Brain Manganese Concentrations in Rats Following Manganese Tetroxide Inhalation are Unaffected by Dietary Manganese Intake

David C. Dorman*, Melanie F. Struve, Brian A. Wong
CIIT Centers for Health Research, 6 Davis Drive, P.O. Box 12137, Research Triangle Park, NC 27709-2137, USA

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Abstract

Manganese-deficient individuals have decreased manganese elimination. This observation has prompted suggestions that relative manganese deficiency may increase the risk for manganese neurotoxicity following inhalation exposure. The objective of this study was to determine whether dietary manganese intake influences the pharmacokinetics of inhaled manganese tetroxide ($\text{Mn}_3\text{O}_4$). Postnatal day (PND) 10 rats were placed on either a low (2 ppm), sufficient (10 ppm), or high-normal (100 ppm) manganese diet for 2 months. Beginning on PND 77 ± 2, male littermates were exposed 6 h per day for 14 consecutive days to 0, 0.042, or 0.42 mg $\text{Mn}_3\text{O}_4$/m$^3$. End-of-exposure tissue manganese concentrations and whole-body $^{54}\text{Mn}$ elimination rates were determined. Tissue manganese concentrations were dependent on the dietary intake of manganese, thus confirming that altered hepatic manganese disposition or metabolism occurred. Male rats given 100 ppm manganese diet developed increased manganese concentrations in the femur, liver, and bile and had elevated whole-body $^{54}\text{Mn}$ clearance rates when compared to animals given 2 ppm manganese diet. Male rats exposed to 0.42 mg $\text{Mn}_3\text{O}_4$/m$^3$ had increased manganese concentrations in the olfactory bulb, lung, liver, and bile when compared to air-exposed male rats. A significant interaction between the concentration of inhaled $\text{Mn}_3\text{O}_4$ and dietary manganese level was observed only with the end-of-exposure liver manganese concentration. Our results indicate that animals maintained on either a manganese-deficient or high manganese diet do not appear to be at increased risk for elevated brain manganese concentrations following inhalation exposure to high levels of $\text{Mn}_3\text{O}_4$.

INTRODUCTION

Manganese is an essential trace mineral that may accumulate within the human striatum and globus pallidus under excess exposure resulting in injury to dopaminergic neurons in these structures (Pal et al., 1999). Human exposure occurs from a variety of sources, and for nearly all people diet contributes the most to an individual’s daily intake. Various surveys have shown that average manganese intake of adults eating Western-type and vegetarian diets range from 0.7 to 10.9 mg Mn per day (Freeland-Graves, 1994; Gibson, 1994). For many individuals, manganese intake is below the recommended dietary reference intake level of 1.8–2.3 mg per day (Finley and Davis, 1999; Pennington and Schoen, 1996). Even when dietary levels of manganese are adequate, high dietary levels of fiber, phytate, iron, and calcium can limit manganese bioavailability (Gibson, 1994). Although manganese intake varies dramatically among individuals, diet-related manganese deficiency in humans has not been identified. This observation reflects in...
part the capacity of the hepatobiliary system to respond to decreased manganese intake by reducing biliary excretion, thereby maintaining normal tissue concentrations of this essential element (Keen et al., 2000; Malecki et al., 1996). Pathological conditions that alter this tightly regulated pattern of response can lead to dramatic changes in manganese disposition. For example, human patients with cholestasis, chronic liver disease, or other forms of hepatobiliary disease are at elevated risk for increased manganese accumulation in the brain (Morgan, 1998; Pal et al., 1999; Rose et al., 1999).

The route of exposure can influence the metabolism and disposition of manganese (Andersen et al., 1999; Roels et al., 1997). Inhalation is more efficient than ingestion at delivering manganese to the brain. Pharmacokinetic factors in rats that may contribute to the increased efficiency in brain manganese delivery observed after inhalation exposure include increased manganese absorption from the pulmonary tract (Andersen et al., 1999) and direct transport of manganese to the central nervous system along the olfactory nerve (Brenneman et al., 2000). Inhalation is also the most common route of exposure for humans with manganese neurotoxicity (Pal et al., 1999). Exposure to airborne manganese occurs from a variety of sources, including fugitive dusts, automotive emissions, power plants, municipal waste incinerators, and metal smelting operations (US EPA, 1996). Manganese is also found in the gasoline additive methylcyclopentadienyl manganese tricarbonyl (MMT). Automobiles equipped with catalytic converters that use MMT-containing fuel emit manganese primarily in the phosphate and sulfate (MnSO₄) forms, although lesser amounts of manganese oxides (e.g. Mn₃O₄) may also be discharged (Lynam et al., 1999; Ressler et al., 1997). Although the possibility exists for increased manganese concentrations in the atmosphere with widespread MMT use, actual air manganese concentrations from Canadian cities in which MMT has been widely used for over 10 years remain below the current inhalation reference concentration (0.05 µg Mn/m³) for inhalable manganese set by the United States Environmental Protection Agency (Clayton et al., 1999; Loranger and Zayed, 1997; Pellizzari et al., 1999; Zayed et al., 1999a). The use of MMT in gasoline has prompted considerable debate as to whether chronic exposure to low levels of manganese could result in neurotoxicity or lead to an increased incidence of neurological disease in susceptible humans. Whether individuals with either deficient or excessive manganese tissue burdens may be at increased risk for manganese toxicity following inhalation exposure is of special concern (Weiss, 1999).

Few investigators have examined whether dietary intake can influence the pharmacokinetics of an inhaled metal. We recently completed a 14-day inhalation study demonstrating that manganese body burden does not influence the pharmacokinetics of inhaled MnSO₄ (Dorman et al., 2001b). Moore et al. (1975) performed inhalation studies in which rats were fed either a 5 or 42.2 ppm manganese diet and exposed subchronically (8 h per day for 56 consecutive days) to irradiated exhaust from an automobile engine using gasoline containing MMT. Although not determined directly during this study, the most likely form of manganese in the engine exhaust was the tetroxide Mn₃O₄ (Ter Haar et al., 1975). Moore et al. (1975) reported that the level of manganese present in the diet influenced end-of-exposure brain, liver, and kidney manganese concentrations. However, the results of this study, were not analyzed in a manner to directly determine whether diet and inhalation interactive effects occurred. The present study was undertaken to more thoroughly assess whether different dietary manganese levels can influence the pharmacokinetics of inhaled Mn₃O₄.

MATERIALS AND METHODS

Experimental Design

A schematic representation of the experiment is presented in Fig. 1. This study used a 3 × 3 factorial arrangement of treatments in a split-plot design. One factor was diet at three levels (2, 10, and 100 ppm manganese), and the other factor was exposure to Mn₃O₄ at three inhalation exposure levels (0, 0.042, and 0.42 mg Mn₃O₄/m³ 6 h per day on 14 consecutive days). Main plots were litters (n = 8 litters per diet). Sub-plots were two PND 77 ± 2 male rats from each litter that were assigned on postnatal day (PND) 10 to one of the three dietary groups (n = 8 litters per diet). Sub-plots were two PND 77 ± 2 male rats from each litter that were assigned on postnatal day (PND) 10 to one of the three dietary groups (n = 8 litters per diet). Sub-plots were two PND 77 ± 2 male rats from each litter that were assigned on postnatal day (PND) 10 to one of the three dietary groups (n = 8 litters per diet). Sub-plots were two PND 77 ± 2 male rats from each litter that were assigned on postnatal day (PND) 10 to one of the three dietary groups (n = 8 litters per diet).
Animals

The 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. In all, 50 primiparous pregnant (sperm plug positive = gestation day (GD) 0) Crl:CD\(^{BR}\) rats were purchased on GD 14 from Charles River Laboratories, Inc. (Raleigh, NC) for this experiment. All presumed pregnant rats were initially fed a pelleted, semipurified AIN-93G certified diet from Bio-Serv (Frenchtown, NJ) that nominally contained 10 ppm manganese and 50 ppm iron. Pregnant rats and lactating dams with their pups were individually housed in polycarbonate cages with stainless steel lids (Laboratory Products, Inc., Rochelle Park, NJ) with Alpha-Dri\textsuperscript{TM} cellulose-fiber chip bedding (Shepherd Specialty Papers, Kalamazoo, MI). Following parturition, 24 litters with at least 6 male and 4 female F\(_1\) rats per litter were assigned on PND 10 to one of three AIN-93G certified diets (Bio-Serv, Frenchtown, NJ) containing 2, 10 or, 100 ppm manganese and 50 ppm iron. All diets were fed ad libitum except during inhalation exposures. Reverse-osmosis purified water containing <0.002 \(\mu\)g Mn/ml was also available ad libitum. Parental (F\(_0\)) female rats were euthanized after weaning on PND 23 \pm 2. Weaned F\(_1\) animals were individually housed in suspended stainless steel cages and were maintained on their respective diets throughout the duration of the study. The animal rooms and exposure chambers were maintained at 22 \pm 2 \({}^\circ\)C, relative humidity of 30–70%, and an air flow rate sufficient to provide 1–15 air changes per hour. Fluorescent lighting was controlled by automatic controls (lights on 06:00–18:00). The general condition of all animals was checked daily. Clinical examinations and body weights were recorded at least once weekly.

Manganese Exposures

The 1 m\(^3\) stainless steel and glass inhalation exposure chambers (Lab Products, Seaford, DE), airflows, chamber temperature and humidity monitoring procedures, animal rotation schedule, and methods used to check for uniformity of distribution of the aerosol have been previously described (Vitarella et al., 2000). The Mn\(_3\)O\(_4\) atmospheres were generated using a commercially available dry powder (Aldrich Chemical Co., Milwaukee, WI) and characterized using methods described by Dorman et al. (2001a). A Wright Dust Feeder packed to 1000 psi with a 25 psi air delivery pressure was used to generate the initial aerosol. Nominal Mn\(_3\)O\(_4\) exposure concentrations were 0, 0.042, and 0.42 mg Mn\(_3\)O\(_4\)/m\(^3\), corresponding to 0, 0.03, and 0.3 mg Mn/m\(^3\). The target particle size distribution was 1.5–2 \(\mu\)m mass median aerodynamic diameter (MMAD) with a geometric standard deviation (GSD) \(<\)2. Control groups were exposed to HEPA-filtered air only.

Based upon optical particle sensor results, the actual Mn\(_3\)O\(_4\) chamber concentrations (mean \pm S.D.) were 0.045 \pm 0.004 and 0.45 \pm 0.02 mg/m\(^3\) for the target concentrations of 0.042 and 0.42 mg Mn\(_3\)O\(_4\)/m\(^3\).
respectively. As determined with the aerosol particle sizing spectrometer, the count median aerodynamic diameter in the 0.042 and 0.42 mg Mn$_3$O$_4$ inhalation chambers were 0.93 (G.S.D. = 1.37 μm) and 1.02 (G.S.D. = 1.47 μm), respectively. The calculated MMADs in the 0.042 and 0.42 mg Mn$_3$O$_4$ inhalation chambers corresponded to 1.25 and 1.59 μm, respectively. Daily mean chamber temperatures during exposures ranged from 19.5 to 25.9 °C, and the relative humidity in the 1 m$^3$ inhalation chambers ranged from 40 to 58%.

**Tissue Collection and Manganese Analysis**

Manganese concentrations in tissues were determined by neutron activation analysis. Tissue collection methods and neutron activation analysis procedures have been previously described (Dorman et al., 2001b). Striatal and liver manganese concentrations were determined in CO$_2$-euthanized F$_1$ female rats on PND 63 ± 2 and 77 ± 2 (n = 1 rat per litter per time point). On PND 91 ± 2, the remaining F$_1$ female rats (n = 1 rat per litter) were fasted overnight and then anesthetized with pentobarbital (50 mg/kg, i.p.); bile (~0.5–1 ml) was collected from the cannulated bile duct for approximately 1 h prior to necropsy. The F$_1$ male rats were then euthanized by exsanguination, and serum, striatum, olfactory bulb, cerebellum, lung, liver, femur, and ovary samples were collected for tissue manganese determination. Bile, serum, striatum, olfactory bulb, cerebellum, lung, liver, femur, and testes samples were similarly collected from Mn$_3$O$_4$-exposed F$_1$ male rats immediately following their last (i.e. 14th) inhalation exposure.

**$^{54}$Mn Tracer Studies**

Immediately after the last Mn$_3$O$_4$ inhalation exposure, the remaining F$_1$ male rats from each exposure group (n = 1 rat per litter per Mn$_3$O$_4$ exposure group) were given 0.5 μCi of $^{54}$MnCl$_2$ (in sterile saline) by tail vein injection (0.4 ml injection volume). Whole-body gamma spectrometry was performed immediately after injection and at 1, 2, 4, 6, 9, and 12 weeks after $^{54}$Mn tracer administration using previously described methods (Dorman et al., 2001b). Pharmacokinetic estimates were made after fitting a non-linear regression curve to the individual whole-body $^{54}$Mn concentration–time profiles data using a two-compartment model and commercially available software (WinNonlin, Pharsight Corp., Cary, NC). Pharmacokinetic parameters were calculated from standard kinetic formulas (Shargel and Yu, 1985) and included estimates of the intercepts for the two compartments (A and B), total body clearance (Ct), apparent volume of distribution at steady state (Vss), peak body concentrations (Cmax), initial and terminal phase elimination half-lives (t1/2a and t1/2b), and the area under the curve (AUC$_{0→∞}$).

**Statistics**

Details concerning statistical methods and procedures have been previously published (Dorman et al., 2001b). Briefly, experimental data obtained from Mn$_3$O$_4$-exposed F$_1$ male rats were analyzed by an analysis of variance appropriate for a split-plot experimental design to test for the main effects of diet and exposure and also their interactions. This analysis is similar to a standard ANOVA except that a nested analysis is used to account for the litter (split-plot) as a source of error thereby increasing the power of the statistical test. If the diet main effect was significant, a Tukey’s honestly significant difference test was used to determine which diet levels were different. If the exposure main effect was significant, a Dunnett’s test was used to compare the two Mn$_3$O$_4$ exposure levels to the air-exposed controls. Statistical analyses were performed using SAS Statistical Software (JMP, SAS Institute, Inc., Cary, NC). Unless otherwise noted, data presented are for mean values ± standard error of the mean (S.E.M.). A probability value of 0.01 was used for tests of homogeneity (Levene’s test), while P < 0.05 was used as the critical level of significance for all other statistical tests.

**RESULTS**

**Body Weight Gain, Clinical Signs, and Necropsy Findings**

Mean body weights of PND 77 ± 2 male rats given 2, 10, and 100 ppm diets were 348.4 ± 4.2, 347.7 ± 33 and 362.6 ± 2.7 g, respectively. PND 77 ± 2 male rats given 2 and 10 ppm diets had significantly lower body weights than those observed in rats given 100 ppm manganese diet. Mean body weights of PND 77 ± 2 female rats given 2, 10, and 100 ppm diet were 228.2 ± 2.7, 221.2 ± 2.7, and 228.4 ± 2.1 g, respectively. There was no significant effect of dietary manganese intake on female body weights. Short-term (14-day) inhalation exposure to Mn$_3$O$_4$ did not affect body weight (data not shown). No treatment-related (diet or Mn$_3$O$_4$ inhalation) clinical effects were observed.
Tissue Manganese Concentrations in F₁ Female Rats Following Dietary Manipulation

Mean liver manganese concentrations in PND 63 female animals given 2, 10, and 100 ppm diets were $1.26 \pm 0.05$, $1.53 \pm 0.10$, and $1.87 \pm 0.10 \mu g \text{ Mn/g}$, respectively. Liver manganese concentrations in female animals from all dietary treatment groups increased from PND 63 through PND 91. Female rats given diets containing 100 ppm manganese developed consistently higher liver manganese concentrations when compared with female rats given 2 ppm manganese diet. PND 63 and PND 77 female rats given 10 ppm manganese diet had significantly higher liver manganese concentrations than those rats given 2 ppm manganese. Mean striatal manganese concentrations in PND 91 female animals given 2, 10, and 100 ppm diet were $0.60 \pm 0.06$, $0.54 \pm 0.06$ and $0.66 \pm 0.05 \mu g \text{ Mn/g}$, respectively, and were unaffected by dietary manipulation. Increased liver, bile, and femur manganese concentrations were observed in PND 91 female rats given 100 ppm diet when compared to female rats given 2 ppm diet (Fig. 2). Increased manganese concentrations in bile were also observed in female rats given 100 ppm manganese diet when compared with female rats given 10 ppm manganese diet. Serum manganese concentrations decreased in female rats given 10 ppm diet compared with female rats given 2 ppm diet. Lung and ovary tissue manganese concentrations were unaffected by dietary manipulation. None of the examined brain regions (cerebellum, striatum, or olfactory bulb) were affected by dietary manipulation.

Tissue Manganese Concentrations in F₁ Male Rats Following Dietary Manipulation and Mn₃O₄ Inhalation

End-of-exposure mean tissue manganese concentrations are presented in Table 1. A statistically significant interaction between dietary manganese level and manganese inhalation was observed for end-of-exposure liver manganese concentration ($P = 0.002$). This interaction resulted in higher liver manganese concentrations than would have occurred following either dietary or inhalation alone and appears to be additive in nature.

An overall main treatment effect related to inhalation exposure was observed at some tissue manganese concentrations (Table 2). Male rats exposed to 0.042 mg Mn₃O₄/m³ had decreased striatal and olfactory bulb manganese concentrations when compared to air-exposed male rats. Male rats exposed to 0.42 mg Mn₃O₄/m³ had increased lung, liver, olfactory bulb, and bile manganese concentrations when compared to air-exposed male rats. An overall main treatment effect related to diet was also observed in some tissues (Table 3). Rats given a marginally manganese-deficient diet (2 ppm) had lower femur, bile, and liver manganese concentrations compared with levels observed in rats given the high-normal manganese diet (100 ppm). Male rats given the marginally deficient manganese

Fig. 2. Mean (±S.E.M.) tissue manganese (Mn) concentrations (μg Mn/g tissue wet weight) in PND 91 female rats given a 2, 10, or 100 ppm Mn diet beginning on PND 10: (∗) indicates 100 > 2 ppm Mn; (∨) indicates 100 > 10 ppm Mn; (†) indicates 2 > 10 ppm Mn, $P \leq 0.05$). Lung data for one animal from the 100 ppm manganese dietary group was excluded as a statistical outlier.
diet (2 ppm) also developed lower liver manganese concentrations when compared with animals given the 10 ppm manganese diet.

54Mn Tracer Pharmacokinetics Following Mn3O4 Inhalation

The whole-body 54Mn elimination data collected in our study could be described using a bi-exponential equation similar to a two-compartment open pharmacokinetic model that assumes first-order transfer of 54Mn between a central and tissue compartment. Mean values for the pharmacokinetic parameters are presented in Table 4, while the whole-body 54Mn results are presented in Fig. 3. A statistically significant interaction between dietary manganese level and manganese inhalation was observed for the estimate of the intercept of the central compartment \( (P = 0.001) \) and the AUC \( (P = 0.031) \). A marginally significant interaction between dietary manganese level and manganese inhalation was also observed for the estimate of the intercept of the second (tissue) compartment \( (P = 0.083) \). Interactions between dietary manganese level and manganese inhalation exposure concentration were not observed for the other 54Mn tracer elimination pharmacokinetic parameters. An overall diet-related main treatment effect was observed for some pharmacokinetic parameters (Table 5). Rats given either 10 or 100 ppm manganese diet had significantly lower AUCs, and shorter terminal elimination half-lives.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Exposure concentrations (mg Mn3O4/m3)</th>
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</thead>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2 ppm Mn diet</td>
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<tr>
<td>Striatum</td>
<td>0.70 ± 0.13</td>
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<tr>
<td>Olfactory bulb</td>
<td>0.97 ± 0.23</td>
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<tr>
<td>Cerebellum</td>
<td>0.60 ± 0.06</td>
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<tr>
<td>Lung</td>
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<tr>
<td>Liver</td>
<td>1.36 ± 0.08</td>
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<tr>
<td>Bile</td>
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<tr>
<td>Testes</td>
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</tr>
<tr>
<td>Femur</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>Serum</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>10 ppm Mn diet</td>
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<td>Striatum</td>
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<tr>
<td>Olfactory bulb</td>
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<td>Cerebellum</td>
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<td>100 ppm Mn diet</td>
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<td>Femur</td>
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</tr>
<tr>
<td>Serum</td>
<td>0.16 ± 0.02</td>
</tr>
</tbody>
</table>

\( ^a \) n = 9 for all treatment groups except \( n = 10 \) for 2 ppm diet animals exposed to 0.042 mg Mn/m3.

\( ^b \) One missing bile sample.

### Table 1
Mean (±S.E.M.) tissue manganese concentrations (μg Mn/g tissue wet weight) in Mn3O4-exposed male rats after 14 days of exposure

### Table 2
End-of-exposure overall mean tissue manganese concentrations (μg Mn/g tissue wet weight) used to test for an inhalation-related main effect

### Table 3
End-of-exposure overall mean tissue manganese concentrations (μg Mn/g tissue wet weight) used to test for a diet-related main effect
(t_{1/2B}) when compared with rats given 2 ppm manganese diet (Table 5). Rats given either 10 or 100 ppm manganese diet also had increased manganese clearance rates, higher apparent volume of distribution at steady state (V_{ss}), and a larger intercept value associated with the tissue compartment when compared with rats given 2 ppm manganese diet (Table 5). Rats given 100 ppm manganese diet had significantly lower AUCs, increased manganese clearance rates, shorter terminal elimination half-lives (t_{1/2B}), and a higher intercept for the central compartment when compared with rats given 10 ppm manganese diet (Table 5). An overall main treatment effect related to inhalation exposure was observed for the estimates of the intercepts associated with the two compartments (Table 6). Rats exposed to 0.42 mg Mn_{3}O_{4}/m^{3} had a significantly higher value for the intercept of the central compartment and a lower intercept value for the tissue compartment when compared with air-exposed control rats (Table 6). Rats exposed to 0.42 mg Mn_{3}O_{4}/m^{3} also had a significantly larger apparent volume of distribution at steady state when compared with air-exposed control rats (Table 6). No other overall main treatment effects related to inhalation exposure were observed for any other examined pharmacokinetic parameter.

**DISCUSSION**

Our laboratory has recently shown that dietary intake of manganese did not influence tissue manganese concentrations in male rats repeatedly exposed to manganese sulfate (MnSO_{4}) (Dorman et al., 2001b). Also, animals exposed to MnSO_{4}, a soluble form of manganese, developed higher brain and liver manganese concentrations than did animals exposed similarly.
to either the tetroxide or phosphate forms of manganese (Dorman et al., 2001a; Vitarella et al., 2000). Knowing that particle solubility plays a key role in determining manganese distribution to the brain, we chose to examine whether dietary manganese intake influences the pharmacokinetics of inhaled Mn$_3$O$_4$, a considerably less soluble form of manganese. We fed neonatal rats diets containing a marginally adequate level of manganese (2 ppm), an adequate level of manganese (10 ppm) that meets the current National Research Council guideline for rodent diets (NRC, 1995), or a high-normal level of manganese (100 ppm). Dietary manipulations were initiated prior to the time when rat pups began to ingest solid food and before they fully developed the ability to excrete manganese in the bile (Keen et al., 1981; Miller et al., 1975; Rehnberg et al., 1982). Similar aerosol concentrations, particle sizes, and diets were used in our two studies to facilitate direct comparison of results among experiments.

The results of this study are qualitatively similar to our previous 14-day Mn$_3$O$_4$ inhalation experiment conducted in male CD rats maintained on an open diet that contained approximately 100 ppm manganese (Dorman et al., 2001a). In this earlier study, we showed that male rats exposed to 0.42 mg Mn$_3$O$_4$/m$^3$ developed increased lung and olfactory bulb manganese concentrations when compared to air-exposed male rats (Dorman et al., 2001a). As in our earlier study (Dorman et al., 2001a), lung manganese concentrations were approximately 4–5-fold higher in rats exposed to 0.42 mg Mn$_3$O$_4$/m$^3$ than in animals exposed to air. Olfactory bulb manganese concentrations were likewise 42% higher in rats exposed to 0.42 mg Mn$_3$O$_4$/m$^3$ than in animals exposed to air, a result virtually identical to that observed in our previous Mn$_3$O$_4$ inhalation study (Dorman et al., 2001a). Our current study showed that male rats exposed to 0.42 mg Mn$_3$O$_4$/m$^3$ also had increased liver and bile manganese concentrations when compared to air-exposed male rats. Neither liver nor bile manganese concentrations were affected by the inhalation of 0.042 mg Mn$_3$O$_4$/m$^3$ in our previous study (Dorman et al., 2001a). This finding suggests that liver metabolism of manganese differed between the two 14-day inhalation studies and may reflect the use of an open diet in our previous study and a defined diet in the current experiment. It is well known that the formulation of a diet will influence the physiological processes and health of rats and their response to administered chemicals (Keenan et al., 1997). As with our previous Mn$_3$O$_4$ inhalation study, the lowest tetroxide exposure level (0.042 mg Mn$_3$O$_4$/m$^3$) did not increase manganese concentrations in any tissue.

This experiment used the same diet as that used in our earlier study that evaluated whether dietary intake...
of manganese could alter the pharmacokinetics of inhaled MnSO$_4$ (Dorman et al., 2001b). As expected, we found that manganese concentrations in some tissues were dependent upon dietary manganese intake. We observed increased liver, bile, and femur manganese concentrations in male and female rats given 100 ppm manganese diet when compared with animals receiving either 2 or 10 ppm manganese diet. The magnitude of the increases in tissue manganese concentrations observed following dietary manipulation was moderate. For example, liver manganese concentrations in male and female PND 91 rats given the high manganese diet (100 ppm) were 25–30% higher than those observed in animals given 2 ppm manganese diet. Our observations are qualitatively similar to those detected in our previous inhalation study with MnSO$_4$ (Dorman et al., 2001b), although some differences between the two studies exist. In our earlier study, we only observed elevated liver and bile manganese concentrations in female rats given 100 ppm manganese diet when compared with animals receiving either 2 or 10 ppm manganese diet. In our earlier study, we only observed elevated femur manganese concentrations in male rats given 100 ppm manganese diet when compared with animals receiving either 2 ppm manganese diet (Dorman et al., 2001b). Another difference observed between the two studies was related to body weight gain. In our previous MnSO$_4$ study, we found that rats fed 2 ppm manganese diet had lower body weight gain when compared with animals receiving 100 ppm manganese diet when compared with animals receiving either 2 ppm manganese diet (Dorman et al., 2001b). Another difference observed between the two studies was related to body weight gain. In our previous MnSO$_4$ study, we found that rats fed 2 ppm manganese diet had lower body weight gain when compared with animals receiving 100 ppm manganese diet (Dorman et al., 2001b). In the present study, we found that rats fed either 2 or 10 ppm manganese diet had lower body weight gain when compared with animals receiving 100 ppm manganese diet. This observation likely reflects the growth-promoting effects associated with diets that are high in manganese (Keen et al., 1999). Our failure to duplicate each of our previous results most likely reflects the small sample sizes used in the study and the relatively narrow range of diets used (2–100 ppm) in the two studies.

In our present study, female rats fed 10 ppm manganese diet had lower serum manganese concentrations when compared with animals receiving either the 2 ppm manganese diet. This observation was unexpected and was not observed in male rats from this study. Serum manganese concentrations in both male and female rats were unaffected by dietary treatment in our earlier dietary MnSO$_4$ inhalation study (Dorman et al., 2001b). The failure to observe altered serum manganese concentrations in our earlier study or in male rats from our present study suggests that the decrease in serum manganese concentration observed in the PND 91 female rats fed 10 ppm manganese diet is not treatment-related.

In both studies, we consistently observed that biliary elimination of manganese increased in response to increasing dietary manganese concentration (Dorman et al., 2001b). The primary route of excretion for manganese is bile (Malecki et al., 1996) and enhanced biliary elimination occurs in animals and humans in response to increasing dietary levels of manganese (Britton and Cotzias, 1966; Mahoney and Small, 1968; Papavasiliou et al., 1966; Sato et al., 1996). Our observation that bile manganese concentrations increased as dietary manganese concentration rose confirms that the different dietary manganese intakes used in our studies changed the rate at which manganese was metabolized and eliminated by the liver, thus meeting a critical criterion for these experiments.

Our analysis of the $^{54}$Mn elimination curves showed that the relative size of the value for the intercept of the central compartment (i.e. the value of $A$) increased in a diet- and MnSO$_4$ exposure concentration-dependent manner. This observation indicates that a larger fraction of the excreted manganese will be cleared via a fast-phase elimination process in response to increased dietary or inhalation exposure to manganese. Our results also indicate that the elimination of the $^{54}$Mn tracer was more sensitive to the influence of diet rather than Mn$_3$O$_4$ inhalation exposure. For example, we observed that rats fed either 2 or 10 ppm manganese diet had lower manganese clearance and higher AUC values when compared with animals receiving 100 ppm manganese diet. In contrast, rats exposed to 0.42 mg Mn$_3$O$_4$/m$^3$ did not develop increased whole-body $^{54}$Mn clearance or altered AUCs when compared to air-exposed control animals. We observed an identical result in our previous 14-day Mn$_3$O$_4$ inhalation study with male rats maintained on an open diet (Dorman et al., 2001a). In this previous study, increased whole-body clearance of manganese was only detected in male rats exposed to 4.2 mg Mn$_3$O$_4$/m$^3$ (Dorman et al., 2001a).

Moore et al. (1975) reported that end-of-exposure liver and brain manganese concentrations observed in rats exposed subchronically to Mn$_3$O$_4$, the likely combustion product found in the irradiated exhaust from an automobile engine using gasoline containing MMT, were influenced by the level of manganese present in the diet. Similar to the Moore et al. (1975) study, we observed a statistically significant interaction between dietary manganese intake and inhaled Mn$_3$O$_4$ concentration on the end-of-exposure liver manganese concentration. This result is in contrast to our earlier
dietary intake which failed to demonstrate that the dietary intake of manganese would influence liver or other tissue manganese concentrations following MnSO₄ inhalation (Dorman et al., 2001b). Unlike Moore et al. (1975), our MnSO₄ and Mn₃O₄ inhalation studies showed that brain manganese concentrations were not influenced by the dietary intake of manganese (Dorman et al., 2001b). These results indicate that individuals with relatively mild manganese deficiency or excess are not likely to develop elevated brain manganese concentrations following short-term inhalation exposure to high levels of manganese.

This experiment lends additional evidence that dose-dependent biliary excretion of manganese serves to regulate the percentage of inhaled manganese retained by manganese-deficient animals, thereby limiting available manganese that could result in increased brain manganese concentrations. Despite being in a relatively manganese-deficient state, rats given 2 ppm manganese diet and exposed to either MnSO₄ or Mn₃O₄ increased their rate of manganese excretion when compared to their respective air-exposed control groups. This observation suggests that, at least initially, animals will not retain inhaled manganese to compensate for relative nutritional deficiencies in this essential metal. Our results further suggest that even moderately manganese-deficient individuals are not likely to develop elevated brain manganese concentrations following short-term inhalation exposure to moderately high levels of manganese. Additional studies evaluating route-to-route differences between the disposition of inhaled and ingested manganese would shed light on conditions under which the adaptive hepatobiliary excretion mechanisms for ingested manganese fail to control systemic concentrations arising from inhaled manganese.

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