ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Toxicology and Applied Pharmacology 191 (2003) 264–271

Toxicology and Applied Pharmacology

www.elsevier.com/locate/taap

Bioaccumulation and locomotor effects of manganese phosphate/sulfate mixture in Sprague-Dawley rats following subchronic (90 days) inhalation exposure

Fariba Salehi,[a,b] Daniel Krewski,[b] Donna Mergler,[c] Louise Normandin,[a] Greg Kennedy,[a] Suzanne Philippe,[a] and Joseph Zayed[a,*]

[a] *TOXHUM (Human Toxicology Research Group) and Department of Environmental and Occupational Health, Faculty of Medicine, University of Montréal, Montréal, Québec, Canada*

[b] *McLaughlin Center for Population Health Risk Assessment, University of Ottawa, Ottawa, Ontario, Canada*

[c] *Centre pour l'étude des interactions biologiques entre la santé et l'environnment (CINBIOSE), Montréal, Québec, Canada*

Received 19 February 2003; accepted 28 April 2003

Abstract

Methylcyclopentadienyl manganese tricarbonyl (MMT) is an organic manganese (Mn) compound added to unleaded gasoline in Canada. The primary combustion products of MMT are Mn phosphate, Mn sulfate, and a Mn phosphate/Mn sulfate mixture. Concerns have been raised that the combustion products of MMT containing Mn could be neurotoxic, even at low levels of exposure. The objective of this study is to investigate exposure-response relationships for bioaccumulation and locomotor effects following subchronic inhalation exposure to a mixture of manganese phosphates/sulfate mixture. A control group and three groups of 30 male Sprague-Dawley rats were exposed in inhalation chambers for a period of 13 weeks, 5 days per week, 6 h a day. Exposure concentrations were 3000, 300, and 30 $\mu g/m^3$. At the end of the exposure period, locomotor activity and resting time tests were conducted for 36 h using a computerized autotrack system. Rats were then euthanized by exsanguination and Mn concentrations in different tissues (liver, lung, testis, and kidney) and blood and brain (caudate putamen, globus pallidus, olfactory bulb, frontal cortex, and cerebellum) were determined by neutron activation analysis. Increased manganese concentrations were observed in blood, kidney, lung, testis, and in all brain sections in the highest exposure group. Mn in the lung and in the olfactory bulb were dose dependent. Our data indicate that the olfactory bulb accumulated more Mn than other brain regions following inhalation exposure. Locomotor activity was increased at 3000 $\mu g/m^3$, but no difference was observed in resting time among the exposed groups. At the end of the experiment, rats exposed to 300 and 3000 $\mu g/m^3$ exhibited significantly decreased body weight in comparison with the control group. Biochemical profiles also revealed some significant differences in certain parameters, specifically alkaline phospatase, urea, and chlorate.

Keywords: Manganese phosphate/sulfate mixture; Inhalation exposure; Sprague-Dawley rats; Bioaccumulation; Locomotor activity

Introduction

Methylcyclopentadienyl manganese tricarbonyl (MMT) is one of the main sources of inorganic manganese (Mn) contamination in urban air, mainly in areas with high traffic density (Joselow et al., 1978). The main combustion products of MMT are essentially Mn-phosphate, Mn-sulfate, and a Mn-phosphate/sulfate mixture (Zayed et al., 1999). Exposure to high concentrations of atmospheric Mn can lead to adverse health outcomes, notably respiratory and neurological effects. Exposure to concentrations of >1 mg of Mn/m^3 among miners and other industrial workers has been shown to persuade adverse respiratory, neurological, and reproductive effects (Iregren, 1999). The clinical syndrome of man-

* Corresponding author. Department of Environmental and Occupational Health, Faculty of Medicine, Université de Montréal, C.P. 6128 Succursale Centre-Ville, Montréal, Québec H3C 3J7, Canada. Fax: +514-343-2200.

E-mail address: joseph.zayed@umontreal.ca (J. Zayed).

0041-008X/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0041-008X(03)00238-2

ganese neurotoxicity (manganism) can be divided into an early phase characterized by obvious mood and behavior changes, and a later stage somewhat similar to Parkinson's disease that is characterized by dystonia and severe gait disorder (Pal et al., 1999). However, little is known about the potential health effects that may result from long-term low-level exposure of populations through ambient air. Certain subpopulations such as children and patients with chronic liver disease could be more susceptible to different levels of Mn contamination.

It is clear that the route of exposure can influence the distribution, metabolism, and potential for neurotoxicity of Mn-containing compounds (Roels et al., 1997). Inhalation exposure is more efficient than ingestion at transporting Mn to the brain. Pharmacokinetic factors that may contribute to the increased efficiency of brain Mn delivery following inhalation include greater Mn absorption from the lungs and slower clearance of absorbed Mn from the circulation (Andersen et al., 1999). Moreover, inhalation exposure to soluble forms of Mn results in higher brain Mn concentration compared with insoluble form of Mn (Dorman et al., 2001). One study has shown that after intratracheal instillation, a surrogate for inhalation exposure, Mn concentrations were higher in brain following the administration of the soluble salt $MnCl_2$, than following the administration of the insoluble oxide MnO_2. Striatal Mn concentrations increased by 205% and 48% following $MnCl_2$ and MnO_2 administration, respectively (Roels et al., 1997).

The main brain target for Mn toxicity is the basal ganglia (caudate nucleus, globus pallidus, and putamen), which is involved in motricity. Disturbances of the basal ganglia can lead to unintentional contraction of the skeletal muscles, such as tremor and muscular rigidity, as in Parkinson's disease. Few studies have been conducted to describe the distribution of brain Mn following inhalation of different Mn species, the main route by which Mn intoxication occurs in workers. It seems likely that the neurotoxicity of inhaled Mn may be related to an uptake of this metal into the brain via olfactory neurons. The olfactory bulb in rats plays a significant role in the uptake of inhaled Mn and subsequent delivery to the brain (Tjälve and Henriksson, 1999). However, the route of delivery of Mn to the brain is not clear in human. The primary objective of this study is to determine the effects of subchronic exposure to an Mn phosphate/sulfate mixture on Mn tissue concentrations and locomotor activity.

Material and method

Chemicals. Manganese phosphate/sulfate mixture, a fine crystalline powder, which includes $Mn_5(PO_4)_2(PO_3(OH))_2 4H_2O$ hureaulite mineral form, and manganese (II) sulfate monohydrate ($MnSO_4 \cdot H_2O$), were obtained from Alfa Aesar (Johnson Matthey Company) and combined 50/50 (wt/wt). The chemistry of the mixture was confirmed by scanning electron microscopy (SEM) and energy dispersive x-ray spectrometry (EDS). Mn in both compounds has the same oxidation state (II). Whereas manganese sulfate is relatively water soluble, manganese phosphate is insoluble.

Animals. A total of 120 six-week-old male Sprague-Dawley rats weighting 125–150 g were purchased from Charles River Laboratories (St. Constant, Quebec, Canada) and marked on the tail for identification purposes. Rats were acclimated for approximately 2 weeks in HEPA-filtered air. Animals were given NIH-07 chow ad libitum when they were not exposed. All experiments were undertaken with the consent of the animal ethics committee of the University of Montreal, and were conducted in accordance with the guidelines set out by the Canadian Council on Animal Care. Rats were individually housed in a polycarbonate cage with stainless steel wire lids under constant conditions of temperature and humidity, with 12:12-h day/night cycles. The subjects were randomly divided into four groups with 30 animals in each group for two different periods. Body weight and food and water consumption were measured weekly.

Inhalation exposure. A control group and three groups of 30 male Sprague-Dawley rats each were respectively exposed to 3000, 300, and 30 $\mu g/m^3$ manganese phosphate/sulfate mixture. Exposure was conducted in two inhalation chambers with a total volume of 1 m^3 each (Hazelton Systems Company, Inc., Kalamazoo, MI) over 13 consecutive weeks, 5 days per week, 6 h/day. The chamber received (HEPA) filtered air and was maintained at a constant temperature (22–25°C) and relative humidity (25–40%) throughout the study. Mn aerosol was generated by a Fluidized Bed Aerosol Generator (Model 3400, TSI Inc., St. Paul, MN) with a flow rate of 200–250 L/min. Stainless steel ASME pressure tanks were used to obtain fine particles. Concentrations were verified continuously by using a Dust Track (Model 8520) aerosol monitor. In both chambers, air samples were collected on a daily basis to monitor Mn concentrations, using a sampling system consisting of Gilian pumps (Gilian Corp., West Caldwell, NJ) with standard three-piece cassettes and 37-mm-diameter filters. The filters were Teflon (manufactured for SKC Inc., Gelman Sciences, Ann Arbor, MI) 0.45-μm pores. Pumps were used at a constant flow rate of 1.5 L/min. The flow rate was calibrated each day with a Gilibrator (Gilian Corp., West Caldwell, NJ). The particle size distribution of manganese phosphate/sulfate mixture was measured by using a six-stage Marple Personal Cascade impactor (Series 290). Rats were individually housed during the nonexposure period and were weighed weekly. Food and water were available ad libitum when the rats were not exposed.

Locomotor activity assessment. Four hours after the last Mn inhalation exposure, the rats were tested for motor activity. A computerized Auto-Track System (Columbus instru-

ments, OH, USA) was used to measure locomotor activity for a period of 36 consecutive hours. This system consists of a 15 × 15 infrared beam array with an interbeam distance of 2.4 cm along the *x* and *y* axes. Data were collected every 0.1 s and the motor activity categorized as distance traveled by rats and resting time. The system was installed in a quiet isolated room with 12-h light/12-h dark cycles.

Tissues concentrations. Following locomotor activity assessment, rats were anesthetized in a glass-walled container containing a pad soaked with metofane, an inhalation anesthetic. As soon as the animals were anesthetized, they were euthanized by exsanguination and the following tissues and organs weighed and analyzed: liver, lung, kidneys, testis, and one hemisphere of the brain (olfactory bulb, globus pallidus, caudate/putamen, frontal cortex, and cerebellum). Blood samples were taken from the abdominal aorta for chemical and biochemical analyses and determination of Mn concentrations.

Chemical analysis. Mn concentrations in blood, food, water, and tissues were measured by INNA (Kennedy, 1990) using a flue nuclear reactor and an EG&G Ortec (Model DSPec) digital gamma-ray spectrometer incorporating a high-resolution large-volume germanium detector. The potential interference from Fe was reduced by irradiating samples in a more thermalized neutron spectrum, verified, and corrected for blood.

Biochemical profile. Blood samples were collected from the abdominal aorta of anesthetized rats. Blood serum was obtained by centrifugation at 4°C for 15 min at 3000 *g*. The biochemical tests were performed by using standard techniques for glucose, urea nitrogen, serum creatinine, bilirubin, transaminases (AST and ALT), and alkaline phosphatases (ALP).

Statistical analysis. The Dunnett T3-test was used to compare among all pair treatment groups for mean concentrations of Mn in blood, kidney, liver, lung, and testis. A similar analysis was conducted for Mn concentrations in different regions of the brain (cerebellum, frontal cortex, globus pallidus, caudate putamen, and olfactory bulb), motricity (distance travelled and resting time), the 10 serum biochemical parameters, and body weight in each of the four treatment groups. Homogeneity of variances for the treatment groups was assessed by using Levene's test. All analyses were performed by using SPSS statistical software (version 11.0). The level of statistical significance was set at $P < 0.05$ for the set of six pairwise comparisons performed among the treatment groups using Dunnett's test. Data are presented as group means ± standard deviation (SD).

Table 1
Percentage of manganese phosphate/sulfate mixture distribution in the inhalation chamber as a function of particle size

Particle size (μm)	Percentage (%)
6.0–9.8	6.6 ± 2.7
3.5–6.0	6.2 ± 0.9
1.55–3.5	7.1 ± 0.1
0.93–1.55	12.7 ± 2.8
0.52–0.93	40.8 ± 5.1
< 0.52	17.3 ± 1.8
B-F[a]	9.3 ± 6.8

[a] B-F, backup filter.

Results

Mn in the inhalation chamber

The average Mn concentrations obtained in this study were 34.8 ± 9.2, 290.8 ± 76.8, and 2841 ± 529 $\mu g/m^3$ for target concentrations of 30, 300, and 3000 $\mu g/m^3$ of Mn phosphate/sulfate mixture, respectively. Based on the cascade impactor, 80% of the Mn phosphate/sulfate mixture particles in the inhalation chamber were smaller than 1.55 μm in aerodynamic diameter (Table 1). Overall mean daily chamber temperatures ranged at 22–25°C, and relative humidity ranged at 25–40%. The results indicated that 33% of particles were found to be agglomerated, while 44% and 23% of free particles were Mn phosphate and Mn sulfate, respectively.

Tissue concentrations

Tissue Mn concentrations are presented in Table 2. Elevated lung Mn concentrations were observed following exposure to 30, 300, and 3000 $\mu g/m^3$ Mn. Mn accumulation in the lung increased in a dose-dependent manner. Increased testis Mn concentrations were observed following inhalation exposure at 300 and 3000 $\mu g/m^3$. Mn concentrations were elevated in blood and kidney only in rats exposed to the highest level of Mn (3000 $\mu g/m^3$). Liver Mn concentrations were unaffected at all exposure levels.

Brain tissue manganese concentrations are shown in Table 3. Mn concentrations in olfactory bulb increased in a dose-dependent manner. In addition, Mn concentrations in the caudate putamen and globus pallidus were significantly increased following exposure to 300 and 3000 $\mu g/m^3$. Mn concentration in the cerebellum and frontal cortex were elevated only in rats exposed to the highest level (3000 $\mu g/m^3$). Mn concentrations were in the range of 0.56–0.83 μg/g in cerebellum, 0.61–1.56 μg/g in the globus pallidus, 0.62–1.19 μg/g in the caudate putamen, 0.58–0.98 μg/g in the frontal cortex, and 0.46–2.32 μg/g in the olfactory bulb. The highest average Mn concentrations in exposure animals

Table 2
Mean manganese concentrations (μg/g ± SD) in different tissues and blood following subchronic (90 days) inhalation exposure to a manganese phosphate/sulfate mixture

Tissue	Exposure concentration			
	Control (n = 26)	30 μg/m^3 (n = 25)	300 μg/m^3 (n = 29)	3000 μg/m^3 (n = 29)
Blood	0.005 ± 0.007	0.006 ± 0.004	0.008 ± 0.002	0.018 ± 0.006[a,b,c]
Kidney	1.01 ± 0.13	1.03 ± 0.20	1.06 ± 0.12	1.37 ± 0.15[a,b,c]
Liver	2.22 ± 0.35	2.23 ± 0.35	2.27 ± 0.38	2.32 ± 0.42
Lung	0.17 ± 0.03	0.42 ± 0.08[a]	2.15 ± 0.5[a,b]	5.97 ± 1.72[a,b,c]
Testis	0.30 ± 0.06	0.34 ± 0.02	0.36 ± 0.03[a]	0.42 ± 0.02[a,b,c]

[a] Significantly different from the control group ($P < 0.05$).
[b] Significantly different from the 30 μg/m^3 group ($P < 0.05$).
[c] Significantly different from the 300 μg/m^3 group ($P < 0.05$).

were observed in the olfactory bulb, and the lowest in the cerebellum.

Body weight

There was a significant reduction ($P < 0.05$) in body weight after 13 weeks at the two highest levels of exposure (Fig. 1). No significant difference in food intake was observed among the different exposed groups. The average Mn concentrations in food and water were 100 μg/g and 0.00002 μg/L, respectively.

Locomotor activity assessment

Locomotor activity was assessed in term of the resting time (RT) and distance traveled (DT). After 36 h, DT was founded to be significantly increased after exposure to 30 and 3000 μg/m^3 Mn, although there was no difference at 300 μg/m^3 Mn. No significant difference in RT was observed among the exposed groups (Table 4).

Blood biochemical analysis

Table 5 summarizes the biochemical test results. Glucose, sodium, chlorate, urea, and ALP levels were significantly different in the animals exposed to highest level of the Mn mixture.

Discussion

Different MMT combustion products are produced depending on fuel combustion and engine and catalytic converter thermodynamics. It is now well accepted that Mn is emitted from the tailpipe primarily as a mixture of Mn phosphate and Mn sulfate particles, with size ranging between 0.2 and 10 μm in aerodynamic diameters (Zayed et al., 1999). In the present study, 100% of the particles were <10 μm, while 87% were <3.5 μm.

In this study, equal weights of Mn phosphate and Mn sulfate were introduced into the inhalation chambers; however, subsequent analysis of air samples by electron microscope showed that 33% of particles were agglomerated, while 44% and 23% of free particles were Mn phosphate and Mn sulfate, respectively. This apparent discrepancy between number of particles and mass can be explained if one considers the different densities of the two compounds; 1.32 g/cm^3 for Mn sulfate and 0.76 g/cm^3 for Mn phosphate (Beaupré et al., 2003). The particle size distributions of the two compounds in the inhalation chamber were found to be approximately the same. Thus, the Mn phosphate particles have lower mass, and more of them are needed to give the same total mass as Mn sulfate. That the ratio of the number of particles of the two compounds in this study was not 50-50 should be of no importance to the effects observed on the rats. Any effects should depend

Table 3
Mean manganese concentrations (μg/g ± SD) in brain regions after subchronic (90 days) inhalation exposure to manganese phosphate/sulfate mixture

Brain region	Exposure concentration			
	Control (n = 26)	30 μg/m^3 (n = 25)	300 μg/m^3 (n = 29)	3000 μg/m^3 (n = 29)
Cerebellum	0.56 ± 0.15	0.57 ± 0.04	0.60 ± 0.04	0.83 ± 0.07[a,b,c]
Frontal cortex	0.58 ± 0.20	0.59 ± 0.08	0.64 ± 0.14	0.98 ± 0.32[a,b,c]
Globus pallidus	0.61 ± 0.15	0.63 ± 0.04	0.81 ± 0.08[a,b]	1.56 ± 0.40[a,b,c]
Caudate putamen	0.62 ± 0.2	0.57 ± 0.03	0.74 ± 0.11[b]	1.19 ± 0.11[a,b,c]
Olfactory bulb	0.46 ± 0.07	0.61 ± 0.05[a]	1.32 ± 0.16[a,b]	2.32 ± 0.25[a,b,c]

[a] Significantly different from the control group ($P < 0.05$).
[b] Significantly different from the 30 μg/m^3 group ($P < 0.05$).
[c] Significantly different from the 300 μg/m^3 group ($P < 0.05$).

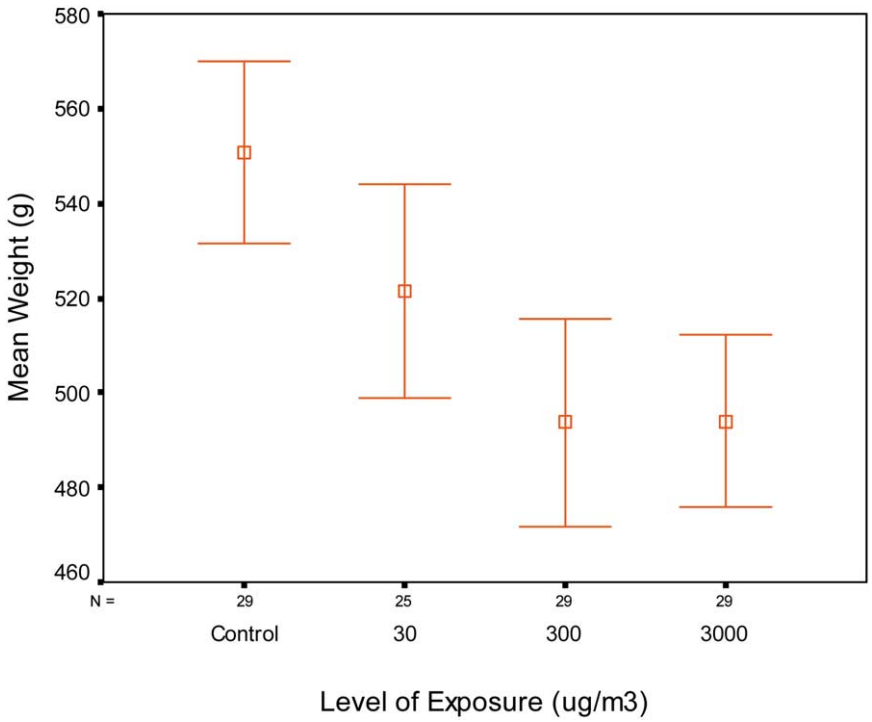

Fig. 1. Mean body weight of rats following subchronic (90 days) inhalation exposure to a manganese phosphate/sulfate mixture.

on the mass of compound absorbed, not on the number of particles inhaled.

Routes of exposure are known to affect the pharmacokinetics of Mn (Anderson et al., 1999), and inhalation is more efficient than ingestion at delivering Mn to the brain region. In rats, increases in brain Mn delivery were observed with inhalation exposure following Mn absorption from the pulmonary tract and direct transport of Mn to the central nervous system along the olfactory nerve (Brenneman et al., 2000). Even if inhalation is not the primary route of exposure to Mn, it can play an important role in central nervous system toxicity. Dorman et al. (2002) have studied whether dietary Mn intake influences the pharmacokinetics of inhaled Mn. The results of their study show that animals maintained on either a Mn-deficient or a high Mn diet do not appear to exhibit elevated brain Mn concentrations following inhalation exposure to a high level of Mn. Also, there is no evidence of adverse effects following dermal exposure to inorganic Mn (ATSDR, 2000)

Rats exposed to Mn in mixture form in our study had lower Mn concentrations in lung compared with levels in rats exposed to either Mn sulfate following inhalation at 3000 $\mu g/m^3$ (Dorman et al., 2001) or Mn phosphate at the same concentration (Vitarella et al., 2000; Normandin et al., 2002). These results suggest that the mixture is more rapidly cleared from the lung than other Mn compounds. The Mn phosphate/sulfate mixture is more water- soluble than Mn phosphate due to the water solubility of Mn sulfate. Accumulation of Mn phosphate/sulfate mixture in the lung is dose dependent, similar to previous reports by Vitarela et al. (2000) and Normandin et al. (2002) after inhalation exposure to Mn phosphate at 3000 $\mu g/m^3$. This characteristic is important since the rate of clearance from the lung is known to influence metal delivery to the brain and other organs. In a study by Rhoads and Sanders (1985), a number of metal compounds that were more quickly cleared from the lungs were retained for longer periods at other sites in the body, from which they could potentially exert their toxic effects.

Table 4
Mean distance traveled and resting time over 36-h period after subchronic (90 days) inhalation exposure to manganese phosphate/sulfate mixture

Locomotor activity	Exposure concentration			
	Control ($n = 26$)	30 $\mu g/m^3$ ($n = 25$)	300 $\mu g/m^3$ ($n = 29$)	3000 $\mu g/m^3$ ($n = 29$)
Distance traveled (m)	875 ± 180	1206 ± 94[a]	928 ± 127	1398 ± 104[a,b]
Resting time (h)	31.9 ± 9	31.7 ± 5	31.9 ± 8	32.4 ± 6

[a] Significantly different from the control group ($P < 0.05$).
[b] Significantly different from the 300 $\mu g/m^3$ group ($P < 0.05$).

Table 5
Biochemical parameters in serum following subchronic (90 days) inhalation exposure to a manganese phosphate/sulfate mixture

Biochemical parameter	Exposure concentration			
	Control ($n = 26$)	30 $\mu g/m^3$ ($n = 25$)	300 $\mu g/m^3$ ($n = 29$)	3000 $\mu g/m^3$ ($n = 29$)
Glucose (mmol/L)	11.6 ± 2.3	9.8 ± 0.97[a]	10.5 ± 1.7	9.8 ± 1.7[a]
Creatinine (μmol/L)	54.2 ± 4.5	54.3 ± 2.1	52.1 ± 3	53 ± 2.9
Sodium (mmol/L)	139.3 ± 1.8	144.8 ± 1.6[a]	143.1 ± 3.1[a]	142.5 ± 2.2[a]
Potassium (mmol/L)	5.1 ± 0.4	4.9 ± 1.1	5.3 ± 1.1	5.8 ± 0.9
Chlorate (mmol/L)	100.9 ± 2.1	104.6 ± 1.7[a]	104.1 ± 1.8[a]	103.2 ± 1.4[a]
Bilirubin (μmol/L)	3.5 ± 0.9	4.0 ± 0.6	3.9 ± 0.7	3.7 ± 1.2
Urea (mmol/L)	5.6 ± 0.7	6.3 ± 0.6[a]	5.7 ± 0.6[b]	6.4 ± 0.6[a,c]
AST (U/L)[d]	77.8 ± 21.2	88.4 ± 18.5	70.5 ± 17.3	72.9 ± 17.2
ALT (U/L)[e]	43.3 ± 8.2	53.8 ± 15.5	48.6 ± 7.3	51.8 ± 15.5
Alkaline Phosphatase (U/L)	113.6 ± 34.7	140.9 ± 42	180.1 ± 49.4[a]	183.8 ± 51.3[a]

[a] Significantly different from the control group ($P < 0.05$).
[b] Significantly different from the 30 $\mu g/m^3$ group ($P < 0.05$).
[c] Significantly different from the 300 $\mu g/m^3$ group ($P < 0.05$).
[d] Asparate aminotransferase.
[e] Alanine aminotransferase.

A significant increase in blood Mn concentration for rats exposed to 3000 $\mu g/m^3$ was observed. The elevated blood Mn concentration following exposure to Mn phosphate/sulfate mixture could be because the mixture is rapidly cleared from the lungs and after enters to the blood stream (Dorman et al., 2001). In comparison with Mn phosphate, testes also displayed a different response after exposure to Mn mixture. Groups exposed to 300 and 3000 $\mu g/m^3$ had significantly higher testes Mn concentrations compared to the control group. This finding is in agreement with the study Dorman et al. (2001) in which animals were exposed to high levels of Mn sulfate. Exposure of laboratory animals to high levels of Mn has been associated with a variety of reproductive effects, including evident histopathologic and biochemical changes in the somniferous tubule (Chandra, 1971; Imam and Chandra, 1975; Murthy et al., 1980).

Studies conducted by Dorman and colleagues (2001) have confirmed that delivery of inhaled Mn to the brain is influenced by particle solubility. Moreover, animals exposed to a high level of the soluble sulfate form had significantly higher brain Mn concentrations compared with levels achieved following exposure to the insoluble tetroxide or phosphate forms. Our data indicate a significant difference in brain Mn concentration in cerebellum, frontal cortex, globus pallidus, caudate putamen, and olfactory bulb in rats exposed to the highest level (3000 $\mu g/m^3$) of Mn compared to the reference group. Mn concentration in the olfactory bulb was dose dependent. Also, Mn concentrations in the globus pallidus and caudate putamen were higher than those reported by Vitarella et al. (2000) following exposure to Mn phosphate (Normandin et al., 2002) and metallic Mn (St-Pierre et al., 2001).

Absorbed Mn is transported to other organs via the iron-binding protein transferrin, α2-macroglobuline, and albumin. Mn readily crosses the blood-brain barrier and is largely distributed to the central nervous system (Aschner et al., 1999). Following chronic exposure to a high level of Mn in humans and other primates, the metal preferentially accumulates in the thalamic nuclei, substantia nigra, pallidum, and other brain regions that also accumulate iron (Barbeau et al., 1976; Hill and Switzer, 1984). In normal plasma concentrations, Mn enters the brain mainly across the cerebral capillaries. Since Mn plasma concentration increases, entry of Mn across the choroids plexus becomes more important (Murphy et al., 1991). Intranasally administered Mn may circumvent the blood-brain barrier and then pass directly into the central nervous system via olfactory pathways (Tjälve et al., 1996), increasing its bioavailability to the brain (Frumkin and Solomon, 1997). A fraction of inhaled Mn is likely to reach cerebral target sites before hepatic clearance (Roels et al., 1997).

Our data show that Mn concentrations in the olfactory bulb and other brain regions were elevated. This is in agreement with Dorman et al. (2001), who showed that an inhaled metal could be delivered directly to the brain via the olfactory nerve. These data indicate that following inhalation of Mn, Mn concentration achieved in the olfactory bulb is significantly higher than that observed in either the striatum or the cerebellum, lending additional support to the direct olfactory transport theory. In the rat, the olfactory bulb comprises a relatively large section of the central nervous system, and the nasal olfactory mucosa cover roughly 50% of the total nasal epithelium (Gross et al., 1982). In humans these structures are proportionately smaller than in rats. It has been suggested that if this route of brain delivery is operative in humans, it might be less important in humans than in rats (Dorman et al., 2001). Nevertheless, our results show that Mn concentration in the olfactory bulb is at least 50% higher than all other brain tissues when rats are exposed to 3000 $\mu g/m^3$. Moreover, after such exposure, tissue concentration for the olfactory

bulb increased by 504%, in comparison to 255%, 191%, 169%, and 148% for the globus pallidus, the caudate putamen, the frontal cortex, and the cerebellum, respectively.

Analysis of the biochemical parameters revealed some significant differences in sodium, glucose, chlorate, and urea in the blood of rats exposed to the highest level of Mn mixture (3000 $\mu g/m^3$), which can lead to levels that can result in kidney failure (Murry et al., 1995). In our study, rats exposed to 300 and 3000 $\mu g/m^3$ also had higher levels of ALP compared to the control group, which is suggestive of liver dysfunction (Murry et al., 1995). However, modification of AST, ALT, and total bilirubin, which are indicative of liver malfunction, was not apparent in this study.

Rats exposed to 300 and 3000 $\mu g/m^3$ Mn phosphate/sulfate mixture exhibited a significant decrease in mean body weight compared to the control group. This finding is quite different from other results reported by our research group for metallic Mn (St-Pierre et al., 2001) and Mn phosphate (Normandin et al., 2002). However, other studies have reported decreasing body weight following Mn exposure (Brenneman et al., 1999). There were no significant differences among groups in total mean food consumption.

Our data indicate a significant effect of Mn on locomotor activity. We observed that exposure led to a significant increase in distance traveled for the groups exposed to 30 and 3000 $\mu g/m^3$ compared to the control group. However, there were no significant differences in resting time. The increase in locomotor activity appeared to be associated with increased Mn concentration in the brain. Similarly, in a study where rats were exposed to 0.1 or 5.0 mg/ml of $MnCl_2$ in drinking water for 8 months, a significant increase in spontaneous motor activity was noted in the first month. Nevertheless, during months 2 to 5 rats showed normal activity, with a significant decrease in motricity in months 6 to 8 (Bonilla, 1984). Rats exposed to Mn orally were significantly more active than controls in the empty open field, and also did not show habituation (Calabresi et al., 2001).

The mechanisms by which Mn exerts its effect on locomotor activity appers to be related to the accumulation of Mn in the brain. Brain Mn deposition is region selective, being predominant in the basal ganglia, which is involved in the control of movement and some cognitive functions. Other than in the olfactory bulb, brain Mn deposition is mainly in the caudate putamen and globus pallidus, which corresponds to the onset of neurological outcomes. The differences in spontaneous motor activity may be explained by the alterations in dopamine transmission associated with Mn toxicity. Acute exposure to Mn is associated with an increase in dopamine neurotransmission, which is also manifested as hyperactivity. Nevertheless, long-term exposure results in a loss of dopamine in the brain, and the concomitant neuronal cell damage could be expressed as an increase in motor activity (Nachtman et al., 1986; Bonilla, 1984). Calabresi et al. (2001) reported that Mn-treated rats exhibited a complex behaviour syndrome in the absence of significant striatal neuronal loss and gliosis, and were significantly more active than controls.

Clinical studies have revealed that psychiatric symptoms might emerge in the early phase of manganism in the absence of motor effects. However, the latter disorders are more commonly reported in the recognized stage. Motor effects have been attributed to basal ganglia dysfunction, whereas the involvement of other motor systems such as cerebellum or cortex is considered unlikely (Calne et al., 1994). The mechanisms underlying early emotional disorders are more difficult to investigate and remain unknown. It is clear that neuronal loss in manganism is most significant in the globus pallidus, which may lead to impaired movement (Olanow et al., 1996). Although the present study has demonstrated an adverse effect of Mn on locomotor activity, the mechanism by which this occurs requires further investigation. In a subsequent article, we will report on the results of ongoing research on Mn damage on neuronal cells.

In conclusion, the present study demonstrated bioaccumulation of Mn in different brain regions, particularly in the basal ganglia, and neurobehavioral changes, similar to those described in cases of human manganism. The American Conference of Governmental Industrial Hygienists is planning to decrease the respirable Mn thresold limit values (TLV) from 200 to 30 $\mu g/m^3$ (ACGIH, 2002), a level at which a significant increase of Mn accumulation in the olfactory bulb and locomotor activity was observed in this study. Structural alteration of brain tissue is known to be associated with increased brain Mn concentrations following inhalation exposure to Mn in animals.

Nevertheless, in experimental animal models, specially rodents, very high concentrations of Mn are necessary to produce neurological effects, suggesting that rodents are less susceptible than humans to Mn neurotoxicity. Extensive use of MMT in gasoline, leading to Mn emissions in the atmosphere, may represent a significant source of exposure to inorganic Mn in urban areas with high traffic density. Since very few studies have been carried out to assess the neurotoxic effects associated with inhalation exposure to Mn, particularly with Mn species related to MMT combustion, additional research is needed to clarify the toxicity of Mn by respiration.

Acknowledgments

This research was supported by the Toxic Substances Research Initiative (TSRI), a research program managed jointly by Health Canada and Environment Canada. Additional support was provided by Dr. D. Krewski, who is the NSERC/SSHRC/McLaughlin Chair in Population Health Risk Assessment at the University of Ottawa, and G. Carrier, Chair of Risk Assessment Toxicology at the University of Montreal.

References

American Conference of Government and Industrial Hygienist (ACGIH), 2002. Threshold limit values for chemical substances and physical agents and biological exposure indices, Cincinnati, OH.

Andersen, M.E., Gearhart, J.M., Clewell, H.J., 1999. Pharmacokinetic data needs to support risk assessments for inhales and ingested manganese. Neurotoxicology 20, 161–171.

Aschner, M., Vrana, K., Zheng, W., 1999. Manganese uptake and distribution in the central nervous system. Neurotoxicology 20, 173–180.

ATSDR, 2000. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Barbeau, A., Inoué, N., Cloutier, T., 1976. Role of manganese in dystonia. Adv. Neurol. 14, 339–352.

Beaupré, L.A., Plamondon, P., L'Espérance, G., Zayed, J., 2003. Physical and chemical characterization of Mn phosphate/sulfate mixture used in an inhalation toxicology study. Environ. Sci. Technol. (Submitted).

Bonilla, E., 1984. Chronic manganese intake induces changes in the motor activity of rats. Exp. Neurol. 84, 696–700.

Brenneman, K.A., Cattly, R., Ali, S., Dorman, D., 1999. Manganese-induced developmental neurotoxicity in the CD rat: is oxidative damage a mechanism of action. Neurotoxicology 20, 477–488.

Brenneman, K.A., Wong, B.A., Buccellato, M.A., Costa, E.R., Gross, E.A., Dorman, D.C., 2000. Direct olfactory transport of inhaled manganese ($MnCl_2$) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion modle. Toxicol. Appl. Pharmacol. 169, 238–248.

Calabresi, P., Ammassari, M., Gubellini, P., Sancesario, G., Morello, M., Centonze, D., Marfia, G., Saulle, E., Passino, E., Picconi, B., Bernardi, G., 2001. A synaptic mechanism underlying the behavioral abnormalities induced by manganese intoxication. Neurobiol. Dis. 8, 419–432.

Calne, D.B., Chu, N.S., Huang, C.C., Lu, C.S., Olanow, W., 1994. Manganism and idiopathic parkinsonism: similarities and differencies. Neurology 44, 1483–1486.

Chandra, S.V., 1971. Cellular changes induced by manganese in the rat testis-preliminary results. Acta Phamacol. Toxicol. 29, 75–80.

Dorman, D.C., Struve, M.F., James, R.A., Marshall, M.W., Parkinson, C.U., Wong, B.A., 2001. Influence of particle solubility on the delivery of inhaled manganese to the rat brain: manganese sulfate and manganese tetroxide pharmacokinetics following repeated (14 days) exposure. Toxicol. Appl. Pharmacol. 170, 79–87.

Dorman, D.C., Struve, M.F., Wong, B.A., 2002. Brain manganese concentrations in rats following tetroxide inhalation are unaffected by dietary manganese intake. Neurotoxicology 23, 185–195.

Frumkin, H., Solomon, G., 1997. Manganese in U.S. gasoline supply. Am. J. Ind. Med. 31, 107–115.

Gross, E.A., Swenberg, J.A., Fields, S., Popp, J.A., 1982. Comparative morphometry of the nasal cavity in rats and mice. J. Anat. 135, 83–88.

Hill, J.M., Switzer, R.C., 1984. The regional distribution and cellular localization of iron in the rat brain. Neuroscience 11, 595–603.

Imam, Z., Chandra, S.V., 1975. Histochemical alteration in rabbit testis produced by manganese chloride. Toxicol. Appl. Pharmacol. 32, 534–544.

Iregren, A., 1999. Manganese neurotoxicity in industrial exposures: proof of effects, critical exposure level, and sensitive tests. Neurotoxicity 20, 315–323.

Joselow, M.M., Tobias, E., Koehler, R., Colemang, S., Bogden, J., Gause, D., 1978. Manganese pollution in the city environment and its relationship to traffic density. Am. J. Public Health 68, 557–560.

Kennedy, G.G., 1990. Trace element determination in polymers by neutron activation. In: Sacher, E., Pireaux, J.-J., Kowalczyk, S.P. (Eds.), ACS Symposium Series. American Chemical Society, Montreal, Quebec, Canada, pp, 128–134.

Murry, R.K., Granner, D.K., Mayes, P.A., Rodwell, V.W., 1995. Précis de Biochimi de Harper, Presses de l'Université Laval, Québec, 8th Ed. 79, 606–886.

Murphy, V.A., Wadhwani, K.C., Smith, Q.R., Rapoport, S.I., 1991. Saturable transport of manganese (II) across the rat blood-brain barrier. J. Neurochem. 57, 948–954.

Murthy, R.C., Srivastava, R.S., Gupta, S.K., Chandra, S.V., 1980. Manganese induced testicular changes in monkeys. Exp. Pathol. 18, 240–244.

Nachtman, J.P., Tubben, R.E., Commissaris, R.L., 1986. Behavioral effects of chronic manganese administration in rats: locomotor activity studies. Neurobehav. Toxicol. Teratol. 8, 711–715.

Normandin, L., Carrier, G., Gardiner, P.F., Kennedy, G.G., Hazell, A.S., Mergler, D., Butterworth, R.F., Philippe, S., Zayed, J., 2002. Assessment of bioaccumulation, neurohistopathology and neurobehavioral following subchronic (90 days) inhalation in rats exposed to manganese phosphate. Toxicol. Appl. Pharmacol. 183, 135–145.

Olanow, C.W., Good, P.F., Shinotoh, H., Hewitt, K.A., Vingerboets, F., Snow, B.J., 1996. Manganse intoxication in the rhesus monkey; a clinical, imaging, pathologic, and biochemical study. Neurology 46, 492–508.

Pal, P., Samii, A., Calne, D.B., 1999. Manganese neurotoxicity: a review of clinical features, imaging and pathology. Neurotoxicology 20, 227–238.

Rhoads, K., Sanders, C.L., 1985. Lung clearance, translocation and acute toxicity of arsenic, beryllium, cadmium, cobalt, lead, selenium, vanadium and ytterbium oxides following deposition in rat lung. Environ. Res. 36, 359–378.

Roels, H., Meiers, G., Delos, M., Ortega, I., Lauwerys, R., Buchet, J.P., Lison, D., 1997. Influence of the route of administration and the chemical form ($MnCl_2$, MnO_2) on the absorption and cerebral distribution of manganese in rats. Arch. Toxicol. 71, 223–230.

St-Pierre, A., Normandin, L., Carrier, G., Kennedy, G.G., Butterworth, R., Zayed, J., 2001. Bioaccumulation and locomotor effect of manganese dust in rats. Inhal. Toxicol. 13, 623–632.

Tjälve, H., Henriksson, J., 1999. Uptake of metals in the brain via olfactory pathways. Neurotoxicology 20, 181–195.

Tjälve, H., Henriksson, J., Tallkvist, J., Larsson, B.S., Lindquist, N.G., 1996. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. Pharmacol. Toxicol. 79, 347–356.

Vitarella, D., Wong, B.A., Moss, O.R., Dorman, D.C., 2000. Pharmacokinetics of inhaled manganese phosphate in male Sprague-Dawley rats following subacute (14-days) exposure. Toxicol. Appl. Pharmacol. 163, 279–285.

Zayed, J., Hong, B., L'Espérance, G., 1999. Characterization of manganese-containing particles collected from the exhaust emissions of automobiles running with MMT additive. Environ. Sci. Technol. 33, 3341–3346.