Examining evidence for a protective occupational exposure limit for inhalable manganese dust below which there are no adverse subclinical nervous system effects.

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DECLARATION

I, Haidee Maxine Williams, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ii

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Contents

Abstract

Introduction

The 1996 American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for inhalable manganese dust (0.2mg/m^3) is currently under review given contradictory findings in the scientific literature with several studies showing no adverse effects at exposures above 0.2 mg/m^3. The ACGIH TLV Committee has requested us to examine the exposure-response relationship for inhalable dust and adverse nervous system effects using mean intensity of manganese as the exposure metric. This was accomplished by analyzing anew data from a previous study which reported on the adverse subclinical nervous system effects of manganese exposure measured as a cumulative exposure index integrating inhalable manganese concentration and duration of exposure across jobs worked. It was not possible to separate the effects of inhalable manganese concentration and duration of exposure to manganese in that analysis.

The aim of this study was to investigate the exposure-response relationship between the concentration of inhalable manganese dust and subclinical nervous system outcomes amongst workers in a South African manganese smelter with a view to identifying any exposure threshold below which adverse effects do not occur.

Methods

The data were derived from a cross sectional study performed in 1999-2000 in a manganese smelting works in South Africa to study the neurological and neurobehavioral effects of manganese exposure using an integrated exposure concentration- time or cumulative exposure index as the exposure metric.

509 workers differentially exposed to manganese and a reference group of 67 unexposed electrical assembly plant workers were included in the study, 442 personal inhalable dust samples were taken. The mean exposure intensity across all jobs was obtained by summing the products of the mean manganese inhalable dust concentration for each job worked by the participant (taken from the job exposure matrix) and the number of years this work was performed. The cumulative exposure thus calculated was divided by the number of years worked to give a mean exposure intensity per participant.

Nervous system outcomes were measured by the Swedish nervous system questionnaire (Q16), World Health Organisation neurobehavioral core test battery (WHO NCTB), Swedish performance evaluation system (SPES), items from the Luria-Nebraska (LN) and Danish product development (DPD) test batteries and a brief directed clinical examination. Potential confounders and effect modifiers included length of service, age, standard passed at school, previous head injury, past jobs with exposure to neurotoxicants, smoking history and alcohol consumption and a proxy variable for ethnicity based on home language and surname. Associations were modelled using multiple linear and logistic regression modelling. A panel of results was obtained for the exposure-response relationship including examining the effect of exposure as a dichotomous variable, as a continuous variable, and with multiple categories with meaningful cutpoints chosen to examine more closely the shape of the exposure response relationship.

Results

The mean inhalable manganese dust concentration was 0.8 mg/m^3 (SD: 1.04, IQR: 0.1- 1) with the exposed participants having worked a mean of 18.2 years (SD:7.6). The results of the 128 outcomes tested could be divided into three groups. Group 1 contained those results showing only an exposure -response relationship with exposure as a dichotomous variable i.e. a difference between the external unexposed referents and all the exposed

and/or between the internal unexposed workers and the rest of the exposed. The Santa Ana Pegboard test and the Benton visual retention test from the WHO NCTB, two SPES tests (endurance tapping and tapping with the dominant hand), one sway test (eyes open and feet insulated), the Luria Nebraska item 2L and questionnaire items irritation and feeling tired fell into this group. Group 2 was the smallest and included exposure-response relationships shown not only with dichotomous exposures but also as a linear trend with exposure as a continuous variable. These results included the digit span and digit symbol test from the WHO NCTB, and the questionnaire item: having less sex than peers. Group 3 comprised those results that either showed no exposure outcome relationships or those that were counterintuitive. This group included the overwhelming majority of the results and included most of the SPES tests, the tremor tests, the sway tests, the Luria Nebraska tests, the questionnaire items and the clinical examination item.

Conclusion

This was essentially a negative study, despite the fact that a large number of workers with a wide range of manganese dust exposures well above and below the current ACGIH TLV were studied. Chance is the most likely explanation for the few, statistically significant exposure-relationships found.

There was a paradoxically steep exposure-response relationship at very low exposures that does not make sense as manganese is an essential trace element. This together with the uniformly flat relationship at exposure levels above 0.7- 1mg/m^3 provided no additional evidence for setting a meaningful protective threshold level in this exposure range below 4mg/m^3, given few exposures higher than this level.

Chapter 1 : Introduction

Manganese (chemical formula - Mn) is a common element in the earth's crust and can be found in soil, rocks, water and food. Elemental manganese is a lustrous pink, grey metal that resembles iron but is more brittle.[1, 2] Manganese is highly reactive and exists in a number of oxidation states with MnO_2 (pyresulite) being it's most common ore. [1,2,3]

South Africa is the second largest producer of manganese ore after China and between 2004 and 2007 18% of the manganese ore imported to the United States originated from South Africa.[4] The major uses of manganese include alloying with metals such as steel to increase hardness, using it as a catalyst, in the production of dry cell batteries and in the manufacture of matches, fireworks and porcelain.[1, 2,5]

Manganese occurs in foods such as nuts, cereals, legumes, grains, rice, fruit, vegetables and tea.[2,3,6] As such it is a normal and essential component of the human diet. The concentration of manganese in foods varies widely and can reach 100mg/kg in rice and wheat.[3] People who consume a predominantly vegetarian diet will have a higher daily intake. Manganese is essential for energy metabolism, nervous system function, reproductive hormone function and as an antioxidant.

Very few cases of toxicity via oral intake have been observed. This is most likely because manganese is not well absorbed orally and is rapidly eliminated. However, people with iron deficiency have an increased rate of absorption.[2] Those cases that have been described have been in people exposed to drinking water contaminated by dry cell batteries buried near a water source,[7] patients receiving total parenteral nutrition [8]and in those with chronic liver failure.[9,10,11,12] Da Silva et al have also described symptoms of manganese toxicity in patients undergoing maintenance haemodialysis. [13]

There have also been case-series reported of intravenous drug users from Russia, Estonia and the Ukraine who inject ephedrone (a recreational drug made from pseudo-ephedrine), developing symptoms of manganism.[14, 15] Sikk et al studied the mixture and it was found to contain 0.6g/ℓ of manganese. [16]

Case reports of manganese deficiency from artificially low dietary intake has been described in humans and the symptoms consist of dermatitis, decreased serum cholesterol levels, slowed growth of nails and hair and decreased clotting protein levels. [6,17]

In contrast to the oral route, it has long been known that manganese can be toxic via inhalation. The first reports of neurological effects were described in 1837 by John Couper amongst workers in a manganese ore crushing plant. He called the syndrome "manganese crusher's disease".[18]

Manganism typically occurs at chronic, high exposures to manganese dust with cases reported at long term exposures ranging from 2 to 22mg total manganese dust/m^3.[2] As a result of this, the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) was 5mg/m^3 from 1982 until 1995 and the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL)[19]as well as the South African occupational exposure limit (OEL)[20]is still set at this level. The Institute for Environment and Health (IEH) in 2003 reported no cases of manganism at levels under 5mg/m^3.[21] In recent years, the occurrence of frank manganism has been rare but the focus of concern is now the possibility of subclinical neurological effects at low levels of manganese exposure.

This study follows a request from the United States based ACGIH. The ACGIH is the premier standard setting body for occupational exposure limits globally. These limits are set in the form of Threshold Limit Values (TLVs) after review by the ACGIH TLV Committee of all available evidence pertinent

to the setting of protective exposure limits. TLVs are defined by the ACGIH as: *"airborne concentrations of chemical substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed, day after day, over a working lifetime, without adverse health effects. TLVs® are developed to protect workers who are normal, healthy adults."*[22] The 1996 TLV for inhalable manganese dust is 0.2mg/m^3.[1] This is currently under review given contradictory findings in the international health literature with several studies showing no adverse effects at exposures above 0.2mg/m^3. The Committees has requested us to produce evidence relating to the exposure-response relationship of inhalable dust and adverse nervous system effects. This was accomplished by analysing anew data from a previous study which reported on the adverse sub- clinical nervous system effects of manganese exposure measured as a cumulative index which integrated manganese concentration and duration of exposure for subjects.[23] It was not possible to disaggregate manganese concentration and duration of exposure in that analysis. A substantial new analysis is required to examine these two effects independently in order to contribute evidence for a suitable TLV which is essentially a pure exposure concentration measure.

Aim

To investigate the exposure-response relationship between the concentration of inhalable manganese dust and subclinical nervous system outcomes amongst workers in a South African manganese smelter with a view to establishing an exposure threshold below which adverse effects do not occur.

Objectives

- To source participants from different exposure profiles ranging from external subjects totally unexposed occupationally to manganese through various degrees of occupational exposure (mild, moderate, high) in a manganese smelter works.

- To describe the nervous system effects within these participants.
- To derive estimates of the exposure-response relationship between inhalable manganese dust concentration and potentially adverse subclinical nervous system effects.
- To adjust estimates for confounders or effect measure modifiers, viz. duration of exposure
- To assess where a threshold exists in the exposure-response relationship below which there is no observable adverse nervous system effects, expressly for inhalable dust.

Glossary of Abbreviations, Symbols, Terms and Definitions

AINT: Mean exposure intensity for manganese dust across all jobs.

Mean: Arithmetic mean

CEI: Cumulative exposure index—an integrated measure comprising job-specific manganese exposure intensity times length of service in each job, summed across all jobs.

DPD: Danish Product Development

GM: Geometric mean – a mathematical formula that tends to dampen the effect of very high or very low numbers which might bias the mean if a straight arithmetic mean is used.

GME: The Government Mining Engineer, South Africa.

Inhalable dust: Dust particles in the inhalable fraction collected by the UK IOM sampling head.

IEH: Institute for Environment and Health

IOM: Institute of Occupational Medicine, Edinburgh, UK.

LOS: Length of service in years on manganese smelters.

Respirable dust: Dust particles in the respirable fraction collected by the Dorr-Oliver cyclone

SD: Standard deviation

SPES: The Swedish Performance Evaluation System neurobehavioral test battery.

TLV: Threshold Limit Value - Concentration in air of a substance to which it is believed that most workers can be exposed daily without adverse effect

Total dust: Dust particles of all sizes collected on an open faced sampling head.

US NIOSH: United States National Institute of Occupational Safety and Health.

WHO-NCTB: World Health Organization neurobehavioral core test battery.

Chapter 2 : Literature Review

This review will be divided into a discussion on assessment of exposure, outcome and a discussion on the current thought on the exposure-response relationship.

Assessment of exposure

Exposure can be assessed via environmental exposure monitoring and absorption through biological exposure monitoring.

Environmental exposure monitoring methods

Sampling manganese concentration in air involves drawing air through a filter sampler at a set rate, and then analysing the amount of manganese left on the filter.

This fairly simple process has been complicated by the use of two categories of filter samplers, namely the Institute of Medicine (IOM) inhalable sampler and a variant on the "Millipore" 37 mm cassette sampler to collect samples of total dust. [21] Unfortunately the sampled concentrations between these two devices will not be directly comparable because of the differences in their sampling efficiencies. The IOM sampler will in almost all cases declare a higher concentration with a ratio ranging from 1.2 to 3 depending on the particle size distribution. The difference is most marked for "coarser" dust.[21,24] Myers et al [25] as part of a study on manganese exposed miners performed a validity study comparing the IOM sampling head to the 37mm cassette sampler. The authors found that the 37 mm sampler measured dust levels systematically lower than the IOM sampler. This systematic difference in dust measurement increased with an increased manganese concentration. Bast-Pettersen et al [26] also performed parallel sampling and in the

manganese-alloy industry studied, the closed- face sampler underestimated exposure by a factor of 2 when compared to the IOM sampler.

The summary work life exposure metric also differs across studies, certain authors use a calculated cumulative exposure index (CEI) whilst others use mean inhalable dust intensity and still others report their findings using the respirable fraction as the exposure metric. The cumulative exposure index is the calculated sum of the measured exposure in each job multiplied by the time spent in that particular job. The CEI is thus an integrated measure of both intensity and time. The mean concentration (or mean grade) is calculated by dividing the CEI by the total time exposed. The respirable fraction is that fraction of the total dust that consists of particles less than 5-7μm in diameter. This fraction penetrates to the gas exchange region of the lung.

The choice of summary measure used depends partly on the relative weight placed on duration of exposure as compared with time of exposure and partly on the biological theories of systemic absorption. Whilst the CEI is a good measure of total "dose" that the worker has been exposed to, it is not possible to easily calculate an occupational exposure limit from this aggregated summary measure of exposure. In circumstances where the mechanism of toxicity is uncertain or controversial it is wise to use more than one summary measure as the results may differ. Using more than one metric also assists with comparing results across different studies.[27]

It has been argued that the respirable fraction of manganese is the "most biologically appropriate measure of exposure to airborne manganese" [21, pg 5] as, the authors argue, much of the inhalable fraction would be swallowed and enter the gastrointestinal tract. The authors' contend that manganese is poorly absorbed by the gastrointestinal tract and therefore the respirable fraction is more closely associated with systemic toxicity. This may well be correct, however the mechanism of action of toxicity via inhalation is poorly

understood and the possibility of nasal deposition and absorption via the olfactory neurones has been postulated. [28] We are also unable to discount the effect of swallowing large quantities of manganese, as is quite likely in very dusty environments like the ferroalloy industry. It therefore continues to be of value to explore the exposure response relationship using the mean inhalable concentration as the exposure metric.

Biological exposure monitoring methods

Many studies have been done to evaluate possible biomarkers for manganese exposure and whether these correlate with adverse health effects. The presence of manganese was investigated in blood, urine, hair and more recently high signal intensities on T1- weighted magnetic resonance imaging (MRI) of the brain.

For a biomarker to be useful it has to be an accurate gauge of environmental exposure levels. This means that the relationship between levels of manganese in the environment and in the body has to be predictable. The utility of the biomarker has to be validated. Validation studies are often difficult as the biomarker has to be specific to external manganese exposure, has to be over and above normal dietary exposures and has to exclude the impact of disease states. Since manganese is an essential nutrient and is present in all tissues, dietary intake as well as vitamin supplements needs to be documented. The levels of manganese in tissues are also altered in certain disease states e.g. liver cirrhosis.[29]

Notwithstanding the above, the most common biomarkers are manganese in blood and urine. Myers et al [30] in their study on the utility of biomarkers found that manganese in urine was not a useful biomarker (manganese is primarily excreted in bile), but that mean whole blood manganese could be used as a screening tool for manganese exposure at the group level. Manganese in blood is not useful at the individual level because of the high intra-individual variability [17,21]and because studies have found that

manganese in blood is not correlated with either length of exposure or concentration of environmental manganese.[31,32]

There has been increased interest in the role of MRI changes as a measure of effect; however studies have postulated that the high T1 signals characteristically found are more appropriately treated as a measure of exposure rather than effect.[33,34]The authors conclude this because the increased signals were highly prevalent in those workers exposed to manganese,[33] the signals were correlated with blood manganese and exposure intensity [34] and the signals disappear after removal from exposure.[33]

Assessment of outcome

There are two main categories of neurotoxic outcomes that have been described. A syndrome called manganism, and an amalgam of subclinical neuropsychiatric, neurophysiological and neurobehavioral signs.

Manganism

Effects at high concentration manganese exposure have long been described. The resultant constellation of symptoms has been called manganism. Manganism is a syndrome that resembles Parkinson's disease but with important differences. Clinically, as compared with Parkinson's, psychiatric symptoms have been noted early in the disease with manganism, there is less frequent resting tremor, dystonia is more pronounced, patients with manganism tend to fall backward and they have a staggering strut like gait "cock walk".[35,2]

The pathological differences include diffuse brain lesions in the case of manganism as compared to lesions concentrated in the pigmented areas in the case of Parkinson's.[2] Magnetic resonance imaging of the brain also differs as patients with manganism have a characteristic accumulation of manganese with little changes in those with Parkinson's.[35] Finally, in terms

of treatment patients with manganism do not usually respond to levo-dopa therapy.[35-37]

A comparison between Parkinson's and manganism has been detailed in Table 2-1.

Table 2-1 Comparison of Parkinson's disease with manganism[1]

Feature	Parkinson's disease	Manganese-induced parkinsonism
Clinical	Resting tremor, unilateral onset	**Early speech and balance dysfunction, symmetric impairment, relative absence of tremor, specific dystonia, early personality changes**
MRI (T1-weighted images)	Normal	**High signal intensity**
FD-PET	Decreased striatal uptake	**Normal**
Response to levodopa	Good	**Poor**
Pathology	**Degeneration of neurons in the substantia nigra pars compacta, locus coeruleus,nucleus basalis of Meynert, dorsal motor nucleus of the vagus, cortex, spinal cord, peripheral nervous system; Lewy bodies**	**Degeneration and gliosis of neurons in globus pallidus, no Lewy bodies.**

Subclinical neurological endpoints.

If we are to expand on the theory of a "continuum of dysfunction" as suggested by Mergler et al[38] we would expect to detect early signs of neurotoxicity. Manganism is not always reversible on cessation of exposure; in fact deterioration of symptoms may occur.[39] It is thus important to be able to detect early changes in neurological dysfunction thereby enabling prevention of progression of dysfunction. To this end various clinical tests have been investigated to identify early adverse nervous system changes.

[1] Based on Olanow [37]and Jankovic[36]

Iregren[40] proposed what he called a 'core' set of tests for the detection of neurotoxic effects of low level manganese exposure. These tests were developed after reviewing the literature and exploring the pattern of effects described. Of the six studies reviewed, motor function and hand steadiness were affected in all and response speed and memory capacity in five. Iregren therefore suggested that the test batteries used to detect early signs of neurotoxicity include tests on motor function, response speed, memory and rating scales for mood and subjective symptoms.

In the review article by Mergler and Baldwin ,[41, pg 98] they expanded on the above by specifying "a pattern of slowing motor functions, increased tremor, reduced response speed, enhanced olfactory sense, possible memory and intellectual deficits, and mood changes." The authors also state that motor tasks that require co-coordinated alternating movements at high speed were the most vulnerable to manganese exposure. Iregren [42] in 1999 added that the ability to repeat simple movements may be particularly sensitive.

Exposure response relationship

Manganism

There are fewer than 500 reported cases of clinical manganism in the literature and almost all of these occurred at very high exposure levels. [43,1,6]

In recent years there has been an epidemic of "manganism" in welders despite a detailed review by Jankovic [36, pg 2026] concluding that "[e]pidemiologic, experimental, or other studies, or standard textbooks of PD [Parkinson's disease] and of other movement disorders, do not provide convincing evidence that welding is a significant risk factor for PD and for parkinsonism". The objectives of this study focus on subclinical nervous system effects, therefore detailed further discussion on manganism in welders is beyond the scope of this paper.

Review of studies focusing on neurotoxicity at low levels of manganese exposure

The effect of low levels of manganese exposure on the nervous system is still controversial despite numerous studies. What clouds the water further is the fact that different parameters of exposure are used and different neurological outcomes assessed.

In 1992 Roels et al [44] performed a study that compared 92 workers exposed to MnO_2 dust in a dry alkaline battery with 101 matched controls. The authors found that the manganese exposed workers performed poorly on the visual reaction time, eye hand coordination and tremor tests as compared with the controls. There was no difference between the groups on the short term memory scores. The geometric mean (GM) of respirable manganese sampled was 215µg/m^3 (range 21-1317). The authors attempted to establish a threshold effect level using tremor as the parameter of interest. They concluded that a CEI of respirable manganese dust of 730µgyear/m^3 or total dust of 3575 µgyear/m^3 may lead to an increased risk of tremor. The outcomes of this study may have been influenced by a selection bias as all the exposed workers were volunteers (102 volunteers out of a workforce of 1100). These workers may have volunteered because they had experienced symptoms of concern and may not have been a representative sample of the exposed worker population.

Bast-Pettersen et al [26] also found that manganese exposed workers had increased postural tremor as compared with unexposed workers. However in contrast to the study by Roels [44] and the expected pattern of effects as postulated by Iregren,[40] she did not find any difference with regard to reaction time. However when comparing only the respirable fraction the exposure level in this study was lower than in the Roels' study.[44] The geometric mean of inhalable manganese was 301µg/m^3 (range 9 to 11,457) and respirable manganese 36 µg/m^3 (range 3 to 356).

A study published by Deschamps, Guillaumot and Raux [32] in 2001 compared manganese exposed workers in an enamels production company with a control group. The current exposure of workers to respirable manganese was much lower than the study performed by Roels,[44] with a mean (the authors do not clarify whether this was a geometric or arithmetic mean) respirable manganese level of 57.2 µg/m^3 (range 10-293). The exposed group had a higher prevalence of symptoms such as asthenia, sleep disturbance and headache than the unexposed controls. These symptoms are all subjective, and as raised by the authors there may have been a biased response. No dummy questions to test the validity of the questionnaire were included. Alcohol use, an important confounder, was not investigated. No difference was found when comparing psychometric tests or on neurological examination. The authors made use of matched controls therefore there was a good comparison of demographics between the exposed and control group. Of concern is that the controls were excluded on the basis of occupation such as painting, chemical use or production therefore they were not representative of the population from which the exposed workers arose. Despite the profound reservations raised about the methodological rigor of this study, it was essentially a negative study amongst workers exposed to low levels of manganese.

Myers et al [23] reported on a cross sectional study comprising 509 smelter workers with varied manganese exposure and 67 unexposed external controls. The smelter mean CEI for inhalable manganese was 16mg year/m^3 (SD 22.4). Myers did find isolated differences between all exposed and external unexposed and/or between internal low exposed group and the rest of the exposed, but these did not show a robust dose-effect relationship. Therefore, despite a range of manganese exposures above and below the ACGIH TLV, large sample size and a wide-ranging array of neurological endpoints tested, the authors concluded that this was essentially a negative study.

Young et al [45] re-analysed the data collected by Myers et al[23] using the respirable fraction of manganese dust as the exposure metric. The median respirable average intensity of manganese exposure was 0.058mg/m^3 with a range from 0 – 0.51 mg/m^3. Despite 29% of workers exposed to levels above 0.1 mg/m^3 (the proposed IEH OEL for respirable manganese dust), the results were similar to those reported in the study by Myers[23] using inhalable dust as the exposure metric.

In 2007 Blond et al [46,47] published two papers on a study they had conducted amongst steelworkers exposed to both lead and manganese. In order to have longitudinal data, only workers who had previously participated in two industry cross sectional studies were recruited. The first paper focused on cognitive function and the second on neuromotor function. Unfortunately 53 participants out of an invited 92 agreed to the cognitive function test and 60 to the neuromotor assessment. The authors had access to past blood manganese samples taken in 1989 and 1995 and compared the non-participants to the participants. The non participants had significantly higher blood manganese than the participants. The exposure measures were those taken presumably as part of the plants' occupational hygiene programme. In the cognitive assessment they found the steel workers to be quicker but less accurate in some tests than the reference group. There was no difference in the neuromotor functioning between the exposed groups and the referents. This study was conducted amongst workers exposed to both lead and manganese, both neurotoxicants, and therefore even if an exposure response relationship had been identified, it would have been impossible to disentangle the two effects

Greiffenstein and Lees-Haley in a recent meta-analysis of the neuropsychological effects of manganese exposure concluded that "it was premature to conclude that occupational exposure to Mn causes early brain damage". [48, pg 124]

To summarize, there is limited and conflicting evidence relating to the exposure-response relationship of inhalable dust and adverse nervous system effects. Studies using different exposure metrics have been reported that show no association between manganese dust levels and neurological outcomes at exposures above the current TLV. A substantial new analysis is required that disaggregates inhalable dust and length of exposure to examine these two effects independently, in order to provide evidence for a suitable TLV which is effectively a pure exposure concentration measure.

Chapter 3 : Methods

Study Design, Population and Sampling

This study data set was obtained from a cross sectional study performed in 1999-2000 in a manganese smelting works in Gauteng, South Africa to study the neurobehavioral effects of occupational manganese exposure.[23]

The manganese smelting production process has been described in detail by Myers et al,[30] therefore only a brief description will be given here. The manganese ore comes into the works by rail and is taken to the raw materials yard. Here it is crushed and sorted, transported to the smelting plant furnaces where the ore is smelted using the Soderberg technique. The Soderberg process is quite an old technique and entails the addition of coke and coal tar pitch to the anode in the smelter. It is generally uses more energy and results in higher worker exposure than newer techniques.[49] The molten ore is then tapped from the bottom of the furnaces and poured into casts. Once the casts have cooled, they are transported to the final product yard where they are crushed, sorted and distributed. The smelter had been in operation for more than 50 years and few changes in exposure control had been made in that time. There were no engineering controls to decrease exposure except for water misters at the raw and final materials handling yard. Personal protective equipment in use consisted of disposable filtering respirators.

For sampling purposes, the production environment at the works was divided into three exposure areas, based on expert opinion, namely high (including three ferro- manganese smelters), medium (including one ferro-silicon smelter, raw and finished manganese materials handling plants), and low exposure areas (including quality control laboratories, administration and security workers, and a chemical plant unrelated to manganese on the periphery of the works). The low exposure group served as an internal exposure control. High inhalable manganese exposure was considered to be

above 2mg/m^3, medium between 2 and 0.1mg/m^3 and low exposure below 0.1mg/m^3. All the plants were in the same works and thus even the low exposure areas had exposure to fugitive manganese dust. For that reason, an external group of workers from an electrical fittings assembly plant in a non-industrial town bordering on Johannesburg and 100km distant from the smelter were included in the study to serve as an external unexposed referent population.

Up to two hundred participants were randomly selected from each of the three exposure groups. Ultimately 509 workers, from a total of 1380 workers at the works, differentially exposed to manganese and a reference group of 67 unexposed electrical assembly plant workers were included in the study. Maintenance workers could not be placed in any of the exposure categories due to the highly variable nature of their jobs and they were therefore excluded from the study.

Exposure measures

The work areas and specific jobs were grouped according to exposure level as discussed above. Participants chosen to wear the sampling pumps originated out of each of these homogenous exposure groups. In order to have 90% confidence that at least one individual from the highest 10% of an exposure group was captured, the NIOSH method[50] of sampling for atmospheric manganese was employed. Full-shift personal breathing zone samples were obtained using the Gilian ® Gilair personal air sampling pumps utilising a flow rate of 2ℓ/min. Institute of Medicine (IOM) sampling heads with 25mm x 1.2 µm pore size, mixed cellulose-ester membrane filters were connected to the pumps by means of Tyvek ® tubing.

All air samples were analysed using a modified version of the NIOSH method 7300 which was designed to optimise the sensitivity of manganese detection.[51] The filters were removed from the IOM cassette, the cassette rinsed with deionised water and this rinsate added to the filter samples. This was done to

ensure that all dust that might have remained in the cassette was removed. The filters were digested with a CEM MARSX microwave digester and a mixture of hydrochloric acid, nitric and hydrofluoric acid added to digest the manganese and any silica compounds. All the analyses were done with a Varian ® Vista simultaneous inductively coupled plasma optical emission spectrometer. In- house quality control was ensured by spiking blank filters with stock manganese solution. These filters were analysed as above.

The sampling method employed ensured that arithmetic mean intensities for different jobs and workplaces were available for all production jobs. These exposure estimates were then used to populate a job exposure matrix (JEM) of the plants by jobs.

The mean exposure intensity (INT) across all jobs was obtained by summing the products of the mean manganese inhalable dust concentration for each job worked by the participant (taken from the JEM) and the number of years this work was performed. The cumulative exposure thus calculated was divided by the number of years worked to give a mean exposure per participant. The formula for INT is as follows:

$$INT = \{(Mn[C]_1 \times yrs_1) + (Mn[C]_2 \times yrs_2) + (Mn[C]_n \times yrs_n)\} / (yrs_1 + yrs_2 + yrs_n)$$

First voided urine samples, venous blood samples and 2 weeks growth of toenail clippings were also collected. Blood specimens were collected daily during the tests with precautions taken to avoid contamination. The samples were sent to independent laboratories in order to test for manganese in urine and blood as well as serum prolactin.

Outcome measures

Nervous system outcomes were measured by questionnaire, neurobehavioral tests and quantitative neurometric testing. A brief clinical examination was also performed.

Questionnaire

Neurological outcomes were evaluated by questions drawn from the Swedish Q16 instrument[52] and the WHO NCTB questionnaire.[53] This questionnaire aimed to elicit autonomic nervous system symptoms, subjective symptoms referable to the nervous system as well as neuropsychiatric questions which intended to measure mood and symptoms.

Questions on neurotoxic exposures in previous work, past medical history of head trauma, and nervous system disease were included. Potential confounders and effect modifiers were measured by questions on age, educational level, home language and alcohol and tobacco use. Dummy questions on ankle swelling and earache were included to ascertain reporting bias.

Neurobehavioral Tests

A review of the literature highlighted certain specific tests to be most appropriate. The battery compiled included 7 items (1, 2, 3, 4, 21, 22, 23) or 13 sub-items if you separate left and right testing motor function from the Luria-Nebraska battery.[54] From the WHO NCTB, the Benton visual retention test for memory, digit-span and the digit-symbol test for cognitive ability, and the Santa Ana pegboard test for motor function were selected. The simple reaction time test (this is an attention task measuring response speed) and finger tapping with the dominant and non-dominant hands (measures the fastest rate of repetitive movement) and finger tapping endurance (the change in finger tapping over time is assessed) for motor function were chosen from the SPES battery.[55]

Neurometric Tests

Quantitative neurometric testing was performed using a device produced by Danish Product Development.[56] These tests comprised the Catsys test for dysdiadochokinesis, the Tremor test for postural tremor and the Sway test on

a force platform for postural sway. The tremor test parameters include tremor intensity (m/s^2), median frequency (F50) standard deviation of F50 (sF50), and the harmonic index (HI) comparing the tremor spectrum with that of a single harmonic oscillation which has a HI=1.00. A characteristic of abnormal tremor is that it tends to consist of more regular oscillations as compared with a physiological tremor.[57]

The Catsys system measures the time between hearing a metronome at slow and fast rhythms and tapping or pronating and supinating the hand. The maximum tempo at which the movement can be maintained is also measured. These tests yielded 20 score parameters.

Depres et al [58] advised 4 conditions for the Sway test: eyes open, no insulation under the feet; eyes shut, no insulation; eyes open with insulation and eyes shut with insulation. For each condition a graph in two dimensions is created and a single composite score variable created from the movement of the stylus on the graph.

Brief Directed Clinical Examination

A brief directed clinical examination to test the glabellar reflex, to observe facial expression, and to observe gait and balance while walking backward on a line was conducted. Participants with gross limb abnormalities were excluded.

Data Management and Analysis

Data management

Categorical exposure variables were coded numerically with the category 0 as the reference or baseline category. The continuous exposure variable AINT (arithmetical mean exposure intensity) was categorised into 5 exposure categories using meaningful cutpoints, chosen to examine an exposure response relationship. These cutpoints were the ACGIH TLV, the lowest observed adverse effect level (LOAEL) which is half of the TLV, the South

African OEL for fume and twice that value. The 5 exposure categories and the unexposed external referents are detailed in Table 3-1.

Table 3-1 Categorisation of exposure variables [30]

Category names	Mean exposure intensity ranges (mg/m^3)	Significance	*n*
0	0	Unexposed external referents	**67**
1	0< x ≤ 0.1	LOAEL =0.1	**105**
2	0.1 < x ≤ 0.2	ACGIH TLV = 0.2	**50**
3	0.2 < x ≤1	SA OEL for fumes = 1	**235**
4	1< x ≤ 2	Company advisors safe level = 2	**59**
5	**>2**		**59**

Statistical analysis

Exploratory data analyses were performed using histograms, means, medians, standard deviations and interquartile ranges for continuous data. Univariate analysis summarized the distribution of each measured variable.

Bivariate analysis was employed to assess the nature of the unadjusted associations between nervous system outcomes or responses and average exposure intensity. Box and whisker plots were used to assess the association between continuous and categorical variables and smoothed plots with locally weighted robust regression [59] when the variables were both continuous.

Significant associations were explored further by adjusting for possible confounders namely age, years of schooling, smoking status, alcohol consumption, past job exposure to neurotoxins, previous head injury and a proxy variable for ethnicity based on home language and surname. Linear and logistic regression analyses using dichotomous exposure variables comparing the exposed participants to the truly unexposed referents

(ACEICAT2) as well as to the internal low exposed referents (AINTCAT2) were performed.

Modelling of the exposure – response relationship using multiple linear or logistic regression for continuous or categorical outcomes respectively was performed adjusting for confounding variables, as listed above, throughout. Exposure was handled as both a continuous variable (AINT) to assess the overall exposure response relationship and a categorical variable (AINTCAT). The latter allowed for additional analyses by examining the adjusted exposure-response relationship across categories of exposure (AINTCAT) to give a better idea of the shape of the exposure- response relationship.

All of the above were considered jointly as a panel of results for each outcome modelled against 4 different ways of treating exposure:

1. As a comparison between all of the exposed in the works with an external truly unexposed control group;
2. As a comparison between internal controls with minimal exposure with the rest of the exposed at the works;
3. As a continuous variable to model the overall (linear or logistic) exposure response relationship;
4. And as a categorical variable with 6 categories in order to examine more closely the shape of the exposure- response relationship across categories of exposure.

Finally this entire panel of results was interpreted for meaningfulness of the exposure response relationship.

This approach allowed for the identification of possible thresholds in the exposure- response relationship below which there might be no substantial risks of adverse neurotoxic effects. It also allowed for the in-depth exploration of the exposure-response relationships with a view to estimating the

existence, strength and statistical significance of any trend with increasing exposure to manganese.

Stata version 10 software was used.[60]

Ethics

Ethical approval for the study generating the data was originally obtained from the Research Ethics Committee of the Health Sciences Faculty at the University of Cape Town in 1999. This study carries out additional analyses on the collected data that have not hitherto been performed. Since no new data was collected and anonymity was preserved during this study, the ethical approval obtained for the original study was extended by the Research Ethics Committee on 12 November 2008 (REC REF212/2003). (Appendix A)

Chapter 4 : Results

Population characteristics

Descriptive information for all participants is detailed in Table 4-1. The table also details the distribution of potential confounding or effect modifying factors that could have influenced the neurobehavioral outcomes tested (age, length of service, standard passed at school, the proxy variable for ethnicity which incorporates the old "race" category –"classc", previous head injury, previous job involving neurotoxins, alcohol consumption and current smoking). As is noted the exposed and unexposed participants differ with regard to age, years worked, standard passed at school, "classc", smoking and alcohol consumption.

Table 4-1 Descriptive characteristics of smelter workers and external unexposed referents

Continuous variables	Exposed participants (n=507) Mean (SD)	Unexposed external referents (n=67) Mean (SD)	Difference (CI)	T- test (p)
Age (years)	45.1(8.4)	38.6 (10.3)	6.5 (4.3;8.8)	**5.7 (p<0.001)**
Std passed at school	4.7 (3.2)	8.0 (2.5)	-3.3 (-2.5;-4.1)	**-8.1 (p<0.001)**
Length of service	18.2 (7.6)	9.4 (7.0)	8.8 (6.9;10.7)	**9 (p<0.001)**
Dichotomous variables	**Percent**	**Percent**	**Difference (%)**	**Chi[2]**
Previous head injury	28	22	6	**0.7 (p=0.4)**
Previous job involving neurotoxins	14	6	8	**3.5 (p=0.06)**
Classc[2]	1	10.3	- 9.3	**5.4 (p=0.02)**
Current alcohol drinker	43	58	-15	**5.4 (p=0.02)**
Current smoker	**38**	**60**	**-22**	**11.1 (p=0.001)**

[2] Classc is a proxy variable for ethnicity based on home language and surname

Workplace Exposure

In total 442 personal inhalable dust samples were measured in homogenous exposure areas enabling the completion of a job exposure matrix (as discussed in chapter 3). The mean exposure intensity, blood manganese, urine manganese and prolactin levels are also detailed in Table 4-2. Both mean blood and urine manganese levels were higher in the exposed population than in the referents.

Table 4-2. Exposure statistics. A comparison of exposed smelter workers and unexposed externals referents

Continuous variables	Exposed participants (n=507) Mean (SD)	Unexposed external referents (n=67) Mean (SD)	Difference (CI)	t- test
Mean intensity of exposure (inhalable fraction) (mg/m^3)	0.8 (1.0) **Median (IQR)** 0.5 (0.1 – 1.0)	0		
Blood manganese (µg/ℓ)	12.5 (5.6)	6.4 (1.7)	6.1 (4.6;7.4)	8.5 (p<0.0001)
Urine manganese (µg/ℓ)	10.5 (20.3)	0.96 (0.8)	9.2 (4.6;14)	3.9 (p=0.0001)
Serum prolactin (µg/ℓ)	6.1 (3.0)	6.2 (2.5)	0.09 (-0.7;0.9)	0.2 (p=0.8)

Mean intensity of exposure was further categorised into 6 categories using specific cutpoints chosen to examine an exposure- response relationship. These categories are listed in Table 4-3 below. Sixty one percent of our participants were exposed to mean manganese concentrations at levels above the ACGIH TLV and 10 percent exposed to more than 2 mg/m^3, a level more than 10 times the TLV.

Table 4-3. Categorisation of mean exposure intensity

Category names	Mean exposure intensity ranges (mg/m^3)	Mean mean exposure intensity(mg/m^3) (CI)	*n*
0	0	0	**67**
1	0< x ≤ 0.1	0.04 (0.03; 0.05)	**105**
2	0.1 < x ≤ 0.2	0.12 (0.11; 0.13)	**50**
3	0.2 < x ≤1	0.54 (0.5; 0.6)	**235**
4	1< x ≤ 2	1.4 (1.3;1.4)	**59**
5	**>2**	**3.4 (3.2; 3.6)**	**59**

All test results presented were selected in order to facilitate a comparison with results previously obtained when analysing this dataset using cumulative inhalable manganese [23] and mean respirable manganese [45] as the exposure metrics and/or because they show an association with exposure using mean inhalable manganese as the exposure metric.

WHO NCTB Tests

The WHO NCTB test results panel is shown below in Table 4-4. Of the selected tests, the Santa Ana pegboard trial, Benton visual retention test, digit span and digit-symbol, the scores of the unexposed external referents differed from the smelter workers. Only the digit span test result differed between the internal exposure referents and the rest of the exposed. While both digit span and digit symbol tests showed a linear trend relationship on regression, the point estimates did not change significantly or monotonically across the multiple exposure categories.

Graphical exploration of all the scores in this battery against mean exposure intensity revealed that the steepest exposure response relationship occurs at very low levels of exposure with a flattening of the curve after approximately $1mg/m^3$. The digit symbol score illustrated this phenomenon clearly and is presented in Figure 4-1.

Table 4-4. Selected WHO NCTB test results panel

Analysis		Santa Ana		Benton		Digit-span (forwards and backwards)		Digit-symbol	
Unexposed referents	Mean Score[a]	49.4		7.1		15.6		33.8	
Internal referents	Mean[b] score	43.1		5.9		12.8		27.8	
		β[c]	***p*[c]**	**β**	***p***	**β**	***p***	**β**	***p***
Dichotomous comparisons	All exposed vs. external referents	-4.3	<0.001	-0.7	0.01	-2.1	<0.001	-2.1	0.04
	Rest of exposed vs. internal referents	0.2	0.8	-0.1	0.5	-1.2	0.001	-1.4	0.08
Analysis of overall linear trend	With INT	-0.1	0.7	-0.1	0.3	-0.6	0.02	-0.9	0.003
Exposure response by INT categories[d]	1	-4.5	<0.001	-0.6	0.05	-1.3	0.03	-1.2	0.3
	2	-4.7	0.001	-0.7	0.04	-2.1	0.002	-2.7	0.06
	3	-4	0.001	-0.7	0.01	-2.4	<0.001	-1.9	0.09
	4	-6.4	<0.001	-0.5	0.2	-2.4	<0.001	-3.8	0.008
	5	-3.1	0.03	-0.9	0.02	-2.7	<0.001	-4.4	0.002

[a] Unadjusted mean score for the unexposed external referents.
[b] Unadjusted mean score for the internal referents
[c] β (adjusted regression coefficient) shows the change, either positive or negative, in the mean for that group compared to the baseline, and the level of significance of that change (*p*)
[d] Comparison of each category (as detailed in Table 4-3) is with the external unexposed referents adjusted for length of service, age, standard passed at school past job exposure to neurotoxins, past head injury, classc, current and previous smoking and current and previous alcohol use.

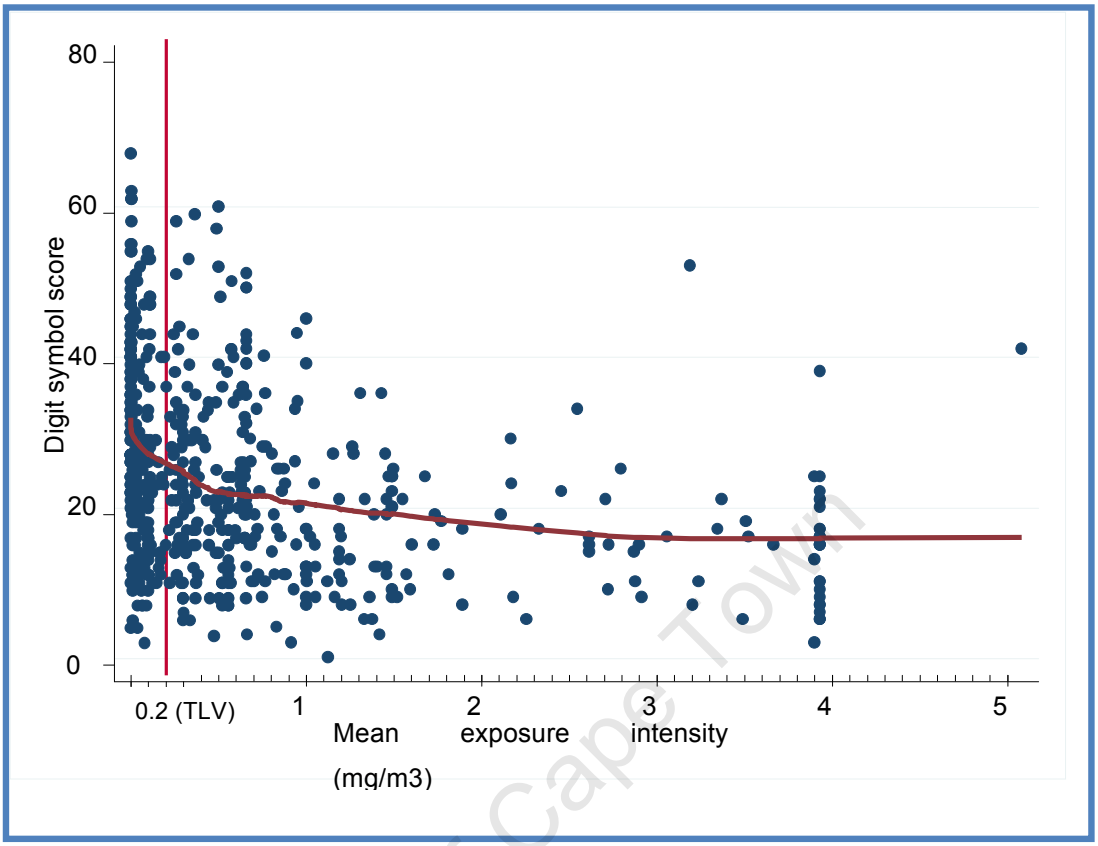


Figure 4-1 Exposure response relationship (crude) for the digit symbol test.

SPES Tests

Of the SPES tests performed tapping endurance and tapping with the dominant hand showed a difference when comparing external referents with all of the exposed. Endurance also showed a difference when comparing internal referents with the rest of the exposed. Whilst certain regression coefficients for isolated INT exposure categories were highly significant when compared to the baseline (unexposed external referents), no significant monotonic exposure-response trend was detectable across increasing categories of exposure. No association between change in mean reaction time and exposure to inhalable manganese was found. The detailed outcome has been tabulated in Table 4-5 below.

Table 4-5 Selected SPES test result panels

Analysis		Endurance		Tapping dominant hand		Tapping non-dominant hand		Mean reaction time	
Unexposed referents	Mean Score	337.1		59.4		54.6		266.4	
Internal referents	Mean Score	322.7		56		52.8		282.8	
		β[a]	***p***	**β**	***p***	**β**	***p***	**β**	***p***
Dichotomous comparisons	All exposed vs. external referents	-14	0.02	-3.5	0.002	-1.9	0.09	10.3	0.09
	Rest of exposed vs. internal referents	-10	0.03	-1.7	0.06	-1.7	0.05	1.7	0.7
Analysis of overall linear trend	With INT	-3	0.1	-0.6	0.07	-0.6	0.1	1.4	0.5
Exposure response by INT categories	1	-6.6	0.3	-2.4	0.06	-0.7	0.6	8.9	0.2
	2	-19.8	0.01	-4.1	0.007	-2.3	0.1	8.7	0.3
	3	-15	0.02	-3.5	0.003	-1.9	0.09	10.7	0.1
	4	-29.2	0.001	-8	<0.001	-5.3	0.001	19.7	0.04
	5	-16.6	0.04	-3.9	0.02	-2.6	0.08	9.3	0.3

[a]β (adjusted regression coefficient) shows the change, either positive or negative, in the mean for that group compared to the baseline, and the level of significance of that change (*p*) adjusted for length of service, age, standard passed at school, past job exposure to neurotoxins, etc.

Graphical exploration of all the SPES test results with mean exposure intensity using smoothed plots were very similar to Figure 4-1 with a steep decline at low exposure and then flattening of the curve at higher exposures.

Danish Product Development Tests

Catsys Coordination

Of the 18 coordination tests analysed only the maximum frequency finger tap (left) (LFTMF) score and auditory reaction time right showed an overall linear trend exposure response relationship. However there were no statistically significant trends visible across the various categories. See Table 4-6

Table 4-6 Selected Catsys coordination tests

Analysis		LFTMF (score)		Auditory reaction time (right)	
Unexposed referents	Mean Score	5.3		0.2	
Internal referents	Mean score	4.9		0.2	
		β[a]	***p***	**β**	***p***
Dichotomous comparisons	All exposed vs. external referents	-0.3	0.3	0.005	0.3
	Rest of exposed vs. internal referents	0.1	0.6	0.002	0.5
Analysis of overall linear trend	With INT	-0.2	0.001	0.003	0.03
Exposure response by INT categories	1	0.3	0.2	0.003	0.6
	2	0.1	0.8	0.01	0.03
	3	0.1	0.6	0.01	0.1
	4	0.4	0.1	0.001	0.8
	5	0.8	0.005	0.002	0.7

[a]β (adjusted regression coefficient) shows the change, either positive or negative, in the mean for that group compared to the baseline, and the level of significance of that change (*p*) adjusted for length of service, age, standard passed at school, past job exposure to neurotoxins, etc.

Graphical exploration of the relationship between LFTMF score and mean exposure intensity in this instance reveals a linear association as shown in Figure 4-2.

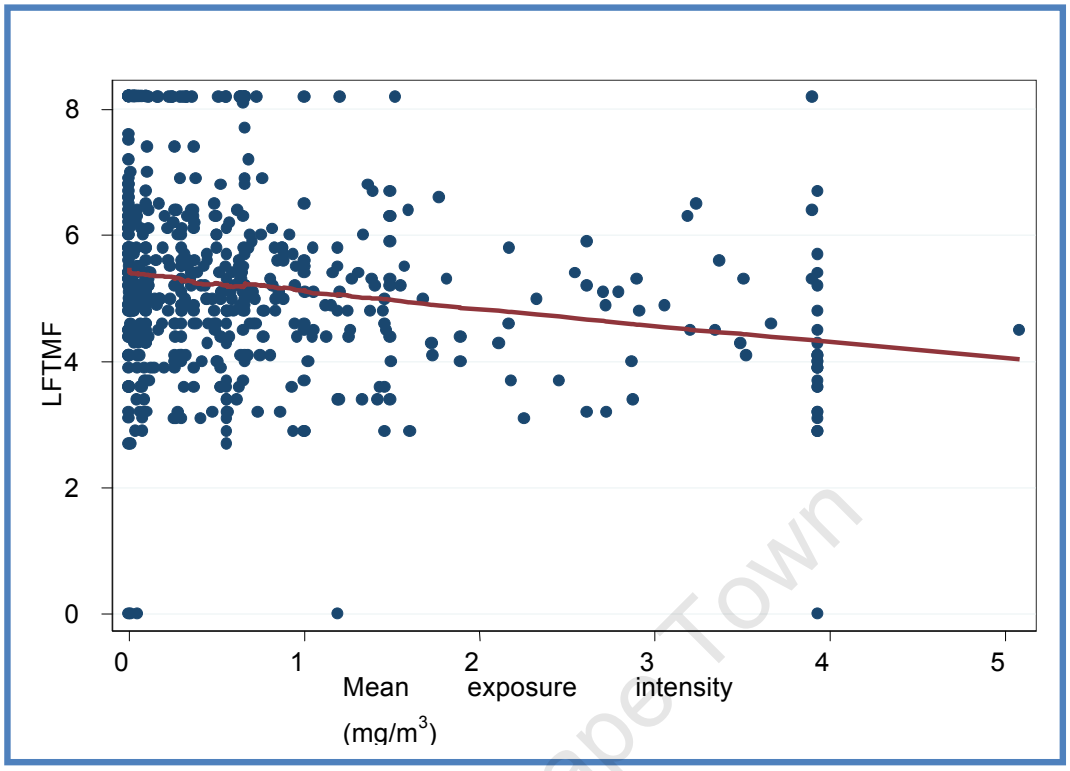


Figure 4-2 Exposure response relationship (crude) for the LFTMF test

Tremor

The eight tremor parameters revealed no differences between external referents and the exposed participants. Left sided mean centre frequency was smaller, and both left and right mean frequency dispersion was higher in the exposed when compared to the internal referents. Left sided mean centre frequency showed a negative trend relationship with increasing exposure intensity (as the exposure increased the mean centre frequency decreased) whereas mean frequency dispersion left and right showed a positive trend relationship. Selected tests are detailed in Table 4-7.

Sway

Of the sway tests the only difference was found when comparing external referents to all exposed for the condition eyes open and feet insulated. There were no differences across the four sway tests, including the most stressed condition – eyes closed, feet insulated, when comparing internal referents to

the rest of the exposed and there was no trend relationship with either continuous or categorical exposure. Selected tests are shown in Table 4-7

Table 4-7 Selected tremor and sway test result panels

Analysis		Left sided mean centre frequency (Hz)		Mean frequency dispersion left (Hz)		Mean frequency dispersion right(Hz)		Sway (eyes open, feet insulated)	
Unexposed referents	Mean Score	9.8		2.7		3		4.4	
Internal referents	Mean score	10.3		2.3		2.7		5.3	
		β[a]	***p***	**β**	***p***	**β**	***p***	**β**	***p***
Dichotomous comparisons	All exposed vs. external referents	0.2	0.2	-0.2	0.2	-0.1	0.4	0.8	0.03
	Rest of exposed vs. internal referents	-0.3	0.02	0.2	0.04	0.2	0.03	0.2	0.5
Analysis of overall linear trend	With INT	-0.1	0.04	0.1	0.02	0.07	0.03	0.1	0.5
Exposure response by INT categories	1	0.4	0.02	-0.3	0.03	-0.2	0.06	0.6	0.1
	2	0.3	0.2	-0.2	0.2	-0.1	0.4	0.5	0.3
	3	0.1	0.4	-0.1	0.3	-0.1	0.5	0.8	0.02
	4	0.1	0.5	-0.04	0.8	-0.1	0.5	1.1	0.02
	5	-0.1	0.6	0.1	0.7	0.1	0.7	0.7	0.1

[a]β (adjusted regression coefficient) shows the change, either positive or negative, in the mean for that group compared to the baseline, and the level of significance of that change (*p*) adjusted for length of service, age, standard passed at school, past job exposure to neurotoxins, etc.

Categorical Outcomes

Luria-Nebraska

Using dichotomised data (0, ≥1); items 22R, 22L, 23L and 23R show a counterintuitive protective effect of exposure when comparing all exposed to external referents. Item 2L shows an effect when comparing internal referents

with the rest of the exposed and also has a negative trend association implying that increased exposure to manganese dust is protective. Items 1R, 2R, 12L, 12R, 23L and 23R showed a significant overall linear trend with a nonsignificant shift from a protective level of exposure to an adverse one across multiple exposure categories. Selected findings to illustrate each aspect are shown in Table 4-8. Items 1L, 3R, 3L, 4R, 4L and 21 showed no exposure effects.

Table 4-8 Selected Luria- Nebraska test outcomes

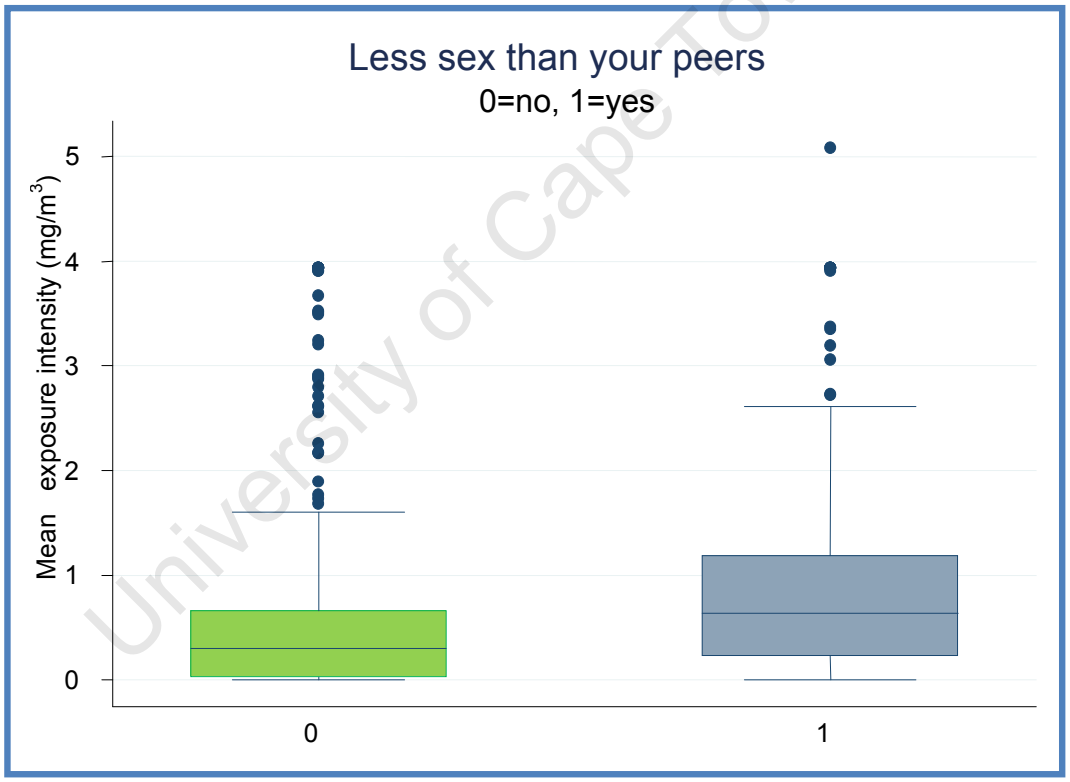
Analysis		LN 22R		LN 23L		LN 2L		LN 1R	
Unexposed referents	Proportion abnormal	0.2		0.4		0.7		0.3	
Internal referents	Proportion abnormal	0.2		0.3		0.7		0.4	
		OR[a]	***p***	**OR**	***p***	**OR**	***p***	**OR**	***p***
Dichotomous comparisons	All exposed vs. external referents	0.3	0.003	0.5	0.04	1	0.9	0.9	0.7
	Rest of exposed vs. internal referents	1	0.9	1.2	0.5	2.4	0.002	1.6	0.07
Analysis of overall trend	With INT	1.2	0.08	1.3	0.01	1.4	0.05	1.4	<0.001
Exposure response by INT categories	1	0.3	0.02	0.4	0.04	0.6	0.1	0.6	0.3
	2	0.4	0.08	0.5	0.1	1.2	0.8	0.5	0.09
	3	0.2	<0.001	0.4	0.01	1.2	0.6	0.9	0.7
	4	0.5	0.1	1.1	0.8	2.6	0.2	2.2	0.1
	5	0.5	0.2	0.9	0.9	1.5	0.5	1.7	0.2

[a] Exposure odds ration (OR)adjusted for length of service, age, standard passed at school past job exposure to neurotoxins, etc.

Questionnaire

Forty two questionnaire items were examined and isolated associations found. The most noteworthy of these is the finding that compared with external referents the exposed had an adjusted odds ratio of 36.2 (4.8; 273.6) for having sex less frequently than their peers. This difference is graphically illustrated in Figure 4-3. There is no convincing trend across exposure categories as the difference seen is mainly between the external referents and all the exposed.

Figure 4-3 Box and whisker plot illustrating the crude association between exposure and the questionnaire item on having sex less than contemporaries



The other isolated differences noted were between the external referents and the exposed for irritation, and between the internal referents and the rest of the exposed for irritation and feeling tired. There were no differences by exposure when answering any of the questions related to memory (short

memory, relatives say that you have a short memory, and notes to help you remember) or change in smell. See Table 4-9 for further detail.

Clinical examination

On clinical examination, two of the participants had an abnormal glabellar reflex, four had gait abnormalities (one had a shuffling gait whilst two were unbalanced) and forty two participants had difficulty walking backwards. None had immobile facies. A composite variable was created combining the clinical features of abnormal balance, gait or glabellar reflex. On using this composite variable there was no significant difference between external referents and all the exposed, and the probability of having a positive clinical test was similarly not significantly increased in the rest of the exposed as compared to the internal controls. Details are provided in Table 4-9.

Table 4-9 Selected questionnaire and clinical outcomes

Analysis		Less sex than peers		Irritation		Feeling tired		Clinical test	
Unexposed referents	Proportion abnormal	0.02		0.2		0.4		0.02	
Internal referents	Proportion abnormal	0.2		0.3		0.4		0.03	
		OR[a]	***p***	**OR**	***p***	**OR**	***p***	**OR**	***p***
Dichotomous comparisons	All exposed vs. external referents	36.2	0.001	2.4	0.02	1.5	0.2	3.8	0.2
	Rest of exposed vs. internal referents	1.8	0.03	1.9	0.01	1.9	0.007	3.5	0.06
Analysis of overall trend	With INT	1.3	0.008	1.2	0.09	1.1	0.4	1.1	0.5
Exposure response by INT categories	1	22.9	0.003	1.5	0. 3	0.9	0.9	1.3	0.8
	2	32.6	0.001	3	0.02	2.2	0.06	2.3	0.5
	3	42.5	<0.001	2.7	0.009	1.6	0.2	4.7	0.2
	4	42.1	<0.001	2.7	0.04	2.6	0.03	5.9	0.1
	5	48	<0.001	3.1	0.01	1.6	0.3	4.6	0.2

[a] Exposure odds ration (OR)adjusted for length of service, age, standard passed at school past job exposure to neurotoxins, etc.

Chapter 5 : Discussion and Conclusion

The study was conducted with a large number of participants who had a very wide range of inhalable manganese dust exposure, from levels well below the current ACGIH TLV [1] of 0.2 mg/m^3 to above 5mg/m^3 (the SA OEL[20]). Within these participants an extensive array of outcomes were tested examining all the neurotoxic effects as postulated by Iregren [40,42] and Mergler and Baldwin.[41] This detailed investigation allowed the move beyond the analyses of just a simple association between a dichotomous exposure variable and an outcome, to a more elaborated study of the exposure- response relationship by examining overall linear trends and the shape of that exposure-response relationship across multiple exposure categories.

The panels helped with interpretation of the large number of results obtained and allowed the organization of the findings into three groups. [23,45]

Group 1 comprised those results that showed only a limited exposure-response relationship in the form of a dichotomous exposure variable - outcome association. These differences were either between the external referents and all the exposed and/or between the internal referents and the rest of the exposed. The Santa Ana Pegboard test and the Benton Visual Retention test from the WHO NCTB, two SPES tests (endurance tapping and tapping with the dominant hand), one sway test (eyes open and feet insulated), the Luria Nebraska item 2L and questionnaire items irritation and feeling tired fell into this group.

Group 2 was the smallest and included those results showing not only a limited association between a dichotomous exposure and outcome, but more elaborated evidence of an exposure-response relationship. This involved the existence of an overall linear trend. None of the results obtained exhibited a convincing monotonic trend relationship across the six exposure categories.

The digit span and digit symbol test from the WHO NCTB and the questionnaire item: having less sex than peers fell into this category.

Group 3 comprised those results that did not show even a limited association between a dichotomous exposure variable and an outcome and/or those results which were counterintuitive. This group constituted the overwhelming majority of the test results. It included the rest of the SPES tests, almost all of the DPD tests (including all of the tremor tests, tests for diadochokinesia, the bulk of the sway tests), the Luria Nebraska tests and the rest of the questionnaire and clinical examination items.

These results, using the mean intensity of inhalable manganese dust as the exposure metric, were thus similar to those obtained by Myers et al [23] who used the inhalable dust based cumulative exposure index (CEI); and Young et al [45] who used mean respirable manganese intensity as the exposure metrics. In the three studies all tests in Group 2 had the same shape of the exposure response relationship as illustrated in Figure 4.1 with the sharpest decline at very low exposures. Only one test, the WHO digit symbol test, could be placed in group 2 in all three studies. Details are provided in Table 5-1.

Myers et al [23] did not find the WHO NCTB item digit span, and the questionnaire item on having sex less frequently than peers to have a trend relationship. These differences might be explained by the influence of length of service on the test outcome in question. When using a mean intensity as the exposure metric, length of service has been adjusted out of the analysis which then focuses only on the mean intensity association. Using an inextricably integrated exposure metric like a cumulative exposure index is therefore likely to produce somewhat different findings.

The study using respirable dust by Young et al [45] could additionally place dominant hand tapping, endurance, the questionnaire item on irritation, the Luria Nebraska item 1R and the clinical test into group 2.

Table 5-1 Comparison of Group 2 tests between results from Myers et al,[23] Young et al[45] and this study.

	Group 2 tests in common to all three studies.	Findings in common to two papers.	Unique findings
This study	WHO digit symbol test.	WHO digit span test and the questionnaire item having less sex than peers.	
Myers et al.	WHO digit symbol test.	Luria Nebraska item 1R	
Young et al.	WHO digit symbol test.	WHO digit span test and the questionnaire item having less sex than peers. Luria Nebraska item 1R	SPES tests dominant hand tapping and endurance; the questionnaire item on irritation, and the clinical test.

Bast-Petersen et al [26] reported on both inhalable and respirable manganese dust concentration in the manganese alloy plant studied. The study used a cross sectional matched pair design with the unexposed referents drawn from industries unexposed to manganese. The arithmetic mean inhalable manganese concentration measured (0.8mg/m^3) in the exposed workers was similar to this study. Similarly there were no differences between the exposed and unexposed participants in tests for either cognitive function or reaction time; in contrast to this study the exposed manganese alloy plant workers had an increased postural tremor with a higher (and opposite to our findings) frequency than the unexposed referents. The authors categorised the exposed workers into low, medium and high exposure based on their blood manganese and compared these groups to the matched unexposed referents. The exposed participants with the highest blood manganese

exhibited more tremor (duration of contacts, number of contacts and tremor frequency dispersion) than their age matched referent counterparts. Interestingly, only the exposed participants in the high blood manganese group had a mean blood manganese level significantly higher than the referents, with the low blood manganese group having a mean blood manganese level significantly lower than the referents. The authors did not perform a trend analysis.

Further direct comparison of results with similar studies proved difficult as most studies used either a cumulative exposure variable [44] or the mean intensity of the respirable fraction [32] to reflect exposure. The study by Blond et al was conducted amongst workers exposed to both lead and manganese and therefore also not strictly comparable.[46,47]

In summary: this was a large study with more than 500 participants, a wide exposure range and 128 neurological outcomes tested. Despite this, only a few, inconsistent and on occasion, counter-intuitive associations were found when using mean intensity inhalable manganese dust as the exposure metric. The few significant associations that were found (Group 2) were similar to those identified in the first two research papers that used CEI[23] and mean respirable manganese[45] as exposure metrics for the same dataset. These significant associations in Group 2 did not provide in this, or any of the three papers, the expected overall picture consistent with manganese intoxication. This was because there was no significant exposure- response relationship across categories of exposure and because the shape of the exposure response relationship was difficult to interpret biologically.

There was no clustering of results around the sensitive neurological outcomes (e.g. slowing motor functions, increased tremor) proposed by Iregren [40,42] and Mergler,[41] and no pattern to the Group 2 results that is consistent with manganese toxicity. Therefore given the few, inconsistent,

isolated and often counterintuitive associations found, the most likely explanation is chance.

Study limitations

Unmeasured and/ or incompletely measured confounding or effect modifying factors could have had an influence on the study. Socioeconomic status (SES) may have been incompletely adjusted for as two pragmatic proxy measures were used. These were standard passed at school and "classc" – a proxy variable for ethnicity based on home language and surname. SES is a complex abstract construct with uncertainties and difficulties in its measurement. [61] These difficulties are further complicated by the history of racial discrimination and inequality in South Africa and its pervasive biopsychosocial impact. The composite variable "classc" attempts to measure aspects of the past inequalities and the standard passed at school measures education. However other aspects such as nutrition, income and access to healthcare were not measured. Bellinger, in a paper on lead neurotoxicity and SES, postulates that co-exposure to neurotoxicants is more likely to occur in children living in poverty. [62] Childhood poverty would therefore not only have an effect on neurological status via poor nutrition and poor access to healthcare, but also through the impact of greater chemical exposures. Residual positive confounding might therefore be expected and whatever Group 2 findings there were would appear stronger than they would in truth be if SES had been adequately adjusted for.

As this was a cross sectional study, premorbid innate intelligence (independent of schooling) of the workforce could not be measured. This may be a potential confounder again with a bias away from the null, as people of lower intelligence may perform less well at the tests measured and as a direct result of their poorer mental ability may be assigned the dustier more manual jobs.

The external referents were significantly better educated, consumed more alcohol and were younger than the exposed workers implying that they were

different from the exposed in ways that make them incomparable to those exposed to the effects of manganese. This pattern of a difference in demographics was highlighted in a meta-analysis by Greiffenstein & Lees-Haley where the authors found that the pooled demographics of the exposed work force was associated with poorer neurobehavioral outcome than the pooled demographic data of the unexposed referents. [48] If this systematic difference was a big influence on the study, a bias away from the null would have been expected. To investigate this possibility, the smoothed bivariate plot was rerun with digit symbol as an outcome (in order to compare with Figure 4-1) excluding the external referents. The linear trend relationship was still significant (albeit smaller) with a 0.8 drop (CI: -1.4; -0.2, p=0.006) in digit symbol score with every 1mg/m^3 increase in mean inhalable manganese concentration. Figure 5-1 illustrates the sharp drop at low exposures (without the external referents) with the flattening of the graph at the higher exposure concentrations. This drop, although not quite as steep as initially seen when all the participants (including external referents) were included, indicates that there is a residual effect which is not all due to having noncomparable external exposure referents.

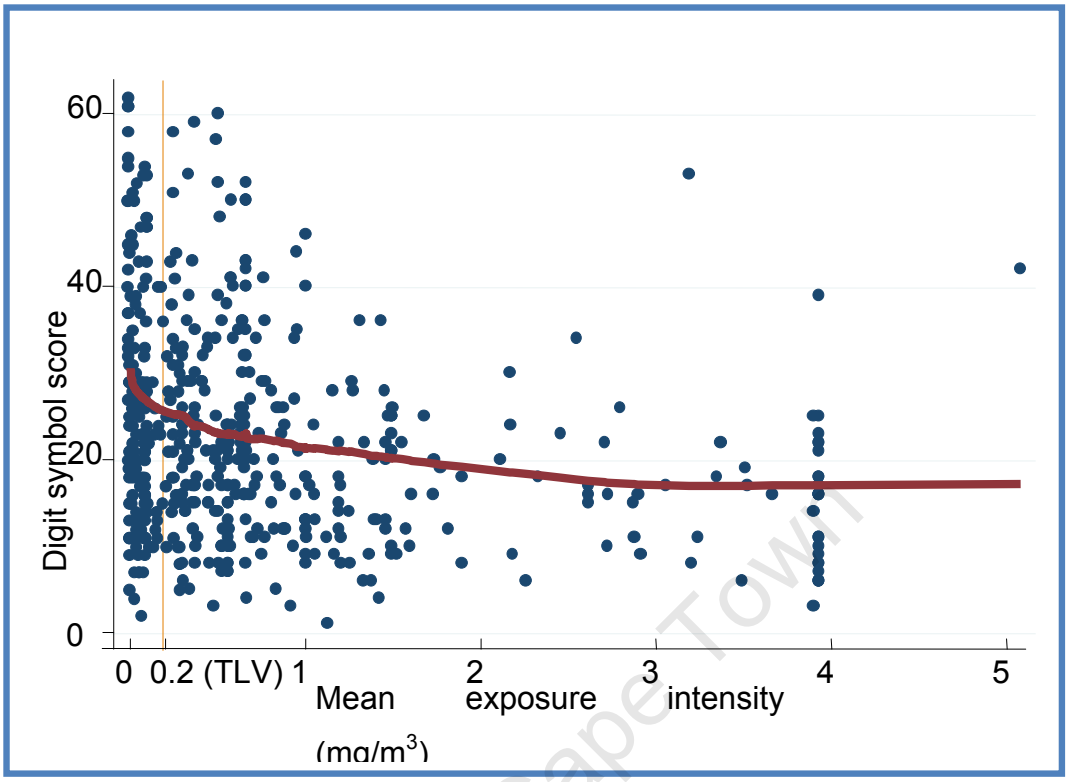


Figure 5-1 Exposure response relationship (without external referents) for the digit symbol test.

A bias in the form of the "healthy worker effect" could be influencing these results if workers experiencing neurological effects left the manganese smelter disproportionately. Such a disproportionate loss is however unlikely to be a powerful explanation as the outcomes examined are sub- clinical and therefore not expected to noticeably or substantially affect a worker's continuation in employment.

Exposure misclassification has to be considered as a possible source of bias. Workers with the same mean inhalable manganese concentration do not necessarily have similar exposure experiences. Some may have low current exposures with high exposure jobs in the past, whilst others may have the opposite exposure history. If recovery of the subclinical neurological effects occurs with reduced exposure, outcome misclassification may have additionally occurred. Possible misclassification of either exposure or outcome would have been non-differential, with a bias towards the null. [45]

A panel of four regression analyses were performed on each outcome resulting in 512 statistical tests performed in total. At the 0.05 significance level, about twenty five tests could have been significant purely by chance. By performing a rough Bonferroni adjustment the significance level drops to 0.0004[3]. Using this tighter significance level would result in all of the current Group 2 tests falling away. Whilst some authors argue that the Bonferroni method is too conservative when tests are dependent [63,64](as in this case), it nevertheless provides some indication of the possible impact of type I error when doing multiple comparisons.

This was a well conducted cross sectional study; however the nature of the design has inherent shortcomings; that of incompletely measured confounders and the question of temporality.

Study strengths

Study strengths include using a wide range of standardised neurobehavioral tools to assess outcome, [23, 65] a large sample size (the largest study of its kind) with a wide range of exposure intensity and the lack of substantial selection bias. The exposed workers were randomly selected from the low and medium exposure groups, to maximize exposure contrast, with 100% recruitment in the highly exposed groups. Additionally the outcome of interest was subclinical therefore reducing the chance of information (recall) bias as well as the possibility of studying a disproportionately healthy worker population (the healthy worker effect).

Lastly the question could be asked as to whether a linear model is appropriate for the exposure –response relationship in the data. [66] The sole use of linear regression would have been inappropriate. Rather the analysis plan in this study was able, by virtue of the large subject numbers, to examine the exposure response relationship across dichotomous exposure categories,

[3] Adjustment: $\alpha/n = 0.05/128 = 0.0004$.
All the outcomes tested (n=128) was used as a conservative denominator. If all tests were treated as independent, the denominator would increase to 512.

as well as multiple exposure categories and graphical exploration of the relationship. The panel of all these results was examined in order to characterise the overall shape and significance of the exposure-response relationship.

Conclusion

There were isolated significant exposure- response relationships, but this was generally steepest at low exposures flattening out at higher exposures. This pattern does not make biological sense as manganese is an essential trace element. The uniformly flat relationship at exposure levels above 0.7- 1mg/m^3 provides no basis for setting a meaningful protective threshold level for the range of exposure below 4 mg/m^3, given few exposures higher than this level.

This was then essentially a negative study, despite the fact that a large number of workers with a wide range of manganese dust exposures from environmental levels to well above the current ACGIH TLV were studied. Performing extensive analyses for each of the outcomes led to not only a thorough investigation of the possible exposure response relationship but also increased the possibility of discovering chance Group 2 associations and hence chance is the most likely explanation for the findings.

The toxicology of manganese induced neurotoxicity is not well understood particularly the role of manganese speciation (Mn^{2+}/Mn^{3+}), [67,68] absorption and transport to the brain [68,69] and that of individual susceptibility. An increased knowledge of toxicokinetics would assist in untangling the contradictory epidemiological findings to date. The impact of particle size e.g. the potential production of nanoparticles during the welding process and the implications for what is the most appropriate exposure metric is of research interest.

The continued inconsistencies in the literature and the limitations of cross sectional studies justify a prospective study where neurological status could

be measured before exposure to manganese, and the true exposure response relationship be more confidently identified.

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Appendix A

UNIVERSITY OF CAPE TOWN

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12 November 2008

REC REF: 212/2003

Dr H Williams &Prof J Myers
School of Public Health & Family Medicine

Dear Dr Williams & Prof Myers

PROJECT TITLE: NEUROLOGICAL EFFECTS OF OCCUPATIONAL EXPOSURE TO MANGANESE

Thank you for your letter to the Ethics Committee dated 10th November 2008.

It is a pleasure to inform you that the Ethics Committee has granted **approval** to use the existing data collected during previous research (REC REF 212/2003) .

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

kmjedi