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Assessment of Bioaccumulation, Histopathology Damage and Neuro-behavioral Status in Portacaval Anastomosis Rats Exposed to Manganese Phosphate Dust: A Pilot Study

par
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Ce mémoire intitulé :
"Assessment of Bioaccumulation, Histopathology Damage and Neuro-behavioral Status in Portacaval Anastomosis Rats Exposed to Manganese Phosphate Dust: A Pilot Study"

Présenté par:
Fariba Salehi

A été évalué par un jury composé des personnes suivantes:

Dr. Robert Tardif	président-rapporteur
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Dr. Ginette Truchon	membre du jury

Mémoire accepté le: _____

Abstract

The use of the additive methylcyclopentadienyl manganese tricarbonyl in unleaded gasoline has resulted in increased attention to the potential toxic effects of manganese (Mn). Hypothetically, people with chronic liver disease may be more sensitive to the adverse neurotoxic effects of Mn. In this work, bioaccumulation of Mn, as well as histopathology and neurobehavioral damage in end-to-side portacaval anastomosis (PCA) rats exposed to Mn phosphate via inhalation was investigated.

The week before the PCA operation, four weeks after the PCA operation and at the end of exposure, the rats were subjected to a locomotor evaluation (day-night activities) using a computerized autotrack system. Then a group of six PCA rats (E) was exposed to 3050 $\mu g\ m^{-3}$ (Mn phosphate) for 8 hr/day, 5 days/week for 4 consecutive weeks and compared to a control group (C). Another group of seven PCA rats were exposed to 0.03 $\mu g\ m^{-3}$. After exposure rats were then euthanaized and Mn content in tissues and organs was determined by neutron activation analysis.

The manganese concentrations in blood (0.05 μg/g vs 0.02 μg/g), lung (1.32 μg/g vs 0.24 μg/g), cerebellum (0.85 μg/g vs 0.64 μg/g), frontal cortex (0.87 μg/g vs 0.61 μg/g) and globus pallidus (3.56 μg/g vs 1.33 μg/g) are significantly higher in the exposed group compared to the control group ($p < 0.05$). No difference was observed in liver, kidney, testes and caudate putamen between the two groups. Neuronal cell loss was assessed by neuronal cell counts. The loss of cells in globus pallidus and caudate putamen in E was significant ($p < 0.01$) as

well as in frontal cortex was significantly higher ($p < 0.05$). Assessment of the locomotor activities did not reveal any significant difference, although E seems to show higher values than C. This study constitutes a first step towards our understanding of the potential adverse effects of Mn in sensitive populations.

Key words: Manganese phosphate, inhalation, Mn bioaccumulation in different organs and tissues, histopatolgical damage of brain, neurological status analysis, portocaval anastomosis rats, and Sprague-Dawley rats.

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Abbreviations

INAA	Instrumental Neutron Activation Analysis
MMT	Methylcyclopentadienyl Manganese Tricarbonyl
Mn	Manganese
RfC	Reference Concentration
U.S.EPA	U.S. Environmental Protection Agency

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CHAPTER I -Introduction

1.1 Methylcyclopentadienyl manganese tricarbonyl

Methylcyclopentadienyl manganese tricarbonyl (MMT, $C_9H_7MnO_3$) is a fuel additive developed in the 1950 to increase the octane level of gasoline and therefore to improve the antiknock properties of the fuel (Davis, 1998). Exposure to MMT is expected to be primarily through inhalation or oral pathways, although occupational exposure for gasoline attendants or mechanics will be more significant via dermal absorption. From the time of its introduction in Canada in 1976, the use of MMT increased substantially until it completely replaced tetraethyl lead in gasoline in 1990 (Hurley *et al.*, 1992). Indeed, Canada is the only country where MMT is used almost exclusively.

In the United Stated, EPA denied Ethyl waiver for the use of MMT until October 1995, when a court decision allowed the company to offer it for sale to refiners for use in unleaded gasoline (Wallace and Slonecker 1997). In 1997 the Canadian government adopted a law (C-29) that banned both the inter provincial trade and the importation for commercial purposes of manganese-based substances, including MMT. MMT is now approved for use in Argentina, Australia, Bulgaria, the United States, France, Russia and conditionally in New Zealand. Nevertheless, these countries are not yet using MMT and they are waiting for strong evidence of the absence of effects on human health. The concentration of MMT approved by the EPA is 31.25 mg of Mn per gallon of gasoline. Usually, 72 mg of MMT, containing 18 mg of Mn, could be added to each liter of gasoline. But the Canadian mean of Mn is approximately 10 mg L^{-1}. However, a recent study shows that Mn and MMT concentrations found in the MMT-added gasoline are respectively 6.5 mg L^{-1} and 25.8 mg L^{-1} (Zayed *et al.*, 1998).

The amount of Mn emitted from the tailpipe of a vehicle varies between 7% and 45% of the Mn consumed, depending on the driving cycle and the type of vehicle. The fraction not emitted to the atmosphere appears to accumulate in the engine (Ardeleanu *et al.,* 1999). Many studies have established relations between traffic density and Mn concentrations in the biotic and abiotic components of ecosystem (Loranger *et al.,* 1996). Nevertheless, exposure of the general population to Mn emanating from MMT seems to be negligible compared to the contribution of the Mn from other industrial and natural sources (Loranger and Zayed, 1997). However, experience with lead in gasoline demonstrated that significant widespread exposure of the general population may occur by adding relatively small amounts of a substance to gasoline (Davis *et al.,* 1996).

1.1.1 Combustion products of MMT

MMT is extremely unstable in light and degrades very quickly in air. Therefore, exposures via gasoline exhausts are likely to be more to the oxidative products of MMT. It is now well accepted that the combustion products of MMT are essentially Mn phosphate, Mn sulfate and a mixture of Mn phosphate / Mn sulfate (Zayed *et al.,* 1999). In fact, it was shown that the frequency of Mn Oxide was only 2% as opposed to 8% for Mn phosphate, 16% for Mn sulfate, and 54% for mixture of Mn phosphate and Mn sulfate. The combustion products of MMT depend on fuel composition, engine and catalytic converter thermodynamics. Modern automobiles equipped with catalytic converters emit mainly the manganese phosphate form and smaller amounts of sulfates and oxides (Lynam *et al.,* 1999).

It was found that Mn is emitted from the tailpipe primarily as a mixture of Mn phosphate and Mn sulfate with particle sizes ranging between 0.2 and 10 μm (Zayed *et al.,* 1994). On average, more than 99 % of Mn particles are in the respirable fraction (< 5 μm) and 86 % were less than 1 μm (Ardeleanu *et al.,* 1999). Then Mn particles have a higher probability of reaching the alveolar region.

1.1.2 Physical and chemical properties of MMT and combustion products

MMT is an organic derivative of Mn produced by Ethyl Corporation and marketed as AK-33X (antiknock agent-33X) or HiTec 3000 (Jaques, 1987). MMT is a yellow, volatile liquid that has a very low vapor pressure at room temperature (0.05mmHg@20°C) and is completely miscible in gasoline and very slightly soluble in water. MMT decomposes rapidly to manganese oxide, CO and unidentified organic compounds, in air in the presence of sunlight with a half-life about 15 seconds (Garrison *et al.,* 1995), and with an herbal odor.

Table 1.1 lists the physical and chemical properties of MMT, manganese phosphate and manganese sulfate. Although the oxidation states of Mn range from 0 to +7, the most stable oxidation state is +2. Inorganic Mn compounds are not volatile, but they can exist in the air as aerosols or suspended particulate matter.

Table 1-1. Physical and chemical properties of Mn, MMT and combustion products

Property	Manganese	MMT[a]	Manganese phosphate[b]	Manganese sulfate[a]
Chemical formula	Mn	$C_9H_7MnO_3$	$Mn_5(PO_4)_2(PO_3(OH))_2$	$MnSO_4$
Molecular weight	54.94	218.09	656.59	151
Boiling point (°C)	1962	233	--	Decomposes at 850
Melting point (°C)	1244	1.5	--	700
Solubility	Decomposes	Soluble in water	Insoluble in water	Soluble

[a] source: adapted from the doctorate theses of Loranger (1994)

[b] source: adapted from paper report of Material Safety Data Sheet, Alfa Aesar

1.2 Sources and level of manganese in the air

The main sources of Mn are industrial emissions associated with ferroalloy production, iron and steel foundries, and power plant and coke oven combustion emissions (Lioy, 1983). Wind erosion of dusts and soils is another important source of atmospheric Mn. Mn is also present in MMT as octane-enhancing fuel additive used in unleaded automotive gasoline. One of the main sources of inorganic Mn contamination in the urban environment is the combustion of MMT, particularly in areas with high traffic density (Joselow *et al.*, 1978).

Although the possibility exists for increased atmospheric Mn concentration to occur with widespread MMT use, actual air Mn concentration from Canadian cities in which MMT has been widely used for over 10 years remain below the inhalation reference

concentration (0.05μg Mn/m^3) for respirable Mn set by the United States Environmental Protection Agency (U.S. EPA). The U.S. EPA calculated a reference concentration (RfC) based on changes in neuropsychological tests (finger tapping, hand steadiness) in an occupational study by Roels (1992).

Mn levels in air are highly variable. According to data summarized by EPA, 80% of U.S. sites assessed have Mn levels well below 0.1 $\mu g/m^3$, and only 4.7% have levels above 0.3 $\mu g/m^3$ (EPA, 1995). Higher ambient levels, up to 5.0 or even 10.0 $\mu g/m^3$ occur near industrial point sources, such as steel plants. Mn levels in certain workplace environments such as garages are affected by the use of MMT in gasoline, as recent Canadian data demonstrate. A recent study in Montreal shows that blue-collar workers are exposed to Mn levels of 0.04 $\mu g/m^3$, while garage mechanics are exposed to 0.43 $\mu g/m^3$ (Sierra *et al.,* 1995). The estimated lowest observed adverse effect level (LOAEL) was 150μg Mn/m^3, and this concentration was adjusted for nonoccupational lifetime exposure and an uncertainty factor (1000) to yield an RfC of 0.05μg Mn/m^3.

The average atmospheric Mn concentrations measured in a high traffic area in Montreal were 0.05 and 0.024 $\mu g/m^3$ for total and respirable fractions, respectively (Loranger and Zayed, 1997; Zayed *et al.,* 1999b). In specific environment, such as house near highway, atmospheric Mn concentration could be higher than RfC (Zayed *et al.,* 1999b). For comparison, the average levels of Mn in ambient air in urban and nonurban areas in the United States during a time when MMT was not in use (i.e., in 1982) were 0.033 and 0.005 $\mu g/m^3$, respectively (ATSDR, 1999).

1.3 Toxicokinetics of Manganese

Limited data are available on the retention and absorption of inhaled particles of Mn. In general, the extent of inhalation absorption is a function of particles size, since size determines the extent and location of particle deposition in the respiratory tract. Particles that are deposited in the lower airway are probably mainly absorbed, while particles deposited in the upper airways may be moved by mucociliary transport to the throat, where they are swallowed and entered the stomach. Thus Mn may be absorbed both from the lungs and in the gastrointestinal tract following inhalation of Mn dust (ATSDR, 1999). Since the solubility of inhaled Mn compounds is poor systemic absorption is restricted to a portion of Mn particles that reach the alveoli and are transferred to the stomach by mucociliary transport. Homeostatic mechanisms control the absorption of Mn from the gut and therefore, the amount of Mn absorbed depends on the amount ingested as well as the existing Mn concentrations.

Tjälve (1996) investigated the uptake of Mn in brain regions of weanling male Sprague-Dawley rats following intranasal administration of 4 μg/kg Mn. The whole body autoradiography of the rats at different time points revealed that the olfactory bulb contained the vast majority of measured Mn at 1, 3, and 7 days post-dosing (90, 69, and 47%, respectively) with values decreasing to a low of 16% at 12 weeks. Significant uptake of Mn by other brain regions was not observed until the third day, when the basal forebrain, cerebral cortex, hypothalamus, and striatum had 21, 2, 3, and 1% of the measured label, respectively. By contrast, liver and kidneys, each contained approximately 1% of measured Mn at 1, 3, and 7 days with values decreasing consistently to 12 weeks (Tjälve *et al.,* 1996).

Roels measured Mn levels in blood following intranasal administration of 1.22 mgMn/kg as either $MnCl_2$ or MnO_2. Mn levels in blood following $MnCl_2$ dosing reached a maximal of 68% increase, but an increase of only 41% following exposure to MnO_2. Further, following Mn levels were significantly increased over control levels in the cerebellum, striatum, and cortex, with the striatum containing the highest level of Mn (Roels *et al.,* 1997). These data indicate that Mn is more readily absorbed via inhalation in a more soluble chemical form.

Low-protein and iron-deficiency increase the absorption of Mn, whereas increased calcium and phosphorus levels decreases Mn absorption (Murphy *et al.,* 1991). There is some evidence to suggest that Mn absorption is age-dependent. Infants, especially premature infants, retain a higher proportion of Mn than adults (Dorner *et al.,* 1989).

Occupational exposure to high concentrations of Mn or decreased excretion of Mn due to hepatic failure influences its distribution in the central nervous system (Layrargues *et al.,* 1995). Individuals with impaired clearance of Mn resulting from liver dysfunction or portal systemic shunting show increase of Mn concentration in blood. The magnetic resonance imaging (MRI) was used to indicate the increase in Mn absorption (Hauser *et al.,* 1996).

The average 70-kg man carries a total body burden of about 12 to 20 mg of Mn. Mn is rapidly cleared from the plasma, with a half-life of 1.3 to 2.2 minutes (Cotzias *et al.,* 1968). Accumulation of Mn occurs in the brain when absorption exceeds from excretion.

Manganese-dependent proteins play an important role in the production of certain neurotransmitters. Eighty percent of the Mn^{+2} in the brain is associated with the manganoprotein glutamine synthetase, which synthesizes glutamine. The concentration of glutamine synthetase is particularly high in glial cells, which synthesize glutamine for subsequent conversion to glutamate and γ-aminobutyric acid (GABA) in neuronal cells (Wedler and Denman, 1985). Other manganese-dependent enzymes include pyruvate carboxylase and phosphophenol pyruvate carboxykinase. The acute extrapyramidal effects of Mn may involve the irreversible oxidation of dopamine to a cyclized ortho-quinone, resulting in a temporary decrease in brain levels of dopamine. The chronic effects of Mn poisoning have been attributed to the generation of free radicals and cell death via apoptosis (Segura-Aguilar and Lind, 1989).

The β-globulin, transmanganin, transports part of the Mn in the trivalent form to tissue stores. Additionally, some transport of the divalent forms of Mn bound to α-macroglobulin also occurs. The highest concentration of Mn appears in the liver followed by the pancreas and the pituitary gland. Little accumulation of Mn occurs on the body with a majority of an average body burden of 8 mg distributed between muscle and the liver (Sumino *et al.*, 1975). Mn content in brain tissues normally averages 1 to 2 mg/g dry weight, with greater concentration found in adults than in infants (Prohaska, 1987). The distribution of Mn in the brain is not influenced by route of intake (Newland *et al.*, 1989). Free Mn is normally distributed throughout the grey matter of the cerebral cortex and basal ganglia, particularly the caudate nucleus, palladium, and striatum, which have relatively high concentrations of neuromelanin (Olanow *et al.*, 1996).

Mn is excreted in the feces, urine, pancreatic fluid, hair, and breast milk. Excretion of inorganic Mn from the body is rapid and does not require active metabolism; inorganic Mn does not pass through the glucuronidation pathway. Most absorbed inorganic Mn rapidly appears in the bile and feces, a portion undergoes enterohepatic recirculation. Blood levels of Mn are increased in patients with impaired Mn clearance due to liver dysfunction (Hauser *et al.,* 1996). Roels has found differences in Mn concentrations in blood and brain regions depending on the oxidation state of metal (Roels, 1997). The biological half-life in blood ranges from about 12 days in healthy miners to approximately 40 days in healthy volunteers (Mahoney *et Small,* 1968).

Animal studies suggest that renal excretion is small (<0.1 % over 5 day). Following the administration of Mn in rats, some Mn crosses directly from the blood to the bile (Thompson *et al.,* 1982). Experiments in animals indicate that the elimination of Mn from the brain, in particular from the cerebellum, is much slower compared with the whole body (Newland *et al.,* 1989).

1.4 Toxicity and health effects of Mn

The two major routes of Mn exposure are ingestion and inhalation. Ingestion, in turn, poses potential exposure from both food and water. Exposure through the respiratory route has a special significance. While ingested Mn is rapidly cleared by the liver, inhaled Mn escapes this mechanism, and may therefore be more bioavailable to the nervous system and other target organs. The implication is that inhaled Mn may be more toxic than ingested Mn (Sandstrom *et al.,* 1986).

Studies in animals and humans indicate that inorganic Mn compounds have very low acute toxicity by any route of exposure. Acute inhalation exposure to high concentrations of Mn dust can cause an inflammatory response in the lung, which can lead to impaired lung function. However, this response is characteristic of nearly all inhalable particulate matter (EPA 1985) and is not dependent on the manganese content of the particle. Acute duration exposure studies in animals exposed to MMT via inhalation, or via a dermal pathway are lacking. The dermal pathway is very important, because MMT in gasoline that may be spilled on the skin could penetrate and become absorbed.

Intermediate-duration inhalation exposure of humans to Mn compounds can lead to central nervous system effects (Rodier 1955). Intermediate-duration inhalation studies in animals have yielded NOAELs or LOAELs values for biochemical and neurobehavioral effects, but the range of exposure levels associated with these effects is too wide to define a threshold. Although neurological effects were observed in animals, symptoms characteristic of Mn toxicity (e.g., ataxia, tremor, etc.) are not typically observed in rodent species. Also other rodent studies reported decreased motor activity (Komura and Sakamoto, 1991), increased activity, aggression (Chandra, 1983) and delayed reflexes (Ali *et al.,* 1983), these effects are not consistent and are observed over a wide dose range.

Studies in humans make clear that the main health effect following chronic inhalation exposure is nervous system toxicity (Mena *et al.,* 1967; Wennberg *et al.,* 1991) although adverse respiratory effects are also seen (Akbar-Khanzadeh, 1993). Exposure to high

concentrations of atmospheric Mn can lead to numerous health problems, especially in the respiratory and neurological systems. Chronic human exposure to high concentrations of Mn containing dust (>1mg Mn/m^3) has been shown to induce adverse neurological, respiratory and reproductive effects (Iregren, 1999). The clinical syndrome of Mn induced neurotoxicity can be divided into an early phase distinguished by marked mood and behavior changes, and a later stage resembling Parkinson disease that is characterized dystonia and severe gait disturbances (Pal *et al.,* 1999).

Certain subpopulations such as patients with chronic liver disease could be more vulnerable to the neurotoxic effects of Mn since they have a decrease in biliary excretion of Mn. Little is known on the potential health effects that may result from long-term continuous exposure of these patients to Mn compounds in ambient air at low concentrations. We hypothesise that a relatively high exposure to Mn may be tolerated by normal animals and humans but may lead to an overload and neurological problems in those with hepatic dysfunction.

1.4.1 Neurotoxicity

The primary target organs following chronic exposure to Mn are the lung and brain. The nervous system is the critical target organ of Mn. The striatum and associated brain structures are important target sites of Mn neurotoxicity in humans. Using brain magnetic resonance imaging (MRI) techniques, researchers have demonstrated that patients with manganism develop elevated Mn concentrations in their basal ganglia (Nagatomo *et al.,*

1999). Severely affected patients also develop progressive, irreversible loss of dopaminergic neurons in globus pallidus and nigrostriatal pathway (Calne *et al.,* 1994).

High exposure to Mn for long time leading to a classic syndrome known as "manganism." Manganism is characterized by a slow deterioration of neurological function that begins insidiously as non-specific symptoms and subclinical neurological signs. These symptoms include anorexia, apathy, arthralgias, asthenia, headaches, irritability, lethargy, and weakness in the lower extremities. Gradually, dysfunction of the basal ganglia develops that is characterized by alterations of gait, loss of balance, fine tremor, loss of facial expressions, and speech disturbances. Finally, a severe, disabling Parkinson's-like clinical condition develops, characterized by muscle rigidity, a staggering fait, dysphagia and a course intention tremor (Rodier, 1955). It seems that exposure to low level of Mn causes clinically to motor performance and memory. The most consistent findings are impaired function on tasks that require coordinated, sequential, alternating movements at a rapid speed (Mergler *et al.,* 1994).

1.4.2 Pulmonary toxicity

Mn is also a pulmonary toxin. Increases in respiratory symptoms, pneumonia and bronchitis have been reported among workers with occupational exposure and in people living near manganese alloy production facilities. In most cases these effects occurred at exposure levels well above what would be expected from MMT use in fuel (EPA, 1994). One study has suggested a possible increase in prevalence of respiratory illnesses in school children residing near a point source of atmospheric Mn pollution (Nogawa *et al.,* 1973).

1.4.3 Reproductive and developmental toxicity

Male mice exposed to elevated dietary Mn show decreased size of the testicles, seminal vesicles, and preputial glands, even without frank neurological signs such as tremor and ataxia (Gray and Laskey, 1980). Impotence and loss of libido and also decreased number of children are common symptoms in male workers exposed for 1-19 years to Mn dust (Laskey *et al.,* 1982). One small human study in an isolated island population with high manganese intake, found an apparent excess of fatal malformations (Kilburn, 1987).

1.4.4 Specific observation in animal models

Chronic exposure to Mn in rodents has been shown to alter brain dopaminergic neurotransmitters (Eriksson *et al.,* 1987; Rodriguez *et al.,* 1998) and inhibits critical enzymes involved in energy production (Zheng *et al.,* 1998). Mn in rat brain may interact with other essential metal ions and cause oxidative stress in targeted brain areas (Ali *et al.,* 1995; Sloot *et al.,* 1996). Moreover, following exposure to MMT, rodents show significant neuroexcitatory toxicity and altered normetanephrine levels (Fishman *et al.,* 1987; Komura and Sakamoto, 1994).

Studies conducted by our research team have shown that in portacaval anastomosis (PCA) rats not exposed to Mn, Mn content was increased in globus pallidus by 57% and in caudate putamen by 67% compared to a control group. In addition, a marked reduction in activity during the dark period was observed in rats with PCA (Therrien *et al.,* 1995). The relative influence of portal-systemic shunting and cholestasis on Mn accumulation in the brain was determined in rats with chronic liver failure (Rose *et al.,* 1999).

1.4.5 Specific observation in human with liver disease

Pallidal signal hyperintensity on magnetic resonance imaging (MRI) has been observed in most cirrhotic patients (Pujol *et al.,* 1993). Pallidal samples obtained at autopsy from cirrhotic patients who died with hepatic encephalopathy contain up to 7-fold increased Mn content. Similar MRI have been observed in patients receiving Mn as part of long-term parenteral nutrition, and after occupational Mn exposure sufficient to cause Parkinson's-like extra pyramidal symptoms (Nelson *et al.,* 1993). There is a positive correlation between blood Mn, pallidal index (a measure of degree of magnetic resonance) and the presence of portal-systemic shunting in cirrhotic patients (Spahr *et al.,* 1996).

1.5 Methods for reducing toxic effects of Mn

When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. There is substantial evidence to indicate that an interaction between iron and Mn occurs during intestinal absorption. Low level of iron and calcium as "synergistic factors" that impact on the toxic effects are associated with Mn exposure. In a dietary study investigating the effects of copper, iron, and ascorbate on Mn absorption in rats, these substances were all found to influence Mn absorption, depending in part on their relative concentrations (Johnson and Korynta, 1992). Thus it may be possible to reduce the uptake of Mn and thereby circumvent the potential for toxic effects caused by current and future exposure to excess Mn through specific dietary supplementation. For example, sufficient iron or calcium stores, as opposed to a

deficiency in these or other minerals, may reduce Mn absorption, and thus reduce potential toxicity (ATSDR, 1999).

Reducing body burden is also another method for reducing toxic effects of Mn. Chelation therapy with agents such as EDTA may alleviate some of the neurological signs of manganism, but not all patients show improvement, and some of the improvement may not be permanent. The potential use of calcium disodium ethylenediaminetetracetate ($CaNa_2EDTA$) for the management of heavy metal poisoning was investigated in dogs by Ibin *et al.,* (1992). $CaNa_2EDTA$-treated dogs (without excess Mn exposure) were found to have decreased Mn levels in their hair. It is possible that $CaNa_2EDTA$ could adversely affect the metabolism of Mn.

Interfering with the mechanism of action is also a method for reducing toxic effects of Mn. The oxidation state of Mn may influence both its retention in the body and its toxicity. Therefore, it is possible that interference with the oxidation of Mn could be a method for preventing Mn cellular uptake and toxicity. Regarding retention, one study suggests that clearance is much more rapid for divalent Mn than for trivalent Mn (Gibbons *et al.,* 1976). Regarding neurotoxicity, Mn (III) appears to be more efficient in enhancing the oxidation of catechols than either Mn (II) or Mn (IV) (Archibald and Tyree, 1986). Thus it is plausible that reducing the formation of Mn (III) could possibly both enhance elimination and prevent neurotoxicity (ATSDR, 1999).

1.6 Objectives of this study

The objective of this pilot study is to assess the inhalation toxicity of Mn in PCA animal model. More specifically, it is to evaluate the effects of Mn exposure on the deposition and bioaccumulation of Mn in different tissues and organs, on histological damage to the brain and on the locomotor activity of rats.

CHAPTER II - Manuscript submitted to review '' Hepatology 'Assessment of Bioaccumulation, Histopathology Damage and Neuro-behavioral Status in Portacaval Anastomosis Rats Exposed to Manganese Phosphate Dust: A Pilot Study

Assessment of Bioaccumulation and Neurotoxicity in Rats with Portacaval Anastomosis and Exposed to Manganese Phosphate: A Pilot Study

Short Title: Neurotoxicity of Manganese phosphate

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Abstract

The use of the additive methylcyclopentadienyl manganese tricarbonyl in unleaded gasoline has resulted in increased attention to the potential toxic effects of manganese (Mn). Hypothetically, people with chronic liver disease may be more sensitive to the adverse neurotoxic effects of Mn. In this work, bioaccumulation of Mn, as well as histopathology and neurobehavioral damage in end-to-side portacaval anastomosis (PCA) rats exposed to Mn phosphate via inhalation was investigated. The week before the PCA operation and four weeks after the PCA operation and at the end of exposure the rats were subjected to a locomotor evaluation (day-night activities) using a computerized autotrack system. Then a group of six PCA rats (E) was exposed to 3050 $\mu g\ m^{-3}$ (Mn phosphate) for 8 hr/day, 5 days/week for 4 consecutive weeks and compared to a control group (C). Another group of seven PCA rats were exposed to 0.03 $\mu g\ m^{-3}$. After exposure, the rats were then euthanized and Mn content in tissues and organs was determined by neutron activation analysis. The manganese concentrations in blood (0.05 μg/g vs 0.02 μg/g), lung (1.32 μg/g vs 0.24 μg/g), cerebellum (0.85 μg/g vs 0.64 μg/g), frontal cortex (0.87 μg/g vs 0.61 μg/g) and globus pallidus (3.56 μg/g vs 1.33 μg/g) are significantly higher in the exposed group compared to the control group ($p< 0.05$). No difference was observed in liver, kidney, testes and caudate-putamen between the two groups. Neuronal cell loss was assessed by neuronal cell counts. The loss of cells in globus pallidus and caudate putamen as well as in frontal cortex was significantly higher ($p < 0.05$) for E. Assessment of the locomotor activities did not reveal any significant difference, although E seems to show higher values than C. This study constitutes a first

step towards our understanding of the potential adverse effects of Mn in sensitive populations.

Key words: Liver disease - Toxicity - Locomotor activities - Tissue manganese concentration - Inhalation exposure.

INTRODUCTION

One of the main sources of inorganic Mn contamination in the urban environment is the combustion of the gasoline additive methylcyclopentadienyl manganese tricarbonyl (MMT), particularly in areas with high traffic density.[1] It is now well accepted[2,3] that the automotive combustion products of MMT are essentially Mn phosphate, Mn sulfate and a mixture of Mn phosphate / Mn sulfate. Modern automobiles equipped with catalytic converters emit mainly the manganese phosphate form and smaller amounts of sulfates and oxides.[4] The combustion products of MMT depend on fuel composition and engine and catalytic converter thermodynamics. Particle sizes range between 0.2 and 10 μm.[5] On average, more than 99 % of the particles were in the respirable fraction (< 5 μm) and 86 % were less than 1 μm.[6]

Exposure to high concentrations of atmospheric Mn can lead to numerous health problems, especially in the respiratory and neurological systems. Chronic human exposure to high concentrations of manganese containing dust (>1mg Mn/m^3) has been shown to induce adverse neurological, respiratory, and reproductive effects.[7] Certain subpopulations such as patients with chronic liver disease could be more vulnerable to the neurotoxic effects of Mn since they have a decrease in biliary excretion of a variety of trace metals, including Mn. Little is known on the potential health effects that may result from long-term continuous exposure of these patients to Mn compounds in ambient air at low concentrations. We hypothesise that a relatively high exposure to Mn may be tolerated by normal animals and humans but may lead to an overload and neurological problems in those with hepatic dysfunction.

Studies conducted by our research group[8] have shown that in portacaval anastomosis (PCA) rats not exposed to inhaled Mn, Mn content was increased in globus pallidus by 57% and in caudate putamen by 67% compared with the control group. The relative influence of portal-systemic shunting and cholestasis on Mn accumulation in the brain was also determined in rats with chronic liver failure.[8] In addition, a marked reduction in activity during the dark period was observed in rats with PCA.[9] Chronic exposure to Mn in rodents has been shown to alter brain dopaminergic neurotransmitters[10,11] and inhibits critical enzymes involved in energy production.[12] Manganese in rat brain may interact with other essential metal ions and cause oxidative stress in targeted brain areas.[13,14]

The objective of this pilot study is to assess the inhalation toxicity in PCA animal model. More specifically, it is to evaluate the effects of Mn exposure on the deposition and bioaccumulation of Mn in different tissues, on histological damage to the brain and on the locomotor activity of rats.

MATERIALS AND METHODS

A total of 16 Sprague-Dawley rats were purchased from Charles River Laboratories (Saint-Constant, Quebec) at age six weeks. Each rat was initially examined by a veterinarian at St-Luc Hospital and marked with a specific number on its tail. Rats were than housed separately in stainless steel cages for three days of acclimation. For the end-to-side portacaval shunt, surgery was performed according to the method of Therrien and Butterworth.[15] After PCA surgery, three rats died before any exposure. Because of the fact that five weeks after surgery liver function, hemodynamic status and neurologic function are stable, this duration was applied for all rats for acclimation. All procedures involving rats were approved by the Institutional Committee of Animal Care and Use.

Rationale for the choice of animal model

Rats were used in this pilot study because they seem to be a useful model for studying Mn exposure and effects. Indeed, chronic Mn exposure through drinking water[16,17], intrastriatal[18] and intrathecal[19] exposures has been shown to induce motor and other behavioral effects in rats. Inhalation exposure in rats has not been well studied to date, a fortiori in cirrhotic individuals. Furthermore, contrary to mice, a substantia nigra (the region responsible for dopamine synthesis) exists in rats[20] and shows various neurochemical, neuropathological, and neurobehavioral effects resulting from Mn exposure.[21] The animal models described are chosen in view of the fact that they are well-validated models of chronic liver failure which represent the major features of chronic liver

failure in humans. Both the animal models and human cirrhotics manifest a reproducible sequence of neurological impairment starting with altered day-night rhythms[9]

progressing through altered reflexes[22] to stupor and coma.[23] The experimental models chosen result in sustained hyperammonemia[23] and in modifications of astrocytic function including reduced expression of key astrocytic proteins[24] similar to those reported in chronic liver failure in humans. Moreover, of direct pertinence to the present work, both the experimental animal models and human chronic liver failure result in the selective accumulation of Mn in pallidum.[25,26,8]

Inhalation exposure

Two groups of 6 and 7 rats were respectively exposed (E) to a mean of 3050 $\mu g\ m^{-3}$ of manganese phosphate in the mineral form hureaulite ($Mn_5\ (PO_4)_2\ (PO_3\ (OH))_2 \cdot 4H_2O$) obtained from Alfa Aesar (Johnson Matthey Company) in a white fine crystalline powder and to a mean of 0.03 $\mu g\ m^{-3}$ of ambient atmospheric Mn (C), which is similar to the atmospheric Mn concentration found in residences in Montreal. The level of exposure of 3050 $\mu g\ m^{-3}$ is derived from the study of Coulston and Griffin[27] who used 100 $\mu g\ m^{-3}$ for 24 h exposure. Thus, 3000 $\mu g\ m^{-3}$ (the mean concentration at which we aimed) results from multiplying 100 $\mu g\ m^{-3}$ by 3 (24h/8h) and by a correction factor of 10 in consideration of exposure duration. To help in adequately evaluating the dose-response relationship, this first level was then divided by another factor of 10.

For the E group, exposure was conducted in an inhalation chamber over 4 consecutive weeks (duration at which portal-systemic shunting is maximal and at which rats show brain accumulation of Mn and after which the general condition begins to deteriorate), 5 days per week and 8 hours per day. The inhalation chamber is a rectangular stainless steel box (131 cm long, 65 cm wide, 125 cm deep) with a total volume of 1 m^3 (Hazelton Systems Company Inc., Kalamazoo, Michigan). The chamber received filtered air (HEPA) and was maintained at a temperature of 20 ± 2°C and relative humidity 60% (± 12 %) throughout the study.

The Mn aerosol particles were generated by a Fluidized Bed Aerosol Generator (Model 3400 TSI Inc., St. Paul, MN 55164) with a flow rate of 9 L/min throughout the study and the concentrations were verified continuously using a Dust Track (model 8520) aerosol monitor. The research done previously in our laboratory using stainless steel ASME Pressure Tanks, which play the role of a cyclone, allowed us to obtain 95% of Mn particles smaller than 3.5μm as shown with a cascade impactor (Table 1).

Since some variability of the air concentrations in different sections of the inhalation chamber could occur, we rotated the positions of the rats every day of exposure. For the C group, exposure was conducted in a second inhalation chamber (0.5 m^3) in which ambient air (Mn ~ 0.03 μg m^{-3}) was introduced at the same flow rate. For both groups, air samples were collected from the chamber on a routine daily basis to monitor Mn concentration. The sampling system consists of a Gilian pump (Gilian Corp., West Caldwell, N.J.) with standard 3-piece cassettes and 37 mm diameter filters. The filters are

Teflon (manufactured for SKC Inc., Gelman Sciences, Michigan) with 0.45 μm pore size. They were changed each day. Pumps were used at a constant flow rate of 1.5 liters per minute and included a size selective cyclone (York respirable dust Sampler # 1U3799) with a cut off point of 5 μm. The flow rate was calibrated each day with a Gilibrator (Gilian Corp. West Caldwell, N.J.). Once per week, a cascade impactor was used in order to establish the particle sizes. Mn was analyzed by Instrumental Neutron Activation Analysis (INAA).

Each rat was kept in an individual cage during the non-exposed period and was weighed each week. Food and water were available *ad libitum* when the rats were not exposed, thereby minimizing contamination of the diet. Mn concentrations in food and water were determined by INAA.

Locomotor activity, day/night rhythms

Before and after surgery (and before exposure) as well as immediately at the end of exposure, an evaluation of the animals (as reported by Therrien *et al.* [9]) was carried out. The tests included the locomotor activity: day-night rhythms, exploratory activity as well as day time and night time activities. Measurements were done using a computerised autotrack system installed in a quiet room with 12 h light/12 h dark cycles. Individual activity profiles for each rat over the whole 12 h light/dark periods as well as cumulative activity scores were measured as described by Therrien *et al.*[9]

Tissue Mn concentrations

After the exposure and after the locomotor evaluation, blood samples were taken from the caudal vein for the determination of the biochemical profile. Rats were then euthanized 48 hours after the end of exposure with halothane, followed by exsanguination. Target tissues (liver, lung, kidney, testis and brain (frontal cortex, pallidum, caudate putamen, cerebellum and globus pallidus) were removed, weighed and dissected. The two brain hemispheres were frozen separately for chemical, biological and morphological analyses. The right hemisphere parts were frozen in two aliquots in order to measure Mn.

Chemical analysis

The Mn concentrations in tissues, blood, water, food and filter were measured by INAA[5] using the university's stable neutron flux nuclear reactor and an EG&G ORTEC (model DSPec) digital gamma-ray spectrometer incorporating a high resolution large volume germanium detector. INAA uses no reagents and a minimum of sample handling; contamination is thus easily avoided. The potential interference from Fe has been reduced by irradiating samples in a more thermalized neutron spectrum; it was verified and corrected when necessary for all Mn determinations. The detection limit for Mn determination in biological tissues is 4 ng g^{-1} and the precision is 2 ng g^{-1} for concentrations between 4 ng g^{-1} and 40 ng g^{-1}, and 5% of the measured concentration for concentrations above 40 ng g^{-1}.

Biochemical profile

The biochemical tests were performed by standard techniques for blood glucose, blood urea nitrogen, serum creatinine, bilirubin, transaminases (AST, ALT), alkaline phosphatases, and blood hematocrit and serum iron.

Histological evaluation

The parameter investigated in this study was neuronal cell loss, which was determined by neuronal cell counts. Brains were sliced at -20° C in a cryostat and adjacent 20 μm sections mounted on gelatinized slides. For studies of neuronal cell counts, slides were fixed in 10% formalin for 5 min at room temperature and stained with creosyl violet according to previously published protocols.[28] Cell counts were obtained from caudate nucleus, putamen, globus pallidus and frontal cortex (control region) by counting by two investigators and averaging two 1.75 mm^2 grid areas.

Statistical analysis

The data for quantitative and continuous variables were compared for the exposed and control groups by tests of homogeneity of variance (Levene's test), one way analysis of variance (ANOVA), T Test for independent comparison and multiple comparison procedure for significant ANOVA (Tukey; Duncon for equal variances assumed: Temphane T2 and T3 for unequal variances assumed). Non parametric tests were achieved as well when Levene's test for homogeneity ($p < 0.01$) indicated the data to be non homogeneous. The later results for the significant differences between groups were

the same as ANOVA (data not shown). The probability value of less than 0.05 was used as the critical level of significance within each statistical test. Statistical analyses were performed using SPSS Statistical Software.

RESULTS

Tissue Mn concentrations are presented in Table 2. Mn concentrations in blood, lung, cerebellum, frontal cortex and globus pallidus were significantly higher in the E group ($p< 0.05$), but no differences were observed in liver, kidneys, testes and caudate-putamen between the two groups. This was also the case for the biochemical tests, where no significant differences were observed (Table 3).

No significant difference in locomotor activities was observed (Fig. 1), although E seems to show higher values than C (3978 vs 2714 cm). The results of the last dark period were similar to those of the first dark period. For resting time also, no significant difference was observed between the two groups, but both groups showed a significant increase in resting time after PCA and before exposure to Mn (Fig. 2).

Finally, the number of neuronal cells in globus pallidus (800 vs. 510), caudate putamen (840 vs. 500) and frontal cortex (680 vs. 450) were significantly different, $p< 0.05$ (Fig. 3). This aspect can be observed in the photos (Fig. 4), showing neuronal cells in globus pallidus.

DISCUSSION

In this study, hureaulite was chosen because Ressler[3] found that the Mn related to MMT combustion exists in a form similar to hureaulite, which is commercially available in a highly purified form.

The main observation of this study was related to tissue Mn concentrations in brain, mainly in globus pallidus, cerebellum and frontal cortex. The cerebellum seems to be a target site as already observed by Vitarella *et al.*[29], even if for Pal *et al.*[30] it is not considered as a site of action for Mn induced neurotoxicity.

These general observations are similar to those of Pomier-Layrargues *et al.*[25] and Butterworth *et al.*[26] who demonstrated that Mn deposition occurs in the brain of human cirrhotic patients. The mechanisms of brain Mn deposition include the following: decrease of biliary excretion of Mn in the presence of liver insufficiency, increase of systemic availability of Mn due to the presence of portosystemic collateral bypassing the liver; changes in the blood-brain barrier permeability to Mn.[31] Besides Mn absorption through the respiratory tract, intranasal deposition of Mn may result in initial uptake of the metal through the primary olfactory neurons following inhalation exposure, affecting astrocytes.[32] In contrast to other metals such as cadmium, Mn has been reported to migrate into most parts of the brain following intranasal instillation in rats via transneuronal transport.[33] Mn can thus circumvent the blood-brain barrier and gain direct access to the central nervous system as was recently observed by Vitarella *et al.*[29] where

Mn concentration in the olfactory bulb was significantly higher than in the striatum. Unfortunately, the olfactory bulb was not analyzed in our study.

In our study, lung concentrations (average of 1.32 μg Mn/g) differ considerably from those of Vitarella *et al.* [29] (average of 23.01 μg Mn/g). This difference is quite surprising since the two inhalation studies involved the same chemical Mn species at about the same level (≈ 3 mg/m^3) and the same duration of exposure per day. A possible explanation could be related to a non instantaneous transfer rate between lung and blood. For instance, if the t_2 in this transfer rate is in the range of 15 to 30 min, the concentration in the lung at equilibrium would be dose dependant as observed by Vitarella *et al.* [29]. Consequently, few hours after the end of exposure would lead to a significant decreased of lung concentration. In fact, in the study of Vitarella *et al.*[29], tissues were collected immediately following the last inhalation exposure while in our study in our study, tissue collection took place 36 hours after the end of exposure. The delay of 36 hours was required for the assessment of motor activities.

The measurement of day and night time activities provides an evaluation of the day-night rhythm and of locomotor activities, parameters that could be altered in the presence of manganese intoxication or liver insufficiency. In rats with PCA we have observed a marked increased of activity during the dark period because of the surgery even if the difference is not statistically significant. Moreover, at the end of exposure, the activity of E seems to be higher during night even it is not statistically significant. This may be due to the small sample size.

A previous report[34] described a positive relationship between pallidal hyperintensity and tremor score in a similar group of cirrhotic patients. Chronic inhalation of manganese in nonhuman primates results in decreased concentrations of dopamine D_1 binding sites in basal ganglia. There is a substantial body of evidence to suggest that effects of manganese on the dopamine system may be responsible for the neurological effects.

Mn exposure also produced a significant effect on loss of cells in globus pallidus and putamen as well as in frontal cortex. It is possible that manganese can move relatively easily between different constituents within the neuronal cells. Donaldson[35] showed a localization of Mn in various cell-rich areas at the base of the cerebral hemisphere, including the basal ganglia. Manganese has an affinity for melanin, which might promote uptake of Mn in the pigmented neurons in the substantia nigra. Therefore, the observed disappearance of cell bodies is evidence of the damage to the substantia nigra.

It has been suggested that combustion of the organomanganese compound MMT (used in gasoline) may be a significant source of exposure to inorganic Mn in urban areas. Mn contamination resulting from the widespread use of MMT leads to higher atmospheric Mn concentrations and could lead to toxic effects especially for susceptible populations with liver disease. It is difficult with this pilot study to arrive at any firm conclusions. Nevertheless, the results represent the first step towards enhancing our knowledge on the contribution of an increase of atmospheric Mn contamination to nervous system deficits in susceptible populations.

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Table 1 Mn particle size in the inhalation chamber

Particle size (μm)	Percentage (%) ± SD
> 6.0 and < 9.8	1.5 ± 0.2
> 3.5 and < 6.0	3.5 ± 0.8
> 1.5 and < 3.5	4.5 ± 1.3
> 0.93 and < 1.5	13.0 ± 3.1
> 0.52 and < 0.93	47.0 ± 7.6
< 0.52	20.0 ± 5.2
Backup filter	10.5 ± 2.2

n = 5

Table 2 Tissue Mn concentrations

Tissue	Control group[1] μg/g ± SD	Exposed group[2] μg/g ± SD
Blood	0.02 ± 0.01	0.05 ± 0.01*
Kidney (left)	1.25 ± 0.16	1.43 ± 0.13
Kidney (right)	1.30 ± 0.14	1.45 ± 0.14
Liver	2.57 ± 0.28	2.26 ± 0.23
Lung	0.24 ± 0.02	1.32 ± 0.61*
Testis (right)	0.45 ± 0.05	0.45 ± 0.14
Testis (left)	0.48 ± 0.08	0.43 ± 0.13
Brain		
Cerebellum	0.64 ± 0.06	0.85 ± 0.05*
Frontal cortex	0.61 ± 0.08	0.87 ± 0.07*
Globus pallidus	1.33 ± 0.72	3.56 ± 0.80*
Caudate putamen	1.50 ± 0.06	2.18 ± 0.92

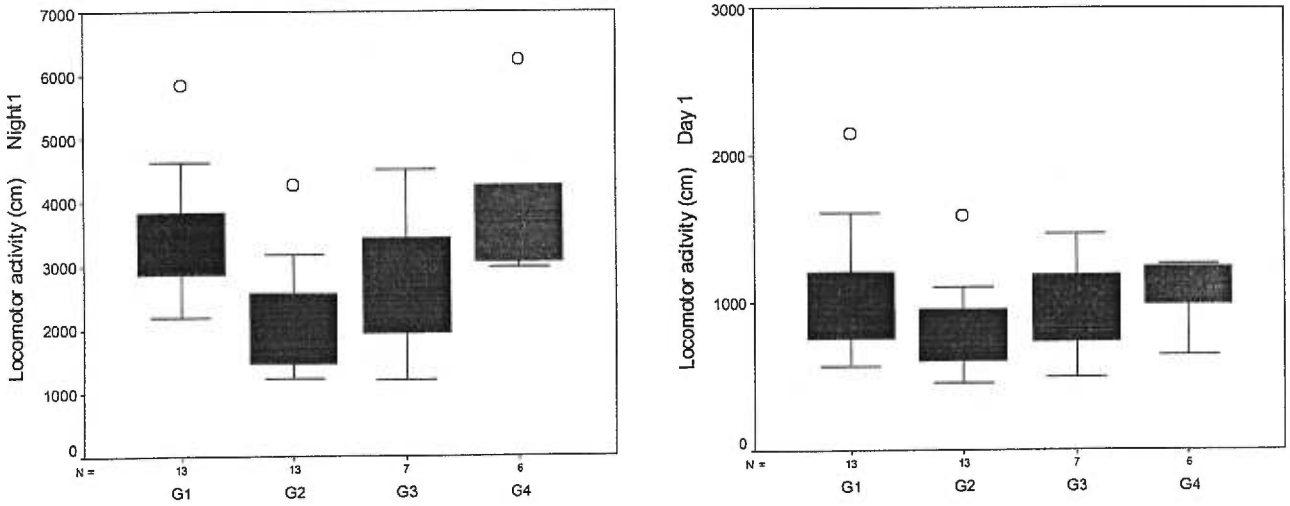
* Significantly different from control ($p < 0.05$)
1 : n = 7
2 : n = 6

Table 3 Biochemical parameters

	Control group[1] Mean ± SD	Exposed group[2] Mean ± SD
Urea (mmol/L)	4.41 ± 0.59	4.17 ± 0.24
Glucose (mmol/L)	6.67 ± 1.32	7.86 ± 1.17
Creatinine (μmol/L)	47.14 ± 2.91	47.50 ± 3.15
Bilirubin (μmol/L)	4.86 ± 1.07	4.17 ± 0.75
Alkaline Phosphate (U/L)	243.43 ± 94.69	243.83 ± 59.06
AST (U/L)*	112.14 ± 25.87	114.67 ± 19.22
ALT (U/L)**	53.00 ± 15.06	50.50 ± 7.01
Sodium (mmol/L)	140.00 ± 0.16	139.33 ± 1.86
Potassium (mmol/L)	4.64 ± 0.55	4.67 ± 0.23
Hematocrit (%)	47.86 ± 1.68	47.67 ± 2.50

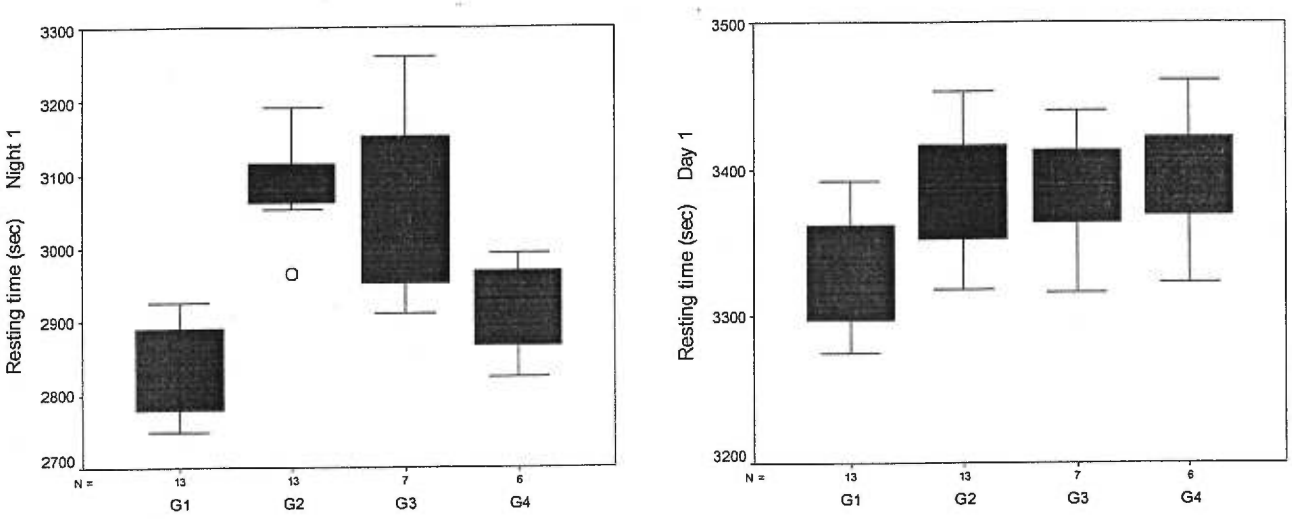
* Aspartate aminotransferase
** Alanine aminotransferase
1 : n = 7
2 : n = 6

Figure 1 Locomotor activity (distance traveled in cm, Mean ± SD) for 12-hour dark period and 12-hour light period



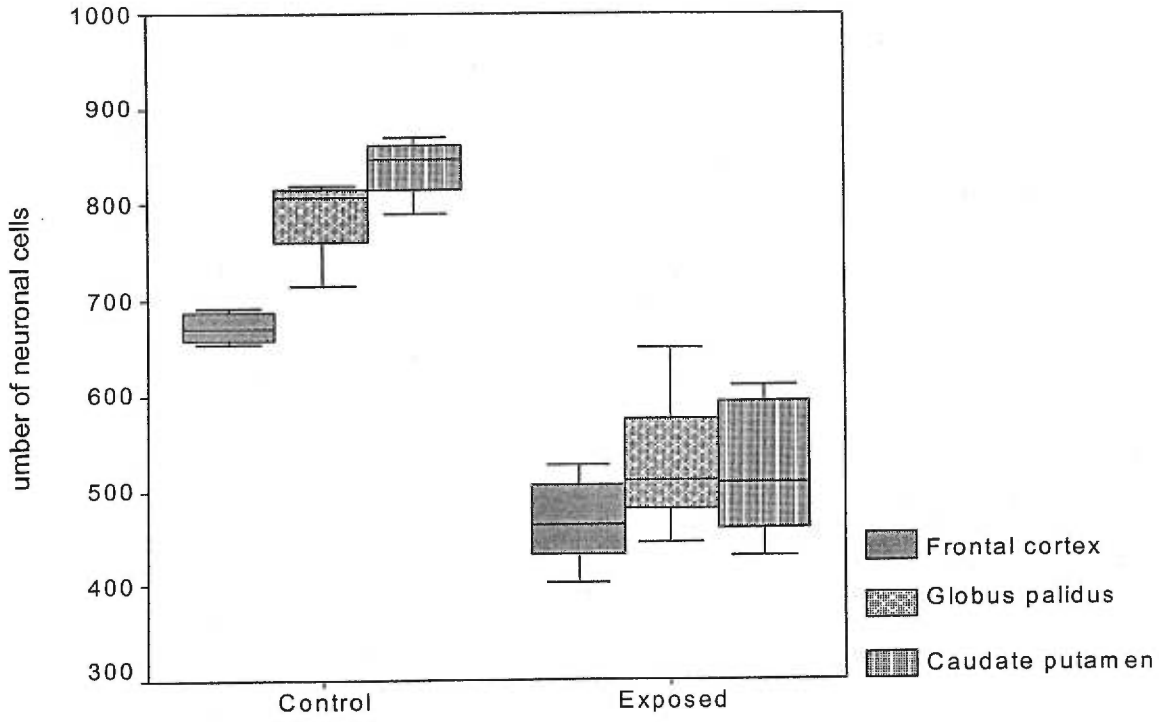
G1 = Exposed and control groups before surgery (PCA) and before exposure (n =13)
G2 = Exposed and control groups after PCA and before exposure (n = 13)
G3= Control group four weeks after exposure (n = 7)
G4= Exposed group four weeks after exposure. (n = 6)
White circle: outlier

Figure 2 Resting time (sec, Mean ± SD) for 12-hour dark period and 12-hour light period



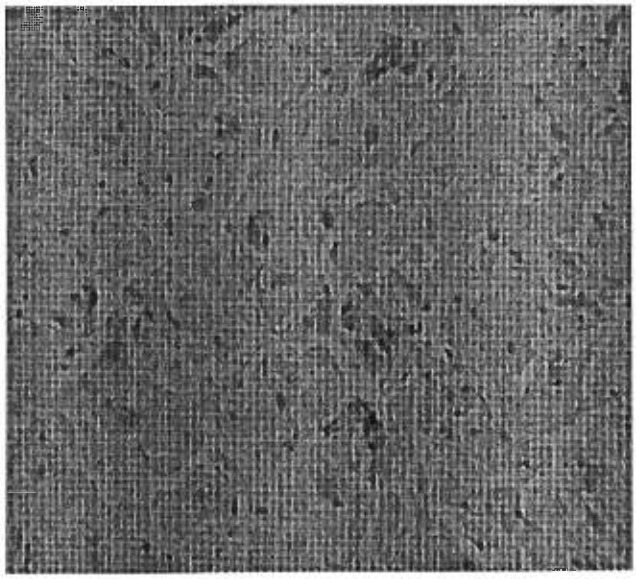
G1 = Exposed and control groups before surgery (PCA) and before exposure (n = 13)
G2 = Exposed and control groups after PCA and before exposure (n = 13)
G3= Control group four weeks after exposure (n = 7)
G4= Exposed group four weeks after exposure (n = 6)
White circle: outlier

Figure 3 Number of neuronal cells in globus pallidus, caudate putamen and frontal cortex (Mean ± SD)

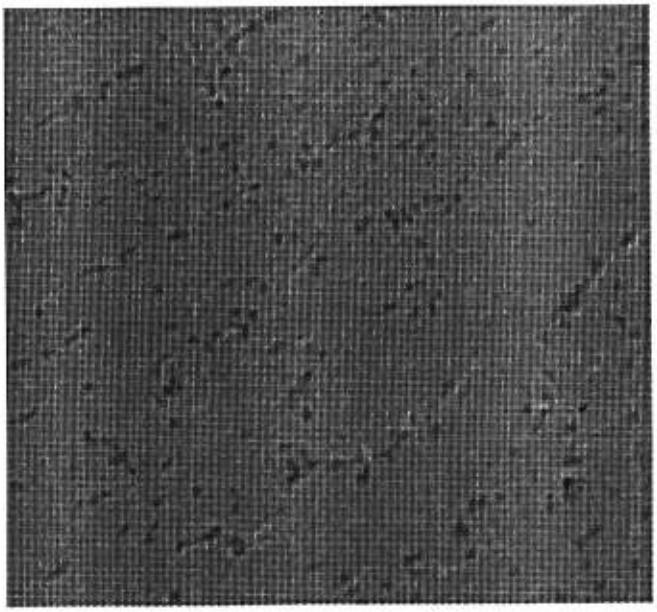


Significant difference ($p < 0.05$) between C and E for all tissues

Figure 4 Neuronal cell in the globus palidus



(A)



(B)

Magnification: 40 x

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CHAPTER III - Discussion and Conclusion

Discussion

The main observation of this study related to bioaccumulation concerned Mn in brain, mainly globus pallidus. The cerebellum and frontal cortex seems also to be a target site as already observed by Vitarella (Vitarella *et al 2000)*, even if for Pal (Pal *et al* 1999) it is not considered as a site of action for Mn induced neurotoxicity.

No significant difference was observed for Mn accumulation in caudate putamen, which was expected from a previous study. This observation is similar to those of Pomier-Layrargues *et al.* (1995) and Butterworth *et al.* (1995) who demonstrated that Mn deposition occurs in the brain of cirrhotic patients. The mechanisms of brain Mn deposition include the following: decrease of biliary excretion of Mn in the presence of liver insufficiency, increase of systemic availability of Mn due to the presence of portosystemic collateral bypassing the liver; changes in the blood-brain barrier permeability to Mn (Spahr *et al.* 1996).

Some neurological symptoms linked to cirrhosis could be related, at least in part, to deposits of Mn in the brain, particularly in the globus pallidus. In cirrhotic patients, a high incidence of extrapyramidal symptoms, in particular rigidity, similar to that observed in Parkinson's disease has been observed (Krieger et al 1997). However, region-selective increases in brain manganese deposition were observed in cirrhotic patients and in rats with portacaval shunts.

The results of this study also show that manganese concentration in globus pallidus is higher than in the cerebella, caudate putamen and frontal cortex. Therefore, manganese deposition

in brain is a region-selective phenomenon. Following high dose exposure, humans and other primates accumulate manganese primarily in the basal ganglia and other regions that have high iron and neuromelanin-binding capacity (Aschner et al., 1999). The intranasal deposition of Mn may result in initial uptake of the metal through the primary olfactory neurons following inhalation exposure. Mn has been reported to migrate into most parts of the brain following intranasal instillation in rats via transneuronal transport (Tjälve *et al.*, 1996).

There is also evidence that the deposition of Mn in the brain of rats may occur by direct olfactory axonal transport to central nervous system (Tjälve and Henriksson., 1999). In rats, intranasal instillation of $MnCl_2$ has been shown to result in direct movement of Mn to the olfactory bulb and the telencephalon via transport in secondary olfactory neurons (Gianutsos et al., 1997). Mn can thus circumvent the blood-brain barrier and gain direct access to the central nervous system, one of the Mn target sites. Furthermore, a fraction of inhaled Mn is likely to reach cerebral target sites before hepatic clearance (Roels *et al.*, 1997).

After oral ingestion, Mn is absorbed in the gut, transported to the liver via the portal vein and then eliminated via biliary excretion. Therefore, for both routes of exposure, the liver has a key role in Mn elimination. Decreased biliary excretion could play a role in manganese overload in blood. As a consequence, the cerebral toxicity of Mn may be enhanced even after respiratory exposure in the presence of liver disease due to the

decrease in the ability of liver to excrete Mn in the bile. This mechanism could explain the neurological effects observed in this study.

Brain Mn deposition is region-selective, being predominant in the basal ganglia which is involved in the control of movements and of some cognitive functions. Mn deposition in brain, mainly in globus pallidus corresponded to onset of the neurological effect.

In rats, the measurement of day and night time activities provides an evaluation of the day-night rhythm and of locomotor activities, parameters that are altered both in the presence of manganese intoxication or/and liver insufficiency. In rats with portacaval shunts we have observed a marked increased of activity during the dark period. However, the relationship between these changes and the brain manganese accumulation also observed in these models is still unclear. As for the locomotor activities and resting time, there were no significant differences, although E seems to show higher values than C, possibly as a result of the small sample size. The results for resting time showed significant differences after shunting but no significant difference was observed after exposure to Mn.

A previous report described a positive relationship between pallidal hyperintensity and tremor score in a similar group of cirrhotic patients (Pujol et al., 1993). Chronic inhalation of manganese in nonhuman primates results in decreased concentrations of dopamine D_1 binding sites in basal ganglia. There is a substantial body of evidence to suggest that effects of manganese on the dopamine system may be responsible for the neurological effects.

Mn exposure also produced a significant effect on loss of cells in globus pallidus and putamen as well as in frontal cortex. It is possible that manganese can move relatively easily between different constituents within the neuronal cells. Donaldson showed a localization of manganese in various cell-rich areas at the base of the cerebral hemisphere, including the basal ganglia (Donaldson, 1987). Manganese intoxication in man may result in degeneration of the substantia nigra. Manganese has an affinity for melanin, which might promote uptake of the metal in the pigmented neurons in the substantia nigra. Therefore, with the observed of disappearance of cell bodies is evidence of the damage to the substantia nigra.

Conclusion

It has been suggested that combustion of the organomanganese compound MMT (used in gasoline) may be a significant source of exposure to inorganic Mn in urban areas. Mn is an essential nutrient, but at high levels of exposure it is well recognized as a neurotoxic. Pulmonary toxicity also occurs at high levels of exposure, and developmental toxicity to fetuses is an important concern based on more limited data. Population exposure to Mn from MMT use is expected to be low in most people, but high in certain subpopulations.

Despite Health Canada's determination that the combustion products of MMT in gasoline do not pose an added health risk to the Canadian population (Wood et al., 1995), it is not known what problems may occur if MMT is introduced widely in gasoline supply (Davis, 1998). While limited evidence indicates that general population exposure to Mn from the use of MMT in gasoline are much lower than those encountered in some occupational settings, more data are needed to assess potential health effects. More research is needed to assess health effects in population that may be especially susceptible to Mn toxicity because of unusually high exposure or physiological vulnerability. Such groups include foetuses, children, certain workers (eg, workers involved with the production of MMT, refinery workers, garage mechanics), and the elderly and people with liver disease.

The results of this study represent the first step to enhancing our knowledge on the contribution of an increase of atmospheric Mn contamination to nervous system deficits.

There is evidence to suggest that chronic liver insufficiency and portosystemic shunting results in accumulation of manganese in the brain and particularly in the basal ganglia. But there is a poor relationship between cognitive abnormalities associated with hepatic encephalopathy and brain manganese overload. However the occurrence of extrapyramidal disorders is increasingly recognized in cirrhotic patient and there is a rational basis to hypothesize a relationship between manganese accumulation in basal ganglia and the presence of certain cognitive and motor symptoms. It has to be done many researches on neurotoxic effects of manganese in patients with liver diseases in the future and also removing manganese and improving neurological dysfunction in these patients.

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