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# Blood manganese concentrations among first-grade schoolchildren in two South African cities ☆

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**Abstract**

Little information exists on the environmental exposures to low levels of organometallic manganese (a principal combustion product from manganese-containing fuel additives) and public health. This study was undertaken to establish biological and environmental levels of manganese among first-grade schoolchildren in the South African cities of Johannesburg and Cape Town. The study was undertaken subsequent to partial introduction of the manganese-containing fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT) to South African petrol in the Johannesburg region only, about 24 months prior to the commencement of the study, and in anticipation of possible future increases in MMT use in the rest of the country.

This study involved the measurement of manganese concentrations in the blood of the total number of 814 of grade one schoolchildren (430 and 384 in Cape Town and Johannesburg, respectively), and in water supplies, soil, and classroom dust at a total number of 21 participating schools. The results indicated higher concentrations of manganese in school soil ($P=0.0007$) and dust ($P=0.0071$) samples from Johannesburg relative to Cape Town. Similarly, the mean blood manganese concentration in Johannesburg study subjects (9.80 μg/L, SD 3.59) was significantly higher than that in Cape Town study subjects (6.74 μg/L, SD 3.47), after allowing for the clustering effect within schools and adjusting for the confounding effect of population group ($P<0.0001$). The blood manganese levels of 4.2% and 12.5% of children in Cape Town and Johannesburg, respectively, equaled or exceeded 14 μg/L, the upper normal reference value specified by the Agency for Toxic Substances Disease Registry (ATSDR, 2000). Importantly, levels of manganese in blood were found to be significantly associated with concentrations of manganese in classroom dust at schools.


*Keywords:* Environment; Blood manganese; Children; South Africa; MMT

## 1. Introduction

When introducing new chemicals into the environment, it is pertinent that short- and long-term environmental and health risk factors are well understood and evaluated. It is thought that certain metals, even in low concentrations, when released into the environment, may exert toxicity to living organisms over a very long time (Nriagu and Pacyna, 1988). A typical example of the enormity of the public health consequences generated by a single toxic metal is exposure to anthropogenic lead. Although its removal from petrol in developed countries significantly reduced overall levels of exposure, it did not remove lead from the environment altogether and the hazard of persistent long-term lead exposure continues to exist.

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A modern compound of concern is the manganese-containing fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT) that was introduced to automobile fuel formulas as an octane boosting and "anti-knock" agent, thus either replacing or reducing the lead content in petrol since 1976 in Canada. In addition, MMT has been officially approved for use by the governments of Argentina, Australia, Bulgaria, the United States, France, and Russia, and conditionally in New Zealand (Health Canada, 2003). However, MMT is not used in these countries intensively as they are waiting for the evidence of the absence of effects on human health.

Biologically, manganese is considered to be an essential metal important to mitochondrial oxidative processes for all living mammals, but may also be toxic at high concentrations. Manganese does not occur as a free metal but exists in 11 oxidation states, with only the manganese (II) and manganese (III) oxidative states being of biological significance (Alessio and Lucchini, 1996). Both deficiency and excess of manganese have been associated with detrimental health effects. Excessive intake of manganese, either through inhalation or ingestion, may result in pathology, particularly to the central nervous system (Keen and Zindenberg-Cherr, 1990). Excessive exposure via inhalation has been shown to cause effects on the lungs and accumulate in the brain, causing irreversible brain disease, to some extent similar to Parkinson's disease. These effects are well documented in occupational settings (Politis et al., 1980, Mergler et al., 1994), where correlations between low doses of exposure, blood manganese levels, and neurological health outcomes have also been reported (Lucchini et al., 1995). The recent review article by Levy and Nassetta (2003) examines numerous published reports of neurological effects of exposure to manganese in occupational and environmental settings. The authors note that an estimated 500,000 to 1.5 million people in the United States have Parkinson's disease, and physicians should consider manganese exposure in its differential diagnosis.

Although studies reported that airborne manganese levels in Montreal (where MMT was used for more than 10 years) were similar to those areas where MMT was not used, the possibility of elevated human exposure to manganese species both by inhalation and ingestion cannot be ruled out (Lynam et al., 1999; Zayed et al., 1999). Recent animal studies performed on rats showed that inhalation of soluble forms of manganese resulted in higher brain manganese levels (Dorman et al., 2001; Brenneman et al., 2000).

The absorption of ingested manganese is regulated by homeostatic mechanisms that may be increased by iron deficiency thought to be prevalent in developing countries (Bothwell, 1995; Szarfarc and de Souza, 1997). It was found that individuals with anaemia absorb almost twice as much manganese as healthy individuals with the same exposure. Infants have a greatly increased absorption and retention capacity for ingested manganese compared to that of adults (Mena et al., 1969). In addition, the blood–brain barrier (BBB), being underdeveloped in infants, may allow for relatively free ingress of manganese into the brain. In young children, contaminated house dust and soil, combined with hand to mouth behaviour, could be major contributors to body burdens of manganese, both by ingestion and respiration (Weiss, 2000; Aschner, 2000). Association between manganese uptake during pregnancy and early psychomotor development of children was also recently reported (Takser et al., 2003).

The elderly and sensitive subpopulations, specifically persons with liver disease, are also thought to be at risk, due to the increased susceptibility of aging brain cells to injury and manganese reaching cerebral target sites before hepatic clearance in the latter group (Silbergeld, 1982; Roels et al., 1997). Animal experiments showed that clearance from the brain, the critical target organ, appears to be slow (Mena et al., 1974). This slow clearance mechanism opens the possibility of accumulation of manganese in tissues under conditions of chronic low exposure.

Considering both essentiality of manganese for the brain and its potential to damage the brain when present in excess, studies are required for examining the mechanisms that mediate both the influx and the efflux of manganese for the maintenance of brain manganese homeostasis. Recent studies using the isotope of manganese ($^{54}$Mn) attempted to characterise transport kinetics of manganese across the BBB and identify manganese species (ligands) responsible for brain manganese homeostasis in rats (Crossgrove et al., 2003; Yokel et al., 2003). The brain influx rates of $^{54}Mn^{2+}$, $^{54}$Mn citrate, and $^{54}$Mn transferrin ($^{54}$Mn Tf) were determined using in situ brain perfusion technique and were found to be faster than can be explained by diffusion. It was found that nonprotein-bound manganese species in blood plasma, namely, manganese citrate, as well as manganese and manganese transferrin, may be the major manganese species entering the brain, suggesting that manganese influx across the BBB is carrier-mediated (Crossgrove et al., 2003). When examining efflux of manganese from the brain, Yokel et al. (2003) established that it is diffusion mediated. The authors concluded that these findings suggest that repeated excessive exposure to manganese might result in manganese accumulation in the brain over time.

The manganese-containing MMT compound, as is the case for tetraethyl lead, is a neurotoxin that can cause agitation and convulsions, as well as pulmonary damage at elevated concentrations. Relatively little is known about possible long-term health consequences of widespread environmental exposure to MMT manganese emission compounds in community settings (Baldwin et al., 1999).

In light of the scarcity of data, the American Medical Association Council on Scientific Affairs has called for research determining possible health effects of long-term, low-dose exposures to MMT and its combustion products (Lyznicki et al., 1999).

As part of a broad process of ongoing automobile technology development, as well as in preparation for the phasing out of lead as an additive in the country's fuels by 2006, South African oil companies have commenced and are currently considering the longer-term addition of MMT to petroleum.

In light of the paucity of epidemiological studies on long-term environmental exposure to low levels of manganese from vehicular emissions and public health, the South African Medical Research Council undertook this study to assess the environmental and biological levels of manganese in different areas of the country, to serve as a baseline for future comparative assessments. With the introduction of MMT to unleaded petrol in the Johannesburg area about 24 months before this study took place, it is most important to establish if measurable differences in manganese levels between different areas in the country can be detected.

If differences are detected and should follow-up, surveys show an increase in environmental loading as well as an increase in children's blood levels, it is intended to expand the investigation to examine possible health effects as called for by the American Medical Association Council.

This paper reports on the findings of a survey of blood manganese levels in two South African cities, namely, Johannesburg and Cape Town, undertaken during 2002.

## 2. Materials and methods

A descriptive cross-sectional analytical study was performed in the cities of Cape Town and Johannesburg. Schools were the primary unit of sampling and were selected from areas expected to have varying levels of exposure. In the city of Cape Town, a total of 11 schools were sampled, including schools in innercity Cape Town (seven schools), the suburban area of Mitchell's Plain (one school), and the less heavily trafficked suburb of Hout Bay (three schools). In Johannesburg 10 schools were included from innercity areas (three schools), the Westbury/Riverlea residential area (three schools), Alexandra (two schools), and Soweto (two schools).

Parents or guardians of all children attending grade one in the selected schools were informed about the study in writing. Those providing written, informed consent for the participation of their children in the study were requested to complete a questionnaire. Structured questionnaires designed to obtain essential information related to, among other factors, socio-economic status, potential sources of lead in the environment, school performance, play activities, general health status, pica, and living conditions were completed by parents or guardians (Mathee et al., 2002).

In Cape Town there was a total 732 children attending grade one, of which 505 (69%) parents/guardians gave consent for blood collection and returned completed questionnaires. The total number of blood samples collected was 430, as in some instances a child was absent from school at the day of the survey or refused to donate blood, or there was a problem with drawing blood. In Johannesburg there was a total 777 children attending grade one, of which 441 (57%) parents/guardians gave consent for blood collection and returned the questionnaire. The total number of blood samples collected was 384.

Venous blood samples were collected from participating children and analysed for manganese content. The total number of blood samples collected in both cities was 814. At each school, samples of drinking water, classroom floor dust (by sweeping 1 $m^2$ in the middle of the classroom), and soil from the school playground (to a depth of 3 cm), were also collected for analyses. Three schools in Cape Town did not have a playground that contained soil. Trace metal-free tubes were used for the collection of blood samples, and acid-washed containers were used for the collection of environmental samples. The manganese content in the blood and water samples was analysed using flameless atomic spectroscopy (Varian Spectr AA-30 with Zeeman background correction). The manganese content in soil and classroom dust was measured by inductively coupled plasma techniques (Varian Vista AX CCD Simultaneous ICP-AES).

Standard quality control procedures were adopted throughout, and certified standards were used to validate the manganese content of blood, water, soil, and dust samples. For blood manganese determinations "Seronorm Trace Elements in Whole Blood" (MR9067 level 2) was used for method validation and as a quality control. The detection limit for manganese in blood equaled 0.0864 μg/L. Certified Reference Materials were used for soil (Montana Soil SRM 2711, National Institute of Standards & Technology (NIST)) with mean recoveries of 90.4%.

### 2.1. Statistical analysis

A comparison of environmental manganese levels between the two centres was carried out using a two-sample $t$ test. The comparison of the mean blood manganese levels between the two centres was made using a linear mixed model. This technique is appropriate to account for the clustering of pupils into schools; i.e., the sampling units were schools, but the observational units were pupils within schools (Donner, 1998; Murray, 1998). These models were also used to adjust for potential

confounders such as population group, as well as to investigate the relationship between blood levels in pupils and the environmental manganese levels.

The prevalence of potential confounding factors, such as proximity to busy roads and walking to school, was compared between the two cities using the Rao and Scott (1981, 1984) correction to the standard Pearson chi-square test statistic for two-way contingency tables, in order to take into account the clustering of children within schools. All statistical analyses were carried out using Stata Version 7 (StataCorp., 2001).

## 3. Results and discussion

### 3.1. Results

Overall characteristics of the study population are shown in Table 1 (only for those pupils for whom we had blood manganese results). The age and gender distributions in the two cities are very similar.

Racial classification under former apartheid legislation created four distinct "population groups"—White, Indian, Coloured, and Black—with unequal access to education, employment, land tenure, and healthcare. Differences in health status between these different groups is pronounced, and they are strongly associated with differences in socioeconomic status and environmental conditions within each of the different areas traditionally "reserved" for the four population groups under the 1966 Group Areas Act (repealed in 1991).

It was found that the distribution of the population groups differed between the cities, with Cape Town having a higher proportion of Coloured (mixed race) children and Johannesburg having a higher proportion of Black African children. Most socioeconomic variables had similar distributions in the two cities; from Table 1, it can be seen that the proportion of homes using electricity for cooking and heating was similar in the two centres. Children in Johannesburg were found to be significantly older than children in Cape Town ($P < 0.0001$), but this finding is artefactual since the survey was carried out later in the year in Johannesburg. In Johannesburg, 82% of children walked to school, compared to 61% in Cape Town, but this difference did not reach statistical significance ($P = 0.069$). Similar proportions of respondents in the two centres lived in houses as opposed to flats or informal houses (58% in Cape Town compared to 61% in Johannesburg, $P = 0.45$) and the proportion living in close proximity to a busy road was similar (51% in Cape Town compared to 56% in Johannesburg, $P = 0.095$). In both cities, parents reported that children were eating nonfood items at the time of the study, with the prevalence of pica being significantly higher in Johannesburg than in Cape Town (39.0% versus 27.7%, $P = 0.014$).

The majority of parents in both centres reported their children to be in good health (98%). Table 2 shows common ill health conditions reported by parents in both cities. Most children were reported to normally have a good appetite.

The mean, standard deviation, median, and range of manganese in the blood of pupils and in the water, soil, and classroom dust of the schools are shown in Table 3. The environmental manganese concentrations were found to be higher in Johannesburg, with the results being statistically highly significant for soil ($P = 0.0007$) and dust ($P = 0.0071$). The concentration of manganese in water was found to be higher in Cape Town but within normal nontoxic levels (National Research Council (US), 1980). These differences were not statistically significant ($P = 0.066$).

The blood manganese concentrations were compared between children in Johannesburg and in Cape Town. The mean level in Johannesburg was 9.80 μg/L

Table 1
Study population

| | Cape Town | Johannesburg |
|---|---|---|
| No. of schools | 11 | 10 |
| No. of children with Mn results | 430 | 384 |
| Gender (%) | | |
| Male | 50.4 | 52.8 |
| Female | 49.6 | 47.2 |
| Mean age in years (SD) | 6.7 (0.55) | 7.1 (0.75) |
| Population group (%) | | |
| African | 35.7 | 64.4 |
| Coloured | 58.4 | 34.2 |
| White | 5.9 | 1.4 |
| How child gets to school (%) | | |
| Walk | 61.0 | 82.4 |
| Public transport | 14.5 | 11.2 |
| Private car | 24.5 | 6.4 |
| Proportion of children perceived to be in good health (%) | 98 | 98 |
| Fuel used for cooking (%) | | |
| Electricity | 88.8 | 85.8 |
| Solid fuel | 0.7 | 2.1 |

Table 2
Reported ill health conditions

| Health symptoms | Johannesburg (%) | Cape Town (%) | *P* value |
|---|---|---|---|
| Abdominal pains | 12.6 | 9.4 | 0.24 |
| Convulsions | 0.9 | 1.5 | 0.43 |
| Anaemia | 0 | 1.6 | 0.0004 |
| Tiredness | 1.7 | 2.9 | 0.16 |
| Headache | 18.8 | 12.5 | 0.046 |
| Constipation | 4.3 | 4.4 | 0.99 |
| Diarrhoea | 1.9 | 2.7 | 0.42 |
| Vomiting | 5.1 | 3.6 | 0.35 |
| Inability to concentrate | 11.5 | 5.4 | 0.0026 |
| Irritability | 3.6 | 5.8 | 0.21 |

Table 3
Comparison of mean blood and environmental manganese levels between Cape Town and Johannesburg

| | Manganese in | | | |
|---|---|---|---|---|
| | Blood (μg/L) | Water (μg/L) | Soil (μg/g) | Dust (μg/g) |
| Cape Town | | | | |
| *N* | 430 | 11 | 8 | 10 |
| Mean | 6.74 (3.47) | 3.4 (4.3) | 31.9 (35.4) | 72.5 (22.6) |
| Median | 6.2 | 1.94 | 18.1 | 77.5 |
| Range | 1.6–32.8 | 1–15.68 | 10.5–115 | 38–99 |
| Johannesburg | | | | |
| *N* | 384 | 10 | 10 | 9 |
| Mean (SD) | 9.80 (3.59) | 0.72 (0.94) | 469.5 (292) | 404 (342) |
| Median | 9.2 | 0.65 | 436 | 314 |
| Range | 3.6–26.5 | 0–3.1 | 117–904 | 17–959 |

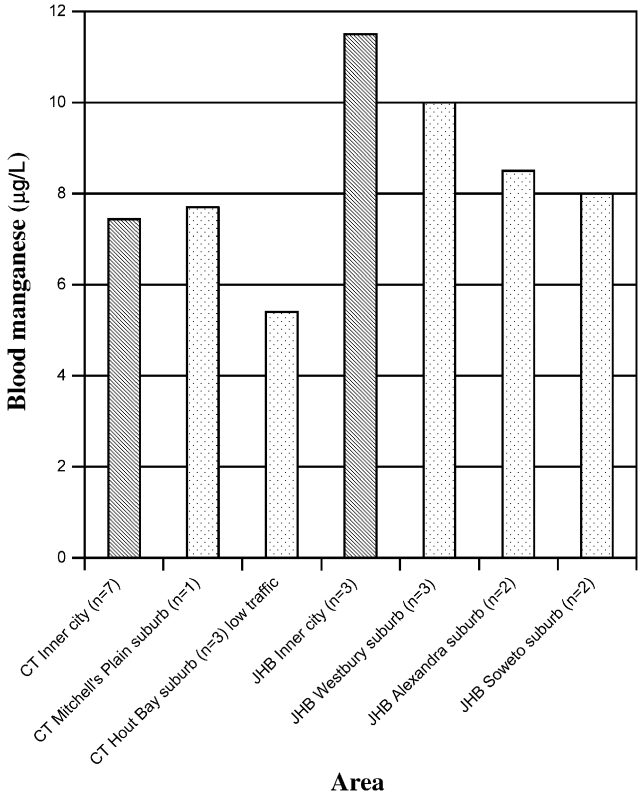


Fig. 1. Mean blood manganese levels by city area (CT, Cape Town; JHB, Johannesburg).

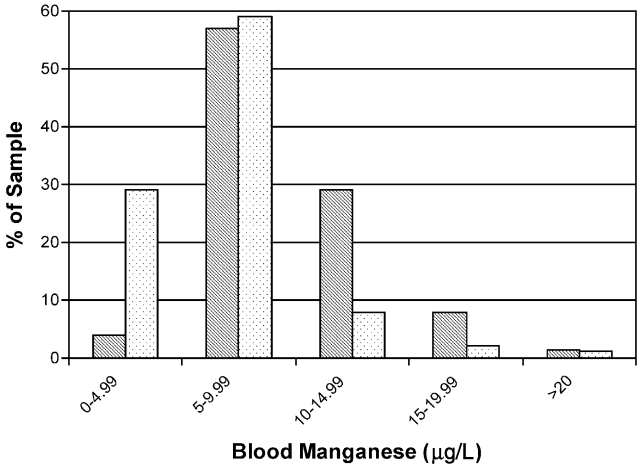


Fig. 2. Distribution of blood manganese levels in Johannesburg (▨) and Cape Town (□).

compared to a mean level of 6.74 μg/L in Cape Town.

Figs. 1 and 2 show the mean blood manganese levels in different areas and the distribution of blood manganese levels in both cities, respectively. Statistically, the concentrations were compared between centres using a linear mixed model to take account of the fact that children were clustered into schools and to adjust for confounders. The potential confounders considered were age, gender, and population group, how the child traveled to school, the fuel used for cooking, and whether or not the child put nonfood items in his/her mouth (pica). The covariates found to be important and retained in the model were age (mean blood manganese levels decreased with age), gender (the mean blood manganese level was slightly higher for females), and population group (Black Africans had a mean blood manganese level lower than that of the other population groups). Adjusting for these, the mean concentration in Johannesburg was 3.44 units higher than that in Cape Town (95% confidence limits for the difference are 1.92–4.96 μg/L). The blood manganese levels of 12.5% of the children in Johannesburg and 4.2% of the children in Cape Town equaled or exceeded 14 μg/L, the upper normal values as specified by the Agency for Toxic Substances Disease Registry (ATSDR, 2000). Further mixed models were fitted to determine the influence of mean environmental manganese levels on children's blood manganese levels. The level of

manganese in dust and the level of manganese in the soil were both found to approach statistical significance ($P = 0.10$); adjusting for these the difference between cities was still highly significant ($P = 0.001$).

## 4. Discussion

To date, the existing scientific knowledge related to the use of MMT has not adequately addressed the specific question of public health risk resulting from chronic, low-level environmental exposure to MMT and its manganese-containing combustion products (Zayed, 2001).

With the planned phase-out of the use of leaded petrol in South Africa by 2006, and the introduction of MMT having already commenced in certain inland areas (including Johannesburg), this investigation constitutes the first baseline study undertaken in South Africa to assess childhood manganese exposure in two of its largest cities, using both environmental and biological indices. The distance between the two cities is approximately 1500 km, reducing the likelihood of manganese transportation between the two cities.

It is of importance that this study found measurable differences in environmental and biological indices between the two cities, as well as among schools within the same geographical area. Highly significant differences in manganese concentrations in soil and classroom dust were measured, these being higher in Johannesburg than in Cape Town. A further, important consideration is the commencement in the Johannesburg region of the addition of MMT to petrol around 24 months prior to the study. Higher natural manganese levels, combined with industrial activities in Johannesburg, may have contributed to the higher environmental concentrations of manganese found in this city. With soil constituting a primary sink for MMT products (83%), followed by water (15%) and sediments (2.5%), it can be expected that young children, due to their hand-to-mouth behaviour, might be particularly sensitive to environmental manganese exposure.

Similarly, levels of manganese in blood were higher in Johannesburg children relative to those in Cape Town children, with 12.5% and 4.2% respectively, having blood manganese concentrations that equaled or exceeded the upper limit reference values of 14 μg/L (ATSDR, 2000). No suitable South African reference data for blood manganese levels in the study age group exist as the only published data are for children around 25 months of age (Subotzky et al., 1992).

In addition to route of exposure, age, diet, and population health status may influence the uptake of manganese and associated health outcomes. As the major route of manganese intake in nonoccupationally exposed subjects, including children, is food, the South African food intake data for the age group of 6–9 years were examined (Report on South African food consumption, 1983–2000). No significant differences in daily diet and types of food intake among children in this age group were found between the two cities. When profiling the environmental fate of manganese combustion products, a number of confounding factors particular to South Africa, and probably other developing countries, need to be considered, including ageing automobile fleets, poor maintenance and high tailpipe emission levels of combustion products, limited use of catalytic converters, inadequate air quality monitoring systems, and weak enforcement of legislation. In addition, the overall health status of the South African population is poorer than that of developed countries. Poor living conditions, high levels of unemployment, and high prevalence rates of communicable diseases, such as TB and HIV/AIDS, render the general population more susceptible to many environmental toxins. Children are at greater risk as they probably have higher absorption rates and have not yet fully developed an excretion mechanism for manganese. These factors indicate that environmental loading of manganese compounds, in the event of widespread use of MMT in petrol in South Africa, has the potential to result in higher emission levels, as well as altered health impacts relative to developed countries.

### 4.1. Conclusion

In this study, statistical differences in environmental and biological manganese loadings were found between the cities of Cape Town and Johannesburg, South Africa. The findings of this investigation will serve as a baseline against which to assess future potential longitudinal changes in environment and childhood blood manganese concentrations in the event of increased or continued use of MMT in petrol in South Africa. Further work is needed to differentiate between natural and anthropogenic sources of manganese.

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## References


Alessio, L., Lucchini, R., 1996. Manganese and manganese compounds. In: Argentesi, F., Roi, R., Sevilla Marcos, J.M. (Eds.), Data Profiles for Selected Chemicals and Pharmaceuticals: 3 2,4-Dinitrophenol, 4 Manganese and Manganese Compounds, 5 Trichloroethylene. Joint Research Centre, Ispra (I), European Commission (S.P.I. 96.59). World Health Org., Geneva, pp. 75–162.

Aschner, M., 2000. Manganese: brain transport and emerging research needs. Environ. Health Perspect. 108 (3), 429–432.

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

Baldwin, M., Mergler, D., Larribe, F., Belanger, S., Tardif, R., Bilodeau, L., Hudnell, K., 1999. Bioindicator and exposure data for a population based study of manganese. Neurotoxicology 20 (2–3), 343–354.

Bothwell, T.H., 1995. Overview and mechanisms of iron regulation. Nutr. Rev. 53, 237–245.

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 ($^{54}MnCl_2$) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. Toxicol. Appl. Pharmacol. 169, 238–248.

Crossgrove, J.S., Allen, D.D., Bukaveckas, B.L., Rhineheimer, S.S., Yokel, R.A., 2003. Manganese distribution across the blood–brain barrier. I. Evidence for carrier-mediated influx of managanese citrate as well as manganese and manganese transferrin. Neurotoxicology 24, 3–13.

Donner, A., 1998. Some aspects of the design and analysis of cluster randomisation trials. Appl. Stat. 47, 95–113.

Dorman, D.C., Struve, M.F., Arden James, R., 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-day) exposure. Toxicol. Appl. Pharmacol. 170, 79–87.

Health Canada, 2003. Assessment of toxicological risks of environmental contamination by manganese. TRSI-109. Toxic Substances Research Initiative, Montreal, Que., Canada.

Keen, C.L., Zindenberg-Cherr, S., 1990. Manganese. In: Brown,, M.L. (Ed.), Present Knowledge in Nutrition. International Life Sciences Institute, Washington, DC, pp. 279–286.

Levy, B.S., Nassetta, W.J., 2003. Neurologic effects of manganese in humans: a review. Int. J. Occup. Environ. Health 9, 153–163.

Lucchini, R., Selis, L., Folli, D., Apostoli, P., Mutti, A., Vanoni, O., Iregren, A., Alessio, L., 1995. Neurobehavioral effects of manganese in workers from a ferroalloy plant after temporary cessation of exposure. Scand. J. Work Environ. Health 21, 143–149.

Lynam, D.R., Roos, J.W., Pfeifer, G.D., Fort, B.F., Pullin, T.G., 1999. Environmental effects and exposures to manganese from use of methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline. Neurotoxicology 20, 145–150.

Lyznicki, J.M., Karlan, M.S., Khan, M.K., 1999. Manganese in gasoline. Council on Scientific Affairs, American Medical Association. J. Occup. Environ. Med. 41, 140–143.

Mathee, A., von Schirnding, YER., Levin, J., Ismail, R., Huntley, R., Cantrell, A., 2002. A survey of blood lead levels among young Johannesburg school children. Environ. Res. 90, 181–184.

Mena, I., Horiuchi, K., Burke, K., Cotzias, G.C., 1969. Chronic manganese poisoning: individual susceptibility and absorption of iron. Neurology 19, 1000–1006.

Mena, I., Horiuchi, K., Lopez, G., 1974. Factors enhancing entrance of manganese into brain: iron deficiency and age. J Nucl. Med. 15, 516.

Mergler, D., Hundel, G., Bowler, R., Iregren, A., Belanger, S., Baldwin, M., Tardif, R., Smargiassi, A., Martin, L., 1994. Nervous system dysfunction among workers with long-term exposure to manganese. Environ. Res. 64, 151–180.

Murray, D.M., 1998. Design and analysis of group-randomized trials. New York, Oxford University Press (Chapter 5).

National Research Council (US), 1980. Drinking Water and Health, Vol. 3. National Academy Press, Washington, DC, pp. 331–337.

Nriagu, J.O., Pacyna, J.M., 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 134–139.

Politis, M.J., Schumburg, H.H., Spencer, P.S., 1980. Neurotoxicity of selected chemicals. In: Spencer, P.S., Schumburg, H.H. (Eds.), Experimental and Clinical Neurotoxicity. Williams Wilkins, Baltimore, MD, pp. 613–630.

Rao, J.N.K., Scott, A.J., 1981. The analysis of categorical data from complex sample surveys: chi-squared tests for goodness of fit and independence in two-way tables. J. Am. Statist. Assoc. 76, 221–230.

Rao, J.N.K., Scott, A.J., 1984. On chi-squared tests for multiway contingency tables with cell proportions estimated from survey data. Ann. Statist. 12, 46–60.

Report on South African food consumption studies undertaken amongst different population groups (1983–2000): average intakes of foods most commonly consumed. Chronic Diseases of Lifestyle Unit, Medical Research Council of South Africa, 2001, webmaster@mrc.ac.za.

Roels, H., Meiers, G., Delos, M., Orbefa, L., 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.

Silbergeld, E.K., 1982. Current status of neurotoxicology, basic and applied. Trends Neurosci. 5, 291–294.

StataCorp., 2001. Stata Statistical Software: Release 7.0, Stata Corporation, College Station, TX.

Subotzky, E.F., De, V., Heese, H., Sive, A.A., Dempster, W.S., Sacks, R., Malan, H., 1992. Plasma zinc, copper, selenium, ferritin and whole blood manganese concentrations in children with kwashiorkor in the acute stage and during refeeding. Ann. Trop. Paediatr. 12, 13–22.

Szarfarc, S.C., de Souza, S.B., 1997. Prevalence and risk factors in iron deficiency and anemia. Arch. Latinoam. Nutr. 47, 35–38.

Takser, L., Mergler, D., Hellier, G., Sahuquillo, J., Huel, G., 2003. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology 24, 667–674.

Weiss, B., 2000. Vulnerability of children and the developing brain to neurotoxic hazards. Environ. Health Perspect. 108 (3), 375–381.

Yokel, R.A., Crossgrove, J.S., Bukaveckas, B.L., 2003. Manganese distribution across the blood–brain barrier. II. Manganese efflux from the brain does not appear to be carrier mediated. Neurotoxicology 24, 15–22.

Zayed, J., 2001. Use of MMT in Canadian gasoline: health and environmental issues. Am. J. Ind. Med. 39, 426–433.

Zayed, J., Thibault, C., Gareau, L., Kennedy, G., 1999. Airborne manganese particulates and methylcyclopentadienyl manganese tricarbonyl (MMT) at selected outdoor sites in Montreal. Neurotoxicology 20, 151–157.