/m3) beginning 28 days prior to breeding and for up to 14 days during the mating period. Presumed pregnant female rats were exposed from gestation day (GD) 0 through GD 19. Inhalation exposures were not conducted when parturition was expected to occur (i.e., after GD 19). Lactating rats and their pups (n = 8–10 litters/exposure concentration) were then concurrently exposed beginning one day after parturition (day of parturition was designated as postnatal day [PND] 0) through PND 18. Litters were selected so that they contained at least 5 male and 5 female rats, and the litters were randomly reduced to four animals per sex on PND 4. Pups were euthanized on PND 1, 14, 19, 45 ± 1, and 63 ± 1, and tissue manganese concentrations determined at these time points. Necropsies on PND 1, 14, and 19 were performed immediately after the end of the 6-h inhalation exposure. Dams were killed on PND 18, and tissue manganese concentrations were subsequently evaluated. Male F0 rats were killed by CO2 after the 2-week breeding period, and tissues were not collected from these animals.

Chemicals. Manganese (II) sulfate monohydrate (MnSO4·H2O) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) with a certified purity of >98%. The material is a relatively water-soluble, white to pale pink crystalline powder that contains 32% Mn by weight.

Animals. This study was conducted under federal guidelines for the care and use of laboratory animals (National Research Council, 1996) and was approved by the CIIT Institutional Animal Care and Use Committee. Sixweek-old male and female Crl:CD1 (SD) BR rats were purchased from Charles River Laboratories, Inc (Raleigh, NC). Randomization of animals to treatment groups occurred prior to the start of the inhalation exposure and was based upon a weight randomization procedure. Animals were acclimated to the facility for approximately 2 weeks prior to the start of the inhalation exposure.

Animal husbandry. Animal rooms were maintained at daily temperatures of 17–23°C, relative humidity of 30–70%, and an air flow rate sufficient to provide 10–15 air changes per h. Fluorescent lighting was controlled by automatic controls (lights on approximately 0700–1900). All animals were housed in CIIT’s animal facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Except during inhalation exposure and breeding periods, male and female F0 rats were individually housed in polycarbonate cages containing cellulose fiber chip bedding (ALPHA-dri®; Shepherd Specialty Papers, Kalamazoo, MI). Animal breedings (typically 1 male: 1 female) were conducted overnight in polycarbonate cages containing cellulose fiber chip bedding. All weaned pups were housed with littermates of the same gender in polycarbonate cages (>3 rats/cage) containing cellulose fiber chip bedding.

A pelleted, semipurified AIN-93G certified diet from Bio-Serv (Frenchtown, NJ) formulated to contain approximately 10 ppm manganese and 35 ppm iron was given throughout the study. Food was available to all animals ad libitum except during inhalation exposures. Reverse osmosis purified water containing <0.222–0.546 μg Mn/l was available ad libitum. Detailed individual animal clinical examinations were conducted and recorded at least once weekly throughout the course of the study, beginning at the start of the inhalation exposures. Adult male (F0) body weights were measured and recorded at least weekly. Maternal body weights were measured and recorded at least weekly, during pregnancy on GD 0, 7, 14, 18, and postnatally on PND 0, 7, and 14. Terminal maternal body weights were collected on PND 18. Average male and female pup body weights were determined for each litter on PND 0, 7, and 14. Individual male and female pup body weights were measured and recorded at least weekly thereafter. All animals had individual body weights determined at necropsy.

Animals were mated at night (female added to male’s cage after each daily exposure, then removed for the next exposure day) for a period of up to 14 days.
with no change in mating partners. Females were examined daily after the cohabitation period for the presence of plug/sperm in the vaginal tract. The observation of a copulation plug or sperm-positive smear was considered evidence of successful mating (sperm positive), and the day was designated as GD 0. The sperm positive female and male from that mating pair were then individually housed, and gestational exposures begun.

**Manganese exposure.** Exposures were conducted for 6 h/day, 7 days/week. Nominal MnSO₄ exposure concentrations of 0, 0.05, 0.5, and 1 mg Mn/m³ were used in this study. Rats were exposed in stainless steel wire cage units contained in four 8-m³ stainless steel and glass inhalation exposure chambers (Lab Products, Maywood, NJ). Airflow through the chambers was adjusted to assure at least 12 air changes per hour (approximately 1600 to 2600 l/min). Temperature and relative humidity inside the 8-m³ chamber were monitored continuously and recorded every 30 min during each exposure. Chamber temperatures were maintained at 22 ± 4°C while the relative humidity was maintained at 40 to 60%. Animal positions within the exposure chambers were rotated during the experiment to minimize experimental error due to any undetected differences in the environment or the manganese aerosol concentration. MnSO₄ atmospheres were generated and characterized using methods described by Dorman et al. (2001). The exposure chamber used to simultaneously expose an individual dam and its litter has been previously described (Vitarella et al., 1998). Air flow through each dam/pup exposure cylinder was controlled by an adjustable metering valve and was maintained at approximately 2.5–3.5 l/min, providing approximately 35 to 50 air changes per h.

**Necropsy procedure.** Milk was collected from lactating animals prior to euthanasia. In order to allow the mammary glands to become engorged with milk, dams were removed from their pups immediately after the end of the 6-h PND 18 inhalation exposure. At least one h later, the dams were anesthetized with ketamine (~40 mg/kg, ip) and xylazine (~5 mg/kg, ip). Approximately 5 min before sample collection, an injection of oxytocin (0.1–0.4 ml of a 10 U/ml solution, im) was given in order to facilitate milk release and collection (Rodgers, 1995). The dam’s ventral surface was cleaned with water and ethanol, and the milk (200–500 µl) sample was manually expressed and collected in glass tubes. Maternal (1–2 ml) blood samples were then collected from the thoracic aorta or heart, and the ketamine-anesthetized dams were euthanized by exsanguination immediately thereafter. Blood samples were placed into heparinized tubes containing ~201U heparin/ml blood and a ≥500 µl aliquot of the heparinized maternal blood was placed into a plastic tube for manganese analysis. The following maternal tissues were collected and analyzed for manganese content: whole blood, milk, lung, pancreas, liver, femur, olfactory bulb, striatum, and cerebellum. Four presumed pregnant animals did not produce a litter and were killed on the equivalent of GD 27. Uteri from these nonpregnant rats were collected and stained with potassium ferricyanide to confirm that implantation had not occurred. Tissues were not collected for manganese evaluation from these animals.

Young pups (<PND 14) were killed by decapitation. PND 14 pups were anesthetized with CO₂ and killed by decapitation. Older pups (PND ≥ 19) were euthanized via CO₂ asphyxiation. The following tissues were collected (n = 1 rat/sex/litter) and analyzed for manganese concentrations: lung, liver, brain, and whole blood. A bone sample was also collected from all pups. Skull cap bone was collected on PND 1, while femur samples were collected thereafter. In addition, two pups per litter (1 pup/sex/litter) with visible milk bands were selected immediately after the end of the first postnatal inhalation day (PND 1) and a sample of stomach contents containing milk was collected and analyzed for manganese concentration. Brain samples collected from older pups (PND ≥ 19) were dissected into olfactory bulb, striatum, and cerebellum samples. Pancreas samples were also collected from the older pups. All tissue samples were transferred to plastic vials, frozen in liquid nitrogen, and stored at approximately −80°C until manganese analysis was performed.

**Tissue manganese analysis.** Tissue collection methods and graphite furnace atomic absorption spectrometry (GFAAS) analysis procedures have been previously described (Dorman et al., 2001). Samples were digested in ~16 M nitric acid using either an Anton Paar Multiwave Sample Preparation System (Perkin Elmer Instruments, Shelton, CT) or a CEM MARS5 Microwave Accelerated Reaction System (CEM, Matthews, NC) prior to manganese analysis. A Perkin Elmer AAnalyst 800 Atomic Absorption spectrometer with AA WinLab software, version 4.1 SP, was used for the determination of tissue manganese concentrations. The analyte was identified using a manganese-specific hollow cathode lamp by the presence of an absorbance signal at the manganese analytical wavelength of 279.5 nm. Quantitation of the analyte (µg Mn/g tissue wet weight) was carried out using an external calibration curve and the AA WinLab software. All samples were analyzed at least twice by GFAAS, and the resulting results were then averaged.

**Statistics.** The data for quantitative, continuous variables were compared for the exposure and control groups by tests for homogeneity of variance (Levene’s test), analysis of variance (ANOVA), and Dunnett’s multiple comparison procedure for significant ANOVA. In the event the Levene’s test was significant, the data were transformed using a natural log (ln) transformation. If the Levene’s test was significant following transformation, then the original data were analyzed using nonparametric statistics (Wilcoxon or Kruskal-Wallis). Individual data that appeared to be outliers were critically evaluated using a Dixon-type test for discordancy for an upper outlier (Barnett and Lewis, 1984). Data collected from neonatal rats were analyzed using an analysis of covariance (using a standard least squares model) to adjust for possible effects associated with the gender of the animal. The data for male and female rats were subsequently pooled when gender effects were not observed. Statistical analyses were performed using JMP software from SAS Institute Inc. (Cary, NC). A probability value of 0.01 was used for Levene’s test, while p < 0.05 was used as the critical level of significance for all other statistical tests. Unless otherwise noted, data presented are mean values ± standard error of the mean (SEM) and reflect comparisons with age-matched air-exposed controls.

**RESULTS**

**Manganese Test Atmospheres**

The overall means (±SD) for the chamber concentrations based on daily optical particle sensor data were 0.001 ± 0.000, 0.157 ± 0.011, 1.50 ± 0.10, and 3.03 ± 0.18 mg/m³ for the target exposure concentrations of 0, 0.15, 1.53, and 3.10 mg MnSO₄/m³, respectively. The particle size distribution was 1.03 µm geometric mean diameter (GMD) and 1.52 geometric standard deviation (GSD), 1.05 µm GMD (GSD = 1.53), and 1.07 µm GMD (GSD = 1.55) for the target concentrations of 0.150, 1.53, and 3.10 mg MnSO₄/m³, respectively. Control groups were exposed to HEPA-filtered air only. The particle size distribution for the control chamber was 0.79 µm GMD (GSD = 1.52), and particles in the control chamber likely represent dander, feed, and other particulate sources.

**Clinical Observations, Pathology, and Organ Weights**

Inhalation exposure to MnSO₄ during gestation and lactation did not affect maternal body weight gain (Fig. 2) or terminal maternal (PND 18) body weight (Table 1). Combined in utero and postnatal inhalation exposure to MnSO₄ did not affect neonatal body weight gain between PND 0 and PND 19 in either female (p = 0.065) or male (p = 0.374) pups (Fig. 3). High-dose MnSO₄ exposure (1 mg Mn/m³) was associated with decreased body weights in PND 19 pups (Table 2). Neonatal body weights were also decreased on PND 1 (p = 0.043) and PND
however, post hoc analysis did not reveal a treatment-related effect. The majority of F₀ and F₁ rats had no observable clinical signs. Clinical signs included alopecia and transient weight loss and were neither clinically relevant nor related to MnSO₄ inhalation. Macroscopic lesions (urinary calculi and secondary hydronephrosis or hydroureter) were observed in 16/35 dams, 56/70 PND 45 pups, and 48/59 PND 63 pups. Chemical analysis of several representative calculi revealed that the calculi were composed of magnesium ammonium phosphate (struvite) or calcium oxalate monohydrate. Urinary tract lesions did not demonstrate a dose-response relationship, were seen in control rats, and were deemed to be unrelated to MnSO₄ inhalation. Klurfeld (2002) and Kankesan et al. (2003) have reported unexpected urinary calculi formation in rats fed AIN-93 diets due to a change in methods used to produce choline bitartrate. Since manganese is minimally excreted in the urine (Andersen, 1999; Vitarella et al., 2000), we anticipate that urinary calculi formation was not a significant confounder in this pharmacokinetic study.

Absolute organ weights for the dams and pups are summarized in Tables 1 and 2, respectively. Manganese exposure did not affect maternal brain, lung, pancreas, or liver weights. High-dose manganese exposure (1 mg Mn/m³) was associated with decreased brain weights in PND 14 pups, PND 19 female pups, and PND 45 ± 1 male pups. Analysis of absolute brain weight (relative to body weight) revealed a slight increase in relative brain weights in PND 19 pups occurred. Mean relative brain weights in air-exposed and high-dose MnSO₄-exposed PND 19 pups were 3.3 ± 0.09 and 3.9 ± 0.17%, respectively. No gender effect on relative brain weight was observed in the PND 19 pups. Relative brain weights in all other treatment groups were unaffected by MnSO₄ exposure. High-dose manganese exposure (1 mg Mn/m³) was also associated with decreased liver weight in PND 19 pups. Male PND 63 ± 1 pups exposed to MnSO₄ at 0.5 mg Mn/m³ had decreased liver weights (13.72 ± 1.07 g) when compared with age-matched air-exposed controls (17.10 ± 0.58 g). All other organ weights measured on PND 63 ± 1 were unaffected by MnSO₄ exposure (data not shown).

### TABLE 1

<table>
<thead>
<tr>
<th>Nominal MnSO₄ concentration (mg Mn/m³)</th>
<th>0</th>
<th>0.05</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1.97 ± 0.03</td>
<td>1.98 ± 0.02</td>
<td>1.98 ± 0.04</td>
<td>1.90 ± 0.04</td>
</tr>
<tr>
<td>Lung</td>
<td>1.47 ± 0.08</td>
<td>1.35 ± 0.06</td>
<td>1.47 ± 0.08</td>
<td>1.36 ± 0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>11.86 ± 0.69</td>
<td>11.94 ± 0.92</td>
<td>11.05 ± 1.14</td>
<td>10.22 ± 0.75</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.84 ± 0.08</td>
<td>0.79 ± 0.08</td>
<td>0.81 ± 0.07</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>Body weight</td>
<td>257.3 ± 8.7</td>
<td>259.5 ± 11.2</td>
<td>257.5 ± 14.9</td>
<td>232.4 ± 8.4</td>
</tr>
<tr>
<td>Group size (n)</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

Note. Data for maternal (PND 18) organ weights (g) are expressed as mean ± SEM.
Maternal tissue manganese concentrations are summarized in Table 3. Increased maternal olfactory bulb tissue manganese concentration was observed following exposure to MnSO₄ at \(0.05 \text{ mg Mn/m}^3\). Increased maternal lung, cerebellum, and striatum manganese concentrations were observed following exposure to MnSO₄ at \(0.5 \text{ mg Mn/m}^3\). Increased maternal milk, liver, and femur manganese concentrations were observed following exposure to MnSO₄ at \(1 \text{ mg Mn/m}^3\). Dams exposed to MnSO₄ at \(1 \text{ mg Mn/m}^3\) also had elevated pancreas manganese concentrations; however, the increase was not statistically significant \((p = 0.062)\).

Manganese concentrations for most pup samples are presented in Figure 4. With the exception of pancreas data for the PND 45 ± 1 pups, no gender-related effects on pup tissue manganese concentrations were observed. Statistical analyses for all other neonatal tissues were performed using pooled male and female pup data. Increased pup blood, liver, skull cap, and stomach content (i.e., milk or partially digested milk) manganese concentrations were observed on PND 1 following exposure to MnSO₄ at \(0.05 \text{ mg Mn/m}^3\). Milk manganese concentrations in the PND 1 pups were \(0.57 ± 0.06, 1.02 ± 0.14, 2.87 ± 0.52, \text{ and } 4.73 ± 0.73 \mu\text{g Mn/g}\) for the 0, 0.05, 0.5, and \(1 \text{ mg Mn/m}^3\) exposure groups, respectively. Increased pup brain, lung, and femur manganese concentrations were observed on PND 14 following exposure to MnSO₄ at \(0.05 \text{ mg Mn/m}^3\). Increased blood and liver manganese concentrations were observed in PND 14 pups following exposure to MnSO₄ at \(0.5 \text{ mg Mn/m}^3\). Increased pup olfactory bulb, cerebellum, and striatum manganese concentrations were observed on PND 19 following exposure to MnSO₄ at \(0.05 \text{ mg Mn/m}^3\). Increased liver, pancreas, and femur manganese concentrations were also observed in PND 19 pups following exposure to MnSO₄ at \(0.5 \text{ mg Mn/m}^3\). Tissue manganese concentrations returned to control values in all tissues collected from the PND 45 ± 1 and PND 63 ± 1 pups.

**DISCUSSION**

Manganese has been shown to be an essential nutrient in several animal species, particularly during gestation and early infancy. Manganese is required for normal amino acid, lipid, protein, and carbohydrate metabolism. Manganese-dependent enzyme families include oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Despite its essentiality, exposure to high levels of manganese during early fetal or neonatal development is a concern.

We recently reported a study designed to evaluate tissue manganese concentrations in GD 20 dams and fetuses following inhalation exposure to MnSO₄ (Dorman et al., in press). The MnSO₄ exposure conditions used in that experiment were identical to those used in the present study. We found that fetal liver manganese concentrations were elevated following *in utero* exposure to MnSO₄ at \(0.5 \text{ mg Mn/m}^3\). Despite increased transfer of manganese from the dam to the fetus, we did not observe any change in fetal brain, blood, or skull cap manganese concentrations following high-dose MnSO₄ exposure. This study extends our previous research efforts by broadening MnSO₄ exposure into lactation. Maternal exposure to MnSO₄ that lasted for approximately 64 consecutive days resulted in dose-dependent increases in maternal manganese.
concentrations when compared with air-exposed controls. For example, maternal olfactory bulb manganese was one of the more sensitive tissues, with significant elevations occurring following exposure to MnSO₄ at 0.05 mg Mn/m³. Increased lung, cerebellum, and striatum manganese concentrations were observed in the dams on PND 18 following exposure to MnSO₄ at 0.5 mg Mn/m³. Increased maternal milk, liver, and femur manganese concentrations occurred following exposure to MnSO₄ at 1 mg Mn/m³. These results are consistent with previous studies that exposed nonpregnant female rats for a similar number of exposure days (Dorman et al., in press).

The results from our present study demonstrate that, relative to air-exposed controls, neonatal rats exposed to manganese (as MnSO₄) during gestation and early lactation develop increased blood and tissue manganese concentrations in an exposure concentration-, tissue-, and age-dependent manner. We found that neonatal blood manganese concentrations were

<table>
<thead>
<tr>
<th>Nominal MnSO₄ concentration (mg Mn/m³)</th>
<th>0</th>
<th>0.05</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group size (n)</td>
<td>16</td>
<td>20</td>
<td>18</td>
<td>16</td>
</tr>
</tbody>
</table>

Note. Mean (SEM) pup organ weights (g) and terminal body weights (g) following in utero and postnatal exposure to manganese. Data from animals that were subsequently analyzed for manganese content.

*aNo gender effect noted.

*bGroup size reflects one male and one female pup per litter.

*cExcludes two outliers.

dExcludes one outlier.

*p < 0.05.

/p = 0.07.
initially increased in PND 1 and 14 pups exposed to MnSO₄ at ≥0.05 mg Mn/m³. By PND 19, blood manganese concentrations in the MnSO₄-exposed rats had returned to levels identical to those seen in control animals. Liver manganese concentrations were also increased in PND 1 and PND 14–19 rats exposed to MnSO₄ at 0.05 mg Mn/m³ and 0.5 mg Mn/m³, respectively. Manganese-exposed neonatal rats also developed increased bone manganese concentrations. Maintenance of high blood, bone, and liver manganese levels in the early neonatal period likely reflect increased manganese retention by neonates (Dorner et al., 1989; Keen et al., 1986), reduced biliary excretion of manganese in preweanling animals (Ballatori et al., 1987; Miller et al., 1975; Rehnberg et al., 1982), and tissue storage (Aschner et al., in press).

Our data show that brain manganese concentrations can change rapidly in neonates exposed to high levels of manganese. Brain manganese concentrations in newborn rats were initially unaffected by the high-dose MnSO₄ exposure that occurred in utero. By PND 14, however, increased brain manganese concentrations were observed in MnSO₄-exposed pups. Brain

TABLE 3
Terminal (PND 18) Maternal Tissue Manganese Concentrations

<table>
<thead>
<tr>
<th>Nominal MnSO₄ concentration (mg Mn/m³)</th>
<th>Tissue</th>
<th>0</th>
<th>0.05</th>
<th>0.5</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>0.08 ± 0.04</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>0.56 ± 0.05</td>
<td>0.71 ± 0.04</td>
<td>1.40 ± 0.07</td>
<td>1.73 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Striatum</td>
<td>0.51 ± 0.02</td>
<td>0.54 ± 0.02</td>
<td>0.74 ± 0.02</td>
<td>0.89 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.50 ± 0.02</td>
<td>0.52 ± 0.02</td>
<td>0.60 ± 0.01</td>
<td>0.61 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.22 ± 0.03</td>
<td>0.37 ± 0.02</td>
<td>0.86 ± 0.07</td>
<td>1.05 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.21 ± 0.15</td>
<td>3.04 ± 0.09</td>
<td>3.37 ± 0.15</td>
<td>4.28 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>Femur</td>
<td>0.62 ± 0.07</td>
<td>0.61 ± 0.04</td>
<td>0.77 ± 0.05</td>
<td>0.89 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.66 ± 0.13</td>
<td>1.80 ± 0.19</td>
<td>1.29 ± 0.28</td>
<td>1.91 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>0.21 ± 0.08</td>
<td>0.20 ± 0.06</td>
<td>0.47 ± 0.06</td>
<td>0.77 ± 0.10</td>
<td></td>
</tr>
</tbody>
</table>

Group size (n): 8 10 9 8

Note. Maternal tissue manganese concentrations (µg Mn/g) following combined gestational and lactational exposure to either air or MnSO₄. Values are reported as mean ± SEM.

*n = 7 (excludes one outlier value).

*p < 0.05.

FIG. 4. continued.
manganese remained approximately two- to three-fold higher than those observed in control animals throughout the remainder of lactation. Manganese exposures ended at weaning, and by PND 45 the brain (and all other tissue) manganese concentrations returned to levels seen in control animals. This observation suggests an apparent half-life of elimination of manganese from the juvenile rat brain is likely on the order of 30 days or less. Takeda and coworkers (1995) and Dorman et al. (2004) reported a biological half-life of manganese in adult rat brain to be approximately 45 to 74 days. A very similar elimination half-time of 53 days has been reported in a macaque monkey given MnCl₂ via an implanted subcutaneous osmotic minipump (Newland et al., 1987). Cotzias and coworkers (1968) reported a brain elimination half-time of 53 days in human beings given intravenous ⁵⁴Mn tracer doses. Actual tissue elimination rates estimated from our study are confounded by the effect of ongoing growth and maturation of the pups.

High-dose manganese exposure during the perinatal period may result in alterations in brain growth and development. Pappas et al. (1997) reported decreased brain weights in neonatal rats exposed to high levels of MnCl₂ (10 mg Mn/ml) in their drinking water. We found that high-dose (1 mg Mn/m³) MnSO₄ inhalation exposure was associated with decreased brain weight in neonatal (PND 14 and 19) rats. Decreased brain weights were also observed in PND 45 ± 1 male pups, suggesting that prolonged alterations in brain size may occur in response to neonatal exposure to high concentrations of manganese. Erikson et al. (2004) showed that identically MnSO₄-exposed PND 45 male rats had more profound changes in brain oxidative stress endpoints than did their female littermates. The changes in pup brain weight observed in the present study may not reflect a permanent effect, since brain weights returned to control values in the MnSO₄-exposed male and female PND 63 ± 1 pups.

The PND 19 rats that were exposed to high-dose (1 mg Mn/m³) MnSO₄ also had a 20% decrease in body weight when compared to control animals. Reduced body weight gain has also been reported in neonatal rats given high doses of oral manganese (Dorman et al., 2000; Pappas et al., 1997).

Even though the PND 1 pups underwent a single 6-h exposure to high levels of MnSO₄, we found that lung manganese...
concentrations were not elevated. Lung manganese concentrations were increased in PND 14 rats exposed to MnSO₄; however, this increase may reflect unusually low manganese concentrations in the air-exposed controls, since lung manganese concentrations were not elevated in the PND 19 pups despite five additional days of MnSO₄ exposure.

Newborn pups were exposed to higher than usual manganese concentrations in the milk. Our analysis of stomach contents taken from PND 1 pups that were removed from the dam immediately after suckling showed that milk manganese concentrations in MnSO₄-exposed pups were approximately 1.8- to 8-fold higher than those observed in air-exposed controls. Milk collected on GD 18 from dams in the high-dose (1 mg Mn/m³) group had manganese concentrations that were approximately 3.5-fold higher (0.77 ± 0.10 μg Mn/g) than control values. A decline in milk manganese concentration near the end of lactation is not unexpected, since manganese levels found in control rat milk vary during lactation, with the highest concentrations found in milk produced during the immediate postnatal period (Keen et al., 1981). Manganese content in human milk also varies with lactation (Casey et al., 1985; Stastny et al., 1984; Vaughan et al., 1979). Our results suggest that milk manganese concentrations remained elevated throughout lactation and contributed to the pup’s total manganese exposure.

The MnSO₄ exposure concentrations used in this study are approximately 2000-fold higher than manganese concentrations found in Canadian cities where MMT has been widely used for the past two decades (Loranger and Zayed, 1997; Pellizzari et al., 1999). Average respirable atmospheric manganese concentrations measured in high and low traffic areas in Montreal were 0.024 and 0.015 μg/m³, respectively (Loranger and Zayed, 1997). Pellizzari and coworkers (1999) showed that the 99th percentile nonoccupational exposure to respirable manganese (PM2.5) in Toronto was 0.0215 μg Mn/m³. Mean personal PM2.5 manganese exposures in Indianapolis, a city where MMT is not used in gasoline is 0.0075 g Mn/g) than control values. A decline in milk manganese concentration near the end of lactation is not unexpected, since manganese levels found in control rat milk vary during lactation, with the highest concentrations found in milk produced during the immediate postnatal period (Keen et al., 1981). Manganese content in human milk also varies with lactation (Casey et al., 1985; Stastny et al., 1984; Vaughan et al., 1979). Our results suggest that milk manganese concentrations remained elevated throughout lactation and contributed to the pup’s total manganese exposure.

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In conclusion, this study provides important new information about the pharmacokinetics of inhaled manganese in lactating dams and their offspring. The results of this study demonstrate that the early postnatal period is especially sensitive to changes in brain and other tissue manganese concentrations following high-dose manganese inhalation. This finding is consistent with previous studies that examined manganese dosimetry in neonatal rats given high oral doses of manganese (Dorman et al., 2001). The enhanced sensitivity of neonatal animals likely reflects the relative immaturity of the hepatobiliary systems that regulate manganese elimination and subsequent systemic delivery to the brain and other tissues. Neonates also have an increased requirement for manganese for optimal brain development (Takeda et al., 1999). Our findings may therefore not necessarily provide evidence for a heightened risk of neurotoxicity in developing animals. This study should also prove very useful for the development of physiologically based pharmacokinetic (PBPK) models to predict disposition of manganese in neonates following inhalation exposure to manganese.

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