OLFACTORY TRANSPORT: A DIRECT ROUTE OF DELIVERY OF INHALED MANGANESE PHOSPHATE TO THE RAT BRAIN

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Experiments examining the dosimetry of inhaled manganese generally focus on pulmonary deposition and subsequent delivery of manganese in arterial blood to the brain. Growing evidence suggests that nasal deposition and transport along olfactory neurons represents another route by which inhaled manganese is delivered to certain regions of the rat brain. The purpose of this study was to evaluate the olfactory uptake and direct brain delivery of inhaled manganese phosphate ($^{54}$MnHPO$_4$). Male, 8-wk-old, CD rats with either both nostrils patent or the right nostril occluded underwent a single, 90-min, nose-only exposure to a $^{54}$MnHPO$_4$ aerosol (0.39 mg $^{54}$Mn/m$^3$; MMAD 1.68 µm, σg 1.42). The left and right sides of the nose, olfactory pathway, striatum, cerebellum, and rest of the brain were evaluated immediately after the end of the $^{54}$MnHPO$_4$ exposure and at 1, 2, 4, 8, and 21 d postexposure with gamma spectrometry and autoradiography. Rats with two patent nostrils had equivalent $^{54}$Mn concentrations on both sides of the nose, olfactory bulb, and striatum, while asymmetrical $^{54}$Mn delivery occurred in rats with one occluded nostril. High levels of $^{54}$Mn activity were observed in the olfactory bulb and tubercle on the same side (i.e., ipsilateral) to the open nostril within 1–2 d following $^{54}$MnHPO$_4$ exposure, while brain and nose samples on the side ipsilateral to the nostril occlusion had negligible levels of $^{54}$Mn activity. Our results demonstrate that the olfactory route contributes to $^{54}$Mn delivery to the rat olfactory bulb and tubercle. However, this pathway does not significantly contribute to striatal $^{54}$Mn concentrations following a single, short-term inhalation exposure to $^{54}$MnHPO$_4$.

Airborne manganese is introduced into the environment from a variety of sources including fugitive dusts and emissions from automobiles, power plants, municipal waste incinerators, metal smelting operations, steel production, and foundries (U.S. EPA, 1996). Manganese is also used in methylcyclopentadienyl manganese tricarbonyl (MMT), an octane-enhancing fuel additive used in some unleaded automotive gasoline. The combustion of MMT by the automobile engine results in the formation of a mixture com-

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posed primarily of manganese phosphates and manganese sulfates and a small amount of manganese oxides (Colmenares et al., 1999; Lynam et al., 1999; Ressler et al., 1999, 2000; Zayed et al., 1999). The presence of the phosphate, sulfate, and oxide constituents is controlled by thermodynamic equilibrium set by the gas temperatures in the engine and exhaust system (Roos et al., 2000).

Under conditions of high occupational exposure, excess manganese accumulates within the human striatum and globus pallidus and produces damage to dopaminergic neurons within these sites (Malecki et al., 1999; Nelson et al., 1993; Pal et al., 1999). The use of MMT in gasoline has raised speculation as to whether chronic exposure to low levels of manganese can result in an increased incidence of human neurological disease (Davis, 1999). A critical first step in understanding whether inhaled manganese plays a role in chronic neurological disease is to determine exposure conditions that lead to increased concentrations of the metal within the central nervous system. This understanding is especially critical for manganese since its mechanism of toxicity is poorly understood and since an elevation in brain manganese levels is one of the few reliable biomarkers available to monitor for excessive exposure (Andersen et al., 1999). Published rodent literature confirms that the age of the animal and the chemical form and route of manganese exposure dramatically influence brain manganese levels (Dorman et al., 2000, 2001; Kontur & Fechter, 1988; Roels et al., 1997). Brain delivery of manganese is higher following inhalation versus ingestion, and pharmacokinetic factors that may contribute to this increased efficiency in brain manganese delivery include increased manganese absorption from the pulmonary tract, slower blood clearance of absorbed manganese, and direct delivery to the brain via the olfactory system (Andersen et al., 1999).

The axonal transport of chemicals in the olfactory system has been demonstrated to occur with solvents, metals, and other chemicals. Metals that have been demonstrated to undergo axonal transport include iron, lead, cadmium, manganese, mercury, thallium, silver, gold, and zinc (for review, see Arvidson, 1994). Olfactory transport of manganese has been demonstrated to occur in the rat, mouse, and freshwater pike following intranasal instillation (Gianutsos et al., 1997; Tjälve & Henriksson, 1999; Tjälve et al., 1995). In rats, intranasal instillation of manganese has been shown to result in direct transport of manganese to the olfactory bulb and the telencephalon through translocation in secondary olfactory neurons. Once in the brain, manganese can continue to move across synaptic connections and along neuronal processes to sites distantly connected to the olfactory center in the brain (Tjälve & Henriksson, 1999). Although olfactory transport has been shown to deliver manganese to brain structures in the olfactory pathway rapidly (Brenneman et al., 2000; Gianutsos et al., 1997; Tjälve et al., 1996), it is relatively slow at delivering manganese to the striatum and other more distant brain structures (Tjälve et al., 1996). Recent data presented by Henriksson and Tjälve (2000) suggest that olfactory uptake may result in
manganese-induced neurotoxicity. These investigators showed that intra-nasal instillation of manganese is associated with alterations in olfactory bulb expression of glial fibrillary acidic protein and S-100b in rats. These proteins are known markers of damage to astrocytes, an important support cell found within the central nervous system. Although nasal instillation studies have clearly demonstrated that olfactory transport of manganese can occur and may mediate cellular responses, questions remain as to the toxicological significance of this route of delivery through inhalation.

Until recently, little was known regarding the olfactory transport of manganese following inhalation exposure. Brenneman and coworkers (2000) conducted studies in rats using short-term (90-min) inhalation exposure to a water-soluble radiolabeled manganese aerosol ($^{54}$MnCl$_2$). They used an animal model in which one nostril was occluded, thus restricting olfactory transport of manganese to the side of the rat brain ipsilateral (i.e., on the same side) to the patent nostril. In this study, direct delivery along the olfactory route accounted for nearly all the $^{54}$Mn found in the olfactory bulb and tract of the rat brain following acute $^{54}$MnCl$_2$ inhalation. Data from longer term rat inhalation studies in which rats were exposed 6 h/day and 7 days/wk (14 exposures) to manganese phosphate, sulfate, or tetroxide at 0, 0.03, 0.3, or 3 mg Mn/m$^3$ further support the role olfactory uptake plays in brain manganese delivery (Dorman et al., 2001; Vitarella et al., 2000b). The manganese concentrations attained in the olfactory bulb following the end of the 2-wk inhalation exposures were significantly higher than those observed in either the striatum or cerebellum, lending additional support to the direct olfactory transport theory. Collectively, these findings suggest that the olfactory route may indeed be a significant pathway by which inhaled manganese gains direct access to certain structures within the rat brain.

The purpose of this study was to determine whether particle solubility influences the olfactory transport of manganese from the nasal cavity to the brain. To answer this question, we used a form of manganese ($^{54}$MnHPO$_4$) that dissolves very slowly in biological fluids (Vitarella et al., 1999a). Inhalation exposures were conducted in CD rats with a unilaterally occluded nostril using exposure conditions similar to those reported earlier by Brenneman and coworkers (2000) for the relatively soluble chloride form. Data obtained from these studies provide an adequate basis for determining the role of particle solubility on the delivery of manganese to the rat brain following inhalation exposure.

**MATERIALS AND METHODS**

**Study Design**

Three replicate, nose-only inhalation exposures of 32 rats to $^{54}$MnHPO$_4$ were conducted. Half of the rats in each replicate had two patent nostrils (control) and were used to estimate the symmetry of delivery of inhaled manganese in normal rats. The second group of rats had one occluded nos-
tril, and data obtained from this group were used to determine the contribution of the olfactory pathway to brain manganese levels. At 0, 1, 2, 4, 8, or 21 d postexposure, rats were anesthetized with sodium pentobarbital (150 mg/kg, ip) and euthanatized by exsanguination. Gamma spectrometry and autoradiography were used to compare the levels of $^{54}\text{Mn}$ found on the left and right sides of the nose and brain to determine the relative contribution of olfactory uptake to brain $^{54}\text{Mn}$ levels.

**Chemicals**

$^{54}\text{MnHPO}_4$ suspended in water (specific activity 0.51 mCi/mg Mn) was custom-synthesized by NEN Life Science Products (Boston). Chemical purity (>98%) was confirmed by the manufacturer using thin-layer chromatography and inductively coupled plasma (ICP) mass spectroscopy analyses. All other chemicals were of reagent grade and were purchased from Sigma Chemical unless otherwise noted.

**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. A total of 96 male CD rats (Charles River Laboratories, Inc., Raleigh, NC), 8-wk-old (~250 g), were used for the study. Animals were acclimated for approximately 2 wk in a HEPA-filtered, mass air-displacement room maintained at 18.5–21.5°C and 40–60% relative humidity in the CIIT animal facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Rats were identified with ear tags and randomly assigned to weight-matched experimental groups. During acclimation and prior to exposure, animals were housed (2–3 rats/cage) in polycarbonate cages with water bottles, stainless steel wire lids, and cellulose fiber chip bedding (ALPHA-dri; Shepherd Specialty Papers, Kalamazoo, MI). After exposure to $^{54}\text{MnHPO}_4$, rats were individually housed in suspended stainless-steel wire cages in biologically clean rooms with HEPA-filtered air and a 12-h (0700–1900) light–dark photoperiod cycle. An NIH-07 pelleted diet (Zeigler Brothers, Gardner, PA) with approximately 100 ppm manganese and filter-purified tap water (NANOpure System; Barnstead, Boston) containing <0.02 µg manganese/ml were available ad libitum.

**$^{54}\text{MnHPO}_4$ Aerosol Generation and Exposure System**

The $^{54}\text{MnHPO}_4$ aerosol was generated using a Collison nebulizer (BGI Incorporated, Waltham, MA), which consisted of a modified MRE-type three-jet Collison nozzle with a precious fluids extension sleeve attached and a precious fluids reservoir. The $^{54}\text{MnHPO}_4$ suspension used to generate the aerosols was placed into the reservoir and contained 3.4 mCi $^{54}\text{Mn}$ in 25 ml water. Air delivery pressure through the nebulizer was maintained at 15 psi during generation. The $^{54}\text{MnHPO}_4$ particles were carried from the nebulizer
into a brass in-line radial diluter (In-Tox Products, Albuquerque, NM) and from there into the nose-only exposure system. At the diluter, the particles were diluted with enough clean air to provide 1.5 times the average minute ventilation of a rat (approximately 0.375 L/min) at each of the 33 open ports on the nose-only exposure system. Rats were exposed on a dynamic, nonre-breathing, Cannon-style (Cannon et al., 1983), nose-only exposure system (Lab Products, Maywood, NJ). During the exposures, the rats were held in open nose-only restraint tubes. The concentration of the exposure atmosphere was determined during each $^{54}$MnHPO$_4$ exposure by mass weight filter gravimetric analysis using 25-mm glass-fiber filters (Osmotics, Inc., Minnetonka, MN). The amount of $^{54}$Mn collected on the filter was also analyzed by counting the radioactivity collected on a small sample (one-eighth) of the filter. An optical particle sizing spectrometer (aerodynamic particle sizer, model 3320, TSI, Inc., St. Paul, MN) was used to measure the particle size distribution of the aerosol.

**Nasal Occlusion Procedure**

The nasal occlusion procedure is described in greater detail elsewhere (Brenneman et al., 2000). Briefly, nasal plugs were made of peroxide-cured silicone plastic tubing (Medical Grade, SF Medical, Hudson, MA) approximately 5 mm in length, into which several longer lengths of smaller diameter polyethylene tubing (Intramedic, Clay Adams, Parsippany, NJ) were inserted. The ends of the polyethylene tubing were melted to form a hard, smooth, rounded seal on both ends of the silicone tubing (Kucharski et al., 1986). The rats in the occluded group were briefly anesthetized with isoflurane approximately 2 h prior to the start of the $^{54}$MnHPO$_4$ exposure. A lubricated (KY Jelly, Johnson & Johnson Medical, Inc., New Brunswick, NJ) plastic plug was gently inserted into the right nasal vestibule and held in place by external application of a few drops of tissue adhesive, octyl-cyanoacrylate (Nexaband Quickseal, Closure Medical Corporation, Raleigh, NC). Control animals were anesthetized with isoflurane for approximately 5 min to mimic the anesthetic regimen used during the nose plugging procedure. The plugging procedure was not associated with any observable adverse health effects.

**Gamma Spectrometry**

Gamma spectrometry was performed on the following harvested and weighed tissue samples (right and left sides of head): nasal olfactory mucosa, nasal nonolfactory (predominantly respiratory) mucosa, olfactory bulb, olfactory tract and tubercle, striatum, cerebellum, and rest of brain. Gamma spectrometry was also performed on the whole lung and representative samples of liver, kidney, testes, and pancreas. The amount of radioactivity ($^{54}$Mn) in a tissue was determined using a Packard Cobra Series auto-gamma counting system model 5003 (Meriden, CT). The final unit of measurement was nCi $^{54}$Mn/g tissue wet weight.
 Autoradiography

Bilateral sagittal sectioning of the frozen heads collected immediately after exposure and at 1, 2, and 4 d after the end of the $^{54}$MnHPO$_4$ exposure was performed at 50-µm intervals at approximately –22°C using a PMV 2250 macrocryostat (LKB Instruments, Rockville, MD). Resulting tape sections were freeze-dried overnight, adhered to Biomax MR-2 film (Eastman Kodak Company, Rochester, NY) in the dark at –20°C for several months, and developed using Kodak GBX reagents according to the manufacturer’s recommendations. Sections were obtained from the right and left sides of the head so that the amount of radioactivity on each side of the nose, olfactory pathway, and striatum could be compared.

Data Analysis

Tissue concentrations of $^{54}$Mn in the occluded and control groups were evaluated statistically using a paired $t$-test analysis of the calculated difference between the $^{54}$Mn concentrations found on the right and left sides of the animal’s head. Time-course data were evaluated by one-way analysis of variance (ANOVA) followed by a comparison with the immediate postexposure group using Dunnett’s test. Statistical analyses were performed using SAS Statistical Software. Pharmacokinetic estimates were made after fitting a nonlinear regression curve to the individual tissue $^{54}$Mn concentration–time profiles data by model-dependent methods (WinNonlin, Pharsight Corp., Cary, NC). Biexponential (two-compartment) curves were used to obtain the best-fit curve. Pharmacokinetic parameters were then calculated from standard kinetic formulas (Shargel & Yu, 1985). The initial and terminal phase elimination half-lives were calculated using 0.693/$\alpha$ and 0.693/$\beta$, respectively, where $\alpha$ and $\beta$ are the rate constants of the initial and terminal phases as determined by linear regression. A $p$ value of <.05 was used as the critical level of significance for all statistical tests. Unless otherwise noted, values presented are means ± standard error of the mean (SEM).

RESULTS

$^{54}$MnHPO$_4$ Test Atmospheres

The average measured mass concentration for the 3 exposures was 0.39 mg $^{54}$Mn/m$^3$. The average calculated particle mass median aerodynamic diameter (MMAD) was 1.68 µm with a geometric standard deviation of 1.42. The calculated and measured characteristics of the $^{54}$MnHPO$_4$ exposure solutions and aerosols generated for each of the three exposures conducted are presented in Table 1.

$^{54}$Mn Nasal Deposition and Brain Delivery

$^{54}$Mn concentrations in the olfactory mucosa, olfactory bulb, olfactory tract and tubercle, striatum, cerebellum, and rest of the brain of rats with
two patent nostrils are shown in Figure 1. Similar levels of $^{54}$Mn were observed between the right and left olfactory mucosa, olfactory bulb, striatum, cerebellum, and rest of the brain from control animals exposed to $^{54}$MnHPO$_4$. Levels of $^{54}$Mn observed in the right olfactory tract and tubercle from control rats were higher than those observed in the left olfactory tract on d 1, 2, 4, and 8 after the $^{54}$MnHPO$_4$ exposure ended. This observation likely reflects random variability since a similar finding was not observed in our previous studies with inhaled manganese chloride (Brenneman et al., 2000). Increased olfactory bulb, olfactory tract and tubercle, and cerebellar $^{54}$Mn concentrations were observed 1 d after the $^{54}$MnHPO$_4$ exposure ended. Striatal $^{54}$Mn concentrations were higher than those observed in either the cerebellum or rest of the brain and were increased 8 d after the $^{54}$MnHPO$_4$ exposure ended. Cerebellar $^{54}$Mn concentrations were approximately 10% of the values observed in the olfactory bulb. $^{54}$Mn concentrations in the residual brain sample were likewise quite low (−5% of peak olfactory bulb values) and were increased between d 2 and 21 after the $^{54}$MnHPO$_4$ exposure ended. Bilateral sagittal head-only autoradiography confirmed the symmetrical distribution of inhaled $^{54}$Mn in rats with patent nostrils and transport of $^{54}$Mn from the nasal cavity to the olfactory bulb and olfactory tract and tubercle (Figure 2).

$^{54}$Mn concentrations in the olfactory epithelium, olfactory bulb, olfactory tract and tubercle, striatum, cerebellum, and rest of the brain of rats with a patent left nostril and an occluded right nostril are shown in Figure 3. As expected, the nostril occlusion procedure markedly reduced deposition of $^{54}$Mn on the olfactory epithelium ipsilateral to the occluded nostril (Figure 4). As with the control animals, increased olfactory bulb and olfactory tract and tubercle $^{54}$Mn concentrations were observed on the side of the head ipsilateral to the patent nostril 1 d after the $^{54}$MnHPO$_4$ exposure ended.

**TABLE 1.** Characteristics of the $^{54}$MnHPO$_4$ Aerosol Generated for Three Rat Nose-Only Inhalation Exposures

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Exposure</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure suspension concentration (mg MnHPO$_4$/ml)</td>
<td></td>
<td>0.74</td>
<td>0.74</td>
<td>0.74</td>
<td>0.74</td>
</tr>
<tr>
<td>Specific activity (mCi/mg Mn)</td>
<td></td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td>Count median diameter$^a$ (µm)</td>
<td></td>
<td>1.16</td>
<td>1.15</td>
<td>1.16</td>
<td>1.16</td>
</tr>
<tr>
<td>Geometric standard deviation</td>
<td></td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
</tr>
<tr>
<td>Mass median aerodynamic diameter$^b$ (µm)</td>
<td></td>
<td>1.68</td>
<td>1.66</td>
<td>1.68</td>
<td>1.68</td>
</tr>
<tr>
<td>Mass concentration (mg $^{54}$Mn/m$^3$)$^c$</td>
<td></td>
<td>0.37</td>
<td>0.38</td>
<td>0.42</td>
<td>0.39</td>
</tr>
</tbody>
</table>

$^a$The count median diameter (CMD) readings from the aerodynamic particle sizer were averaged.
$^b$The mass median aerodynamic diameter (MMAD) was calculated from the CMD and geometric standard deviation.
$^c$Mass concentration of the manganese was determined from the total activity detected on the filter, the airflow rate, sample collection time, and specific activity.
Olfactory bulb $^{54}\text{Mn}$ concentrations on the side ipsilateral to the occluded nostril did not increase during the 21-d experiment. Olfactory tract and tubercle $^{54}\text{Mn}$ concentrations on the side ipsilateral to the patent nostril did not increase until 8 d after the $^{54}\text{MnHPO}_4$ exposure ended. This result suggests that the initial delivery of $^{54}\text{Mn}$ to the olfactory tract and tubercle was...
most likely due to olfactory transport, while delivery occurring at later times may be associated with arterial delivery of manganese that is being redistributed within the body. An alternative explanation may be that delayed delivery of manganese to the side ipsilateral to the occluded nostril reflects transport from the opposite olfactory system. Neurons in the ipsilateral and contralateral anterior olfactory nuclei (a cortical brain structure just caudal to the olfactory bulb) link olfactory networks in the two hemispheres via the anterior commissure (Shipley et al., 1995). Subdivisions of the ipsilateral anterior olfactory nucleus project to the contralateral olfactory bulb, thus manganese delivered to the side ipsilateral to the occluded nostril could come from the opposite olfactory system. Striatal $^{54}$Mn concentrations were increased on d 8; however, equivalent amounts of $^{54}$Mn were observed in the right and left striatal samples, suggesting that olfactory uptake does not contribute significantly to striatal $^{54}$Mn concentrations. $^{54}$Mn concentrations in the residual brain sample were quite low (~2–3% of peak olfactory bulb values) and were increased on the side of the head ipsilateral to the patent

![FIGURE 2. Autoradiographic images and corresponding tissue sections from one side of sagitally sectioned control rat heads at 0, 1, and 4 d following a single 90-min inhalation exposure to $^{54}$MnHPO$_4$. At d 0 (A–B), $^{54}$Mn was visible (white) in the nasal cavity, including on the olfactory mucosa-lined ethmoturbinates (et). By d 1 (C–D), $^{54}$Mn was transported to the olfactory bulb (ob); by d 4 (E–F), $^{54}$Mn was evident in the olfactory tract (ot).]
nostril between d 2 and 21 after the $^{54}$MnHPO$_4$ exposure ended. Cerebellar $^{54}$Mn concentrations were unaffected by $^{54}$MnHPO$_4$ exposure.

**Tissue $^{54}$Mn Concentrations**

$^{54}$Mn concentrations in the lung of rats with either two patent nostrils or an occluded right nostril are shown in Figure 5. Rats with one nostril occluded had lung $^{54}$Mn concentrations that were approximately 50% of the...
control group at the end of the exposure. Similar terminal half-lives of elimination were observed in the lungs from control animals and rats with one occluded nostril, suggesting that the occlusion procedure did not affect $^{54}$Mn clearance from the lung (data not shown). $^{54}$Mn concentrations in the nasal respiratory epithelium of rats with two patent nostrils or an occluded right nostril at the end of the exposure. Similar terminal half-lives of elimination were observed in the lungs from control animals and rats with one occluded nostril, suggesting that the occlusion procedure did not affect $^{54}$Mn clearance from the lung (data not shown). $^{54}$Mn concentrations in the nasal respiratory epithelium of rats with two patent nostrils or an occluded right nostril at the end of the exposure.

**FIGURE 4.** Autoradiographic images and corresponding tissue sections from the left and right sides of sagitally sectioned, right-nostril-occluded male rat heads immediately following a single 90-min inhalation exposure to $^{54}$MnHPO$_4$. Nasal deposition of $^{54}$Mn was virtually eliminated on the side ipsilateral to the occluded nostril (A–D). $^{54}$Mn was visible (white) in the nasal cavity, including on the olfactory mucosa-lined ethmoturbinates (et).

**FIGURE 5.** $^{54}$Mn activity levels (nCi Mn/g tissue wet weight) in the lungs of male CD rats with either both patent nostrils (unoccluded group) or with the right nostril occluded (occluded group) that underwent a single, 90-min, nose-only exposure to $^{54}$MnHPO$_4$. Lung $^{54}$Mn concentrations at 0, 1, 2, 4, 8, and 21 d postexposure are presented. Values are means ± SEM; $n = 7$ rats/group/time point.
nostril are shown in Figure 6. Maximal kidney, liver, testis, and pancreas $^{54}$Mn concentrations were achieved within 1 to 2 d after the end of the 90-min inhalation exposure (Figure 7). Kidney, liver, testis, and pancreas $^{54}$Mn concentrations were elevated between 1 and 8 d after the $^{54}$MnHPO$_4$ exposure ended.

$^{54}$Mn Clearance from the Respiratory System

One goal of this experiment was to determine the role of particle solubility on the delivery of manganese to the rat brain following inhalation. Exposures were conducted in CD rats using methods reported by Brenneman and coworkers (2000) for the more soluble chloride ($^{54}$MnCl$_2$) form. Data from the Brenneman et al. (2000) study have been included for comparison.

A biphasic pattern of elimination was observed in the lung, olfactory epithelium, and respiratory epithelium. Manganese elimination from these sites was characterized by an initial rapid elimination phase followed by an appreciably slower second phase of elimination. Control animals exposed to $^{54}$MnHPO$_4$ had an initial rapid phase ($t_{1/2a}$ of 0.28 ± 0.03 d) and a slower second phase of elimination ($t_{1/2b}$ of 3.1 ± 0.5 d) from the lung. Surprisingly, Brenneman and coworkers (2000) observed similar lung clearance kinetics in control animals exposed to $^{54}$MnCl$_2$ ($t_{1/2a}$ of 0.27 ± 0.09 d; $t_{1/2b}$ of 2.5 ± 0.8 d). Manganese clearance from the olfactory and respiratory epithelium was influenced by particle solubility, and the effect was most pronounced on the terminal elimination half-lives. Animals exposed to $^{54}$MnHPO$_4$ had initial ($t_{1/2a}$) and terminal ($t_{1/2b}$) elimination half-lives from the olfactory epithelium of 0.45 ± 0.15 and 11.4 ± 1.5 d, respectively. Olfactory epithelial elimination half-lives observed in control animals exposed to $^{54}$MnCl$_2$ were somewhat faster ($t_{1/2a}$ of 0.30 ± 0.11 d; $t_{1/2b}$ of 8.3 ± 1.2 d). Animals exposed to $^{54}$MnHPO$_4$ had initial ($t_{1/2a}$) and terminal ($t_{1/2b}$) elimination half-lives from the respiratory epithelium of 0.54 ± 0.08 and 19.0 ± 4.8 d, respectively. Respiratory epithelial elimination half-lives observed in control animals exposed to $^{54}$MnCl$_2$ for both elimination phases were shorter ($t_{1/2a}$ of 0.40 ± 0.02 d; $t_{1/2b}$ of 10.9 ± 1.4 d).

DISCUSSION

This study examined the olfactory transport of inhaled manganese (as $^{54}$Mn) in rats following acute exposure to relatively low aerosol concentrations of manganese phosphate. This experiment relied on a unilateral nasal occlusion procedure developed by Brenneman and coworkers (2000) that prevents the deposition of inhaled manganese on the side ipsilateral to the occluded nostril. The unilateral nasal occlusion model is a useful tool for studying the role of olfactory transport in delivering inhaled toxicants to the brain. By preventing intranasal deposition of the inhaled chemical on one side of the nose, the contribution of olfactory transport to brain levels of the
FIGURE 6. $^{54}$Mn activity levels (nCi Mn/g tissue wet weight) in the respiratory epithelium of male CD rats with either both patent nostrils (unoccluded group) or with the right nostril occluded (occluded group) that underwent a single, 90-min, nose-only exposure to $^{54}$MnHPO$_4$. Respiratory epithelium $^{54}$Mn concentrations at 0, 1, 2, 4, 8, and 21 days postexposure are presented. Values are means ± SEM ($n = 7$ rats/group/time point). Asterisk indicates significantly different from levels measured on the other side of the nose ($p < .05$).
FIGURE 7. Mean (± SEM, n = 7 rats/group/time point) $^{54}$Mn activity levels (nCi Mn/g tissue wet weight) in the liver, kidney, pancreas, and testis of male CD rats with either both patent nostrils (unoccluded group) or with the right nostril occluded (occluded group) that underwent a single, 90-min, nose-only exposure to $^{54}$MnHPO$_4$.
inhaled toxicant is initially eliminated on one side of the brain following inhalation. The side of the brain ipsilateral to the occluded nostril relies predominantly on systemic delivery of the inhaled toxicant to the brain, while toxicant transported to the side of the brain that is ipsilateral to the unoccluded nostril is delivered via both the blood and olfactory routes. A comparison of brain levels of the inhaled toxicant on the unoccluded versus the occluded sides can be used to determine the contribution of the olfactory route, relative to the blood route, to brain levels of the toxicant.

We evaluated manganese delivery to several brain regions of interest: the olfactory bulb and tract, striatum, cerebellum, and the rest of the brain. Marked asymmetry in $^{54}$Mn concentrations within the right and left olfactory epithelia, olfactory bulbs, and olfactory tract and tubercles were observed in animals with an occluded nostril. Elevated $^{54}$Mn concentrations in the olfactory bulb and tract on the side or sides of the head ipsilateral to a patent nostril are consistent with but not definitive evidence for direct olfactory transport of inhaled $^{54}$Mn from the nasal cavity to the brain. Olfactory bulb and tract and tubercle $^{54}$Mn concentrations were consistently lower on the side ipsilateral with the occluded nostril, confirming that the olfactory route contributes a significant portion of total delivery of $^{54}$Mn to these brain structures. Our results demonstrate that inhaled manganese is delivered directly to the rat brain, with peak concentrations observed between 1 and 2 d and 2 and 8 d in the olfactory bulb and tract/tubercle, respectively. Qualitatively similar results demonstrating direct delivery of manganese to the olfactory bulb and tract and tubercle were observed by Brenneman et al. (2000) with the more soluble manganese chloride form.

There is compelling evidence that within the human brain the striatum, globus pallidus, and substantia nigra develop higher tissue manganese concentrations and are the primary target sites for manganese neurotoxicity (for review, see Pal et al., 1999). Our data with inhaled $^{54}$MnHPO$_4$ indicate that direct olfactory transport does not contribute significantly to manganese delivery to the rat striatum. This negative finding occurred despite using a relatively long postexposure delay (21 d) that should have allowed for transport of manganese to the striatum (Tjälve et al., 1996). Our results with manganese phosphate corroborate the findings of Brenneman et al. (2000), who also failed to observe manganese delivery to the striatum following inhalation exposure to the more soluble $^{54}$MnCl$_2$ salt. Likewise, olfactory delivery of $^{54}$Mn to the cerebellum did not occur in either our study or that of Brenneman et al. (2000) following manganese inhalation. To our knowledge, the human cerebellum is not considered a primary site of action for manganese-induced neurotoxicity, and it is unlikely to receive manganese via the olfactory pathway.

Although our experimental results demonstrate that direct transport of inhaled manganese occurs to the olfactory bulb and tract or tubercle, these brain structures have not been implicated in manganese-induced neurotoxicity in humans. Furthermore, the relevance of these findings to human man-
Manganese inhalation exposure and the risks for neurotoxicity are not known and are complicated by interspecies differences in nasal and brain anatomy and physiology. Differences in the relative size of the rat olfactory mucosa and olfactory bulb likely predispose rats, more so than humans, to nasal deposition and olfactory transport of manganese. Although the rat is a good animal model for olfactory transport, it is a poor model for manganese neurotoxicity in humans. The rat fails to selectively accumulate manganese in the striatum and does not demonstrate the behavioral and pathological changes characteristic of maniasis in human and nonhuman primates (for review, see Brenneman et al., 1999).

Besides the brain, other organs also had increased tissue $^{54}$Mn concentrations following manganese phosphate inhalation. Peak liver, kidney, and pancreas tissue $^{54}$Mn concentrations were achieved 1–2 d following inhalation exposure, which is consistent with the data of Brenneman et al. (2000) and of Wieczorek and Oberdörster (1989). As expected, the lung had the highest tissue $^{54}$Mn concentrations. Elimination rate constants for the removal of manganese from the lung observed in our study were lower than those observed by Wieczorek and Oberdörster (1989) in rats following inhalation exposure to the more soluble chloride salt. Surprisingly, Brenneman and coworkers (2000) observed similar lung clearance kinetics in rats exposed to the chloride ($^{54}$MnCl$_2$) form. Clearance of manganese from the lungs following inhalation is affected by particle size and surface area, the species of manganese, particle distribution, solubility and dissolution rate, and toxicity to macrophages; thus, multiple factors could account for differences observed among these experiments (Schlesinger, 1995; Vitarella et al., 2000a). The rate of metal clearance from the lung can influence its delivery to the brain and other organs. We have previously shown that rats develop higher striatal manganese concentrations following repeated inhalation exposure to soluble forms of manganese (Dorman et al., 2001). Rhoads and Sanders (1985) have also shown that metal compounds that were more rapidly cleared from the lungs were retained for longer periods at other sites in the body and could potentially exert their effects at these other sites.

Another goal of this study was to determine whether the solubility of inhaled manganese particles influences direct olfactory delivery of manganese to the rat brain. Our laboratory recently completed a rat inhalation olfactory uptake study with manganese chloride ($^{54}$MnCl$_2$), a relatively soluble form of manganese (Brenneman et al., 2000). Similar aerosol concentrations (0.54 vs. 0.39 mg Mn/m$^3$ for the $^{54}$MnCl$_2$ and $^{54}$MnHPO$_4$ exposures, respectively) and particle sizes (2.51 vs. 1.68 µg for the $^{54}$MnCl$_2$ and $^{54}$MnHPO$_4$ exposures, respectively) were used in these studies to facilitate comparison of our experimental results. Our results indicate that manganese clearance from the olfactory epithelia was slower following exposure to manganese phosphate when compared to the more soluble chloride salt. For example, approximately 48% of the $^{54}$Mn initially deposited on the olfactory epithelium remained 4 d after the end of the $^{54}$MnHPO$_4$ exposure. In con-
trast, only 26% of the $^{54}\text{Mn}$ deposited on the olfactory epithelium remained following a similar short-term $^{54}\text{MnCl}_2$ exposure (Brenneman et al., 2000). In addition, a smaller fraction of the $^{54}\text{Mn}$ initially deposited on the olfactory epithelium was found in the olfactory bulb 1 d after the end of the $^{54}\text{MnHPO}_4$ exposure. The finding of decreased olfactory epithelial clearance and reduced olfactory bulb delivery following manganese phosphate inhalation is consistent with the results of our longer term inhalation experiments with soluble and insoluble manganese salts (Dorman et al., 2001). Dorman and coworkers (2001) showed that inhalation exposure to soluble forms of manganese (e.g., manganese sulfate) resulted in higher olfactory bulb manganese concentrations than those achieved following exposure to a relatively insoluble form of manganese (e.g., manganese phosphate or tetroxide). Data obtained from the present study affirm the importance of direct olfactory delivery of manganese to certain structures within the rat brain and provide evidence for a role of particle solubility in this process.

Species differences in nasal and brain anatomy and physiology confound the extrapolation of our research results obtained for manganese in rodents to humans. Interspecies differences in nasal and brain anatomy and physiology must be considered before a determination is made that this route of delivery is toxicologically important in humans. In the rat, the olfactory bulb accounts for a relatively large portion of the central nervous system, and the nasal olfactory mucosa covers approximately 50% of the total nasal epithelium (Gross et al., 1982). These structures are proportionately smaller in humans; for example, the olfactory mucosa covers approximately 5% of the total nasal epithelium in humans (Gross et al., 1982). These anatomical differences suggest that this route of brain delivery may be less important in humans as compared to the rat. Rats are also obligatory nasal breathers, while humans are oronasal breathers. Up to 16.5% of the inhaled air stream is estimated to reach the olfactory mucosa in the rat, whereas only about 5% of the inhaled air stream reaches the olfactory region of the nose in a human (Kimbell et al., 1997; Proctor & Chang, 1983; Schreider, 1983). These differences probably predispose the rat, more so than humans, to olfactory deposition and potential olfactory transport of manganese. Additional research will be required to better clarify the potential significance of the olfactory route of delivery of manganese to the brain in humans exposed via inhalation.

REFERENCES


