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A tissue dose-based comparative exposure assessment of manganese using physiologically based pharmacokinetic modeling—The importance of homeostatic control for an essential metal

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ABSTRACT

A physiologically-based pharmacokinetic (PBPK) model (Schroeter et al., 2011) was applied to simulate target tissue manganese (Mn) concentrations following occupational and environmental exposures. These estimates of target tissue Mn concentrations were compared to determine margins of safety (MOS) and to evaluate the biological relevance of applying safety factors to derive acceptable Mn air concentrations. Mn blood concentrations measured in occupational studies permitted verification of the human PBPK models, increasing confidence in the resulting estimates. Mn exposure was determined based on measured ambient air Mn concentrations and dietary data in Canada and the United States (US). Incorporating dietary and inhalation exposures into the models indicated that increases in target tissue concentrations above endogenous levels only begin to occur when humans are exposed to levels of Mn in ambient air (i.e. >10 μg/m^3) that are far higher than those currently measured in Canada or the US. A MOS greater than three orders of magnitude was observed, indicating that current Mn air concentrations are far below concentrations that would be required to produce the target tissue Mn concentrations associated with subclinical neurological effects. This application of PBPK modeling for an essential element clearly demonstrates that the conventional application of default factors to "convert" an occupational exposure to an equivalent continuous environmental exposure, followed by the application of safety factors, is not appropriate in the case of Mn. PBPK modeling demonstrates that the relationship between ambient Mn exposures and dose-to-target tissue is not linear due to normal tissue background levels and homeostatic controls.

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1. Introduction

Manganese (Mn) is an essential element required as a cofactor for many enzymes with key biological functions, yet studies demonstrate that adverse health effects occur following exposure to high concentrations. Manganese exposure can occur through the diet; releases from industrial sources such as ferroalloy production plants, iron and steel foundries, alkaline battery manufacture, power plants, and coke ovens; and other sources. Long-term exposures to high concentrations of Mn are neurotoxic, leading to deficits in neuromotor and cognitive domains (Guilarte, 2010, 2013; Roels et al., 2012).

Although risk assessments for Mn have attempted to quantitatively estimate potential toxicity from inhalation exposures including those in the ambient environment, many of these assessments rely largely on the results from occupational cohorts where exposures may be many orders of magnitude higher than ambient air exposures of the general population. Using external concentration as the "dose metric" may not adequately address nonlinearities in complex biological processes (e.g. absorption, metabolism), resulting in target tissue concentrations that are not linearly correlated with external concentrations. Accounting for these nonlinearities is especially important for chemicals that are essential elements, whereby individuals already have substantial background tissue levels resulting from typical dietary intake.

In evaluating target tissue dosimetry following chemical exposures, the application of physiologically-based pharmacokinetic (PBPK) models has been suggested as a valuable tool. These models provide a

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method for integrating toxicological and pharmacokinetic data and provide valuable alternatives to using external exposures in risk assessment (Barton and Clewell, 2000; Clewell and Andersen, 1985; Clewell et al., 1995). Use of PBPK modeling is the preferred approach for estimating internal doses of chemical substances for route and species extrapolation, preferably at the target tissue, that would then be used in dose-response modeling for deriving acceptable regulatory levels (USEPA, 2005, 2006). PBPK models also offer advantages for extrapolation across large differences in exposure concentrations, such as those that exist when attempting to compare the potential for health effects at Mn concentrations from occupational studies to those following exposure to lower ambient air concentrations (Andersen et al., 1999). This investigation relies upon a published human PBPK model developed by Schroeter et al. (2011) to evaluate target tissue concentrations following reported occupational exposures, as well as measured ambient air concentrations of Mn and the potential for health effects.

Traditional approaches to risk assessment for noncancer toxicants use a threshold approach where safety factors are applied to the highest concentration of the toxic agent that does not elicit toxicity (the no observed adverse effect level [NOAEL] or a benchmark dose [BMD]) to estimate an acceptable exposure concentration where even sensitive subpopulations will be protected. Evaluating the "margin of safety" (MOS) is an alternative approach where the maximum amount of exposure producing no measurable adverse effect is divided by the actual amount of human exposure in a population to understand the magnitude of the difference between actual exposures and concentrations associated with potential for health effects. By examining the target tissue Mn concentrations associated with occupational air exposure concentrations for which adverse changes in one or more specific physiological functions have been reported, comparisons can be made to target tissue exposures expected following exposure to Mn from ambient air. The MOS thus reveals how much of an increase in the ambient Mn air concentrations would be required to produce target tissue levels at or near the range where adverse impacts might be expected to occur.

The approach applied in this assessment is grounded in the synthesis of all available data and information on biological mechanisms, examines the application of PBPK models in risk assessment, and provides a comparison to the traditional risk assessment approach that incorporates default adjustments. Our investigation demonstrates that use of a MOS approach for an essential nutrient such as Mn allows for a harmonized biologically-based approach to risk assessment that is more specific to the chemical of interest than the traditional approach that relies on safety factors. Toward this goal, we applied a human adult PBPK model for Mn (Schroeter et al., 2011) to investigate: 1) target tissue and corresponding air concentrations of Mn below which subclinical neurological effects would not be expected to occur, based on observations reported in workers in occupational studies; 2) target tissue concentrations corresponding to ambient air concentrations of Mn; and 3) the range of air concentrations associated with increases of Mn above background levels in the target tissue.

2. Materials and methods

Epidemiological, toxicological, and pharmacokinetic studies of Mn and exposure studies of the levels of Mn in the environment have been conducted, some of which have been reported in the published literature. However, all of these studies have been conducted independently of one another. The approach presented here allows for the integration of epidemiological, toxicological, and exposure data in a quantitative manner, along with pharmacokinetic data to lay the foundation for a new approach to dose-response and exposure analyses for Mn – the estimation of target tissue concentrations.

Epidemiological and toxicological studies that provided quantitative exposure and response data were selected. The epidemiological/toxicological/exposure data were evaluated to determine if the quantitative information could be used in combination with published PBPK models to estimate target tissue concentrations of Mn and at what target tissue concentrations potential health effects would be expected. In addition, data sources from both Canada and the United States (US) were surveyed to provide data that would allow for the estimation of potential exposure to Mn in both the diet and ambient air. Potential exposure from ingestion of drinking water containing Mn was not considered.

2.1. Selection of epidemiological data

Literature involving occupational studies of workers exposed to Mn was identified through searches of PubMed and Google Scholar, as well as review of the citations/reference lists in the studies identified. The identified studies were reviewed to determine if sufficient quantitative information on Mn exposures were provided in order to allow modeling of tissue Mn concentrations in exposed workers. Ideally, the most useful epidemiological studies would include the following: 1) a well-defined exposed population with little or no exposure to other neurotoxicants, 2) a well-defined and appropriate comparison group, 3) objective measures of neurological deficits (e.g. as opposed to self-reported symptoms or measured effects of unknown clinical importance), 4) estimates of personal exposure to respirable fractions of Mn, and 5) neurological measures collected for the same individuals. At a minimum, studies needed to provide quantitative exposure estimates for respirable Mn and objective measures of neurological deficits. The level of detail necessary to model tissue concentrations of Mn requires individual exposure and response data; however, this is often absent from published studies. Therefore, the raw data was relied upon when available.

2.2. Exposure to manganese from ambient air[1]

Air monitoring data were obtained from publicly available databases in both Canada and the US for the years 1991 through 2014, inclusively, to allow estimation of potential Mn concentrations in the ambient air. Sources of air monitoring data were the Canadian National Air Pollution Surveillance Network (NAPS, 2015) and the US Technology Transfer Network Air Quality System (USEPA, 2015). Total suspended particles (TSP) and particulate matter (PM) with aerodynamic diameter less than or equal to 10 μm (PM_{10}), 2.5 μm ($PM_{2.5}$), and between 10 and 2.5 μm ($PM_{10-2.5}$) were measured at these monitoring networks. The Canadian monitoring stations reported Mn concentrations from either $PM_{2.5}$ or $PM_{10-2.5}$, while the US stations generally reported Mn concentrations from TSP, PM_{10}, and/or $PM_{2.5}$, with a small number also reporting $PM_{10-2.5}$. All available Mn measurements from 1991 to 2014 were used to estimate the mean concentration and 95% lower and upper confidence limits (95% CLs) of Mn in ambient air as presented in the Supplemental information. For the purpose of this study, respirable PM was defined as particulate matter ≤10 μm. Estimates of mean concentrations of Mn from respirable particles were calculated as the sum of PM_{10} and $PM_{10-2.5}$ for Canadian data, and PM_{10} for US data. In all cases, the estimated 95% CLs assumed a normal distribution of the data.

2.3. Exposure to manganese the diet[2]

Estimated daily dietary intake of Mn for Canadians was obtained from tables provided by Health Canada (2011). Values reported in microgram per kilogram (μg/kg) body weight/day were converted to total daily intake using comparable body weight data for each age and gender category from US Department of Health and Human Service's 2009–2012 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2010, 2012). Estimated daily dietary intake of Mn for the US population was obtained from NHANES. Two sets of data (NHANES 2009–2010 and NHANES 2011–2012) were combined for

[1] See discussion presented in Supplemental information.
[2] See discussion presented in Supplemental information.

this analysis and the body weights were adjusted so that the combination provided a nationally representative sample of dietary intake (CDC, 2006, 2007). The amount of Mn in the food items consumed was determined using the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNNDS) (USDA, 2012), and the USDA National Nutrient Database for Standard Reference, SR27 (USDA, 2014). The FNNDS database provides the "recipes" which break down the food items in the NHANES database into the individual items in the SR27 database. The SR27 database provides the amount of Mn (in units of mg/100 g) for each food item in a recipe.

Using data from the three databases (NHANES, FNNDS, and SR27), estimates of the amount of Mn consumed daily in the diet were determined for specific age groups and gender combinations. Intake from both food and nutritional supplements was based on data from the NHANES dietary intake interview and the data on supplements provided by the CDC in NHANES 2009–2012.

2.4. Application of the human PBPK Mn model

The development of the current human PBPK model for Mn compounds (Schroeter et al., 2011) builds upon previous models (Andersen et al., 2010; Leavens et al., 2007; Nong et al., 2008, 2009; Teeguarden et al., 2007a, 2007b, 2007c; Yoon et al., 2009a, 2009b, 2011). These models characterize the movement of Mn compounds in the body following intake by oral or inhalation routes, as well as target tissue concentrations of Mn in selected regions of the brain. The Schroeter et al. (2011) model also incorporates direct transport of Mn to the brain via the olfactory nerve (Leavens et al., 2007; Nong et al., 2008, 2009). They build upon integration of pharmacokinetic data for various Mn compounds in rats, monkeys, and humans (Dorman et al., 2012; Taylor et al., 2012), considering the significant physiological and biochemical differences between species (Schroeter et al., 2011).

The Schroeter et al. (2011) human PBPK model was the primary model used for this investigation. This PBPK model has the capability of simulating concurrent exposure to dietary and inhaled Mn. The structure for this model relies on the structure initially developed for the rat and the monkey (Nong et al., 2009). This structure was extended to humans to predict dietary and inhalation exposure conditions, with human tracer studies relied upon for verification. Information for the characterization of the kinetics of Mn in humans was based on ^{54}Mn clearance studies in monkeys and humans (Dastur et al., 1971; Davidsson et al., 1988; Dorman et al., 2006a; Furchner et al., 1966; Mahoney and Small, 1968; Mena et al., 1967; Newland et al., 1987). Clearance behavior of Mn displayed biphasic kinetics, dose-dependent differences in elimination, and dependence on total Mn body burden, either from Mn in the diet or supplemental Mn exposures. The Schroeter et al. (2011) model has adequately simulated the results from ^{54}Mn tracer kinetics from oral and inhalation exposures and also for multiple water-soluble Mn compounds (i.e. Mn sulfate [$MnSO_4$], Mn (II) chloride [$MnCl_2$]) administered by intraperitoneal, intravenous, or subcutaneous administration.

The results of the extensive modeling effort by Schroeter et al. (2011) identified that one of the primary species differences that affect Mn kinetics is dietary exposure. Because Mn is an essential nutrient, dose-dependent biological processes that regulate absorption, tissue storage, and elimination must be considered, with a focus on those external exposures that alter uptake processes in the gut. The current models (Nong et al., 2008, 2009; Schroeter et al., 2011, 2012; Yoon et al., 2009a, 2009b, 2011) have characterized these processes, specifically changes in intestinal absorption and biliary elimination, capturing the homeostatic controls that regulate Mn body burden.

In the Schroeter et al. (2011) model, estimation of the deposition of Mn particles in the lung was conducted using the Multiple-Path Particle Dosimetry Model (MPPD model) particle deposition software; (copyright Applied Reseach Associates Inc., Albuquerque, NM), which considered species-specific lung geometries, ventilatory rates and particle properties. Data for $MnSO_4$, which is a more bioavailable compound than other forms of manganese (i.e. Mn dioxide [MnO_2], Mn [III] phosphate), were relied upon for characterizing lung deposition, and hence, subsequent systemic distribution in the human model based on the available studies for characterizing lung deposition in the monkey (Aschner and Aschner, 2005; Bush et al., 1995; Dorman et al., 2006a, 2006b; Schroeter et al., 2008). The use of the sulfate form assumes the highest absorption and distribution of manganese in the tissues as other forms have lower absorption and are likely to result in lower tissue concentrations (Dorman et al., 2001; Vitarella et al., 2000).

Information regarding the particle size for the distribution of total dust particles of Mn measured in the occupational studies is necessary to incorporate into the MPPD model to estimate the deposition fractions that are used in the PBPK model. However, data on the mean or median particle diameter for the occupational Mn measurements were not provided in the published studies (Gibbs et al., 1999; Lucchini et al., 1999; Myers et al., 2003a; Roels et al., 1987a, 1987b, 1992). The most detailed information characterizing particle size distributions of total Mn in occupational settings were measured using personal air monitors. Therefore, these data were focused upon initially for simulations.

Information on the particle size distribution from measurements of Mn dust gathered at a large alkaline battery plant from 1987 to 1995 (Roels et al., 1992, 1999) was obtained from the author. This database represents approximately 1500 air sampling results (total and respirable dust), and demonstrates a bimodal distribution for the airborne Mn particles with one peak observed at 6.0–6.5 μm, characteristic of respirable particles, and a second peak >8.5 μm, characteristic of the non-respirable fraction. Because no additional information was available on the size distribution of the respirable concentration of Mn dust from any other occupational studies, the median particle diameter for respirable dust was assumed to be approximately 6 μm in diameter. USEPA (1993) notes a similar median diameter cut point for the respirable dust fraction of 5 μm based on information provided by Roels et al. (1992) and Roels (1993). Since information was not available regarding median particle size for the respirable and non-respirable fraction from other Mn-associated occupational cohorts, a Monte Carlo analysis using personal exposure measures from the one occupational cohort study with particle size distribution data available (Roels et al., 1999) was conducted to estimate a potential geometric standard deviation (GSD) that would encompass the respirable range of particles (defined as <10 μm), as well as each of the bimodal distribution peaks. These data (Roels et al., 1999) displayed a respirable particle size distribution with peaks at 5.0 μm (d_{50}) and 7.1 μm (d_{100}). This analysis predicts a median particle size of 6.0 μm (GSD 3.4 μm). While this represents a large variation in the potential particle size distribution, a median particle diameter of 6 μm (GSD 3.4 μm) for simulating occupational exposures was used in the MPPD model.

Additional simulations were conducted to evaluate the potential effect of the assumptions of particle diameter and GSD on the PBPK model estimates of target tissue concentration (e.g. Mn concentration in the globus pallidus) (Table 1). The model was allowed to simulate exposure concentrations ranging from 21 to 1317 micrograms per cubic meter (μg/m^3) corresponding to the exposure range for respirable Mn dust in alkaline battery plant workers (Roels et al., 1992). The mass media aerosol diameter (MMAD) varied from 3 to 9 μm with GSD values of 1.5 μm or 3.4 μm. At the lower concentrations of particles (<100 μg/m^3), relatively little change in target tissue concentration was predicted. At the highest concentration (1317 μg/m^3), the variation in target tissue concentration ranged from 7% less to 6% more than the value estimated using the MMAD of 6 μm (GSD 3.4 μm) for simulating occupational exposures.

2.5. Comparison with results from analyses in nonhuman primates

An analysis similar to that presented here was conducted by Schroeter et al. (2012) using manganese neurotoxicity studies

Table 1
PBPK-predicted concentration in the globus pallidus (μg Mn/g of tissue) after occupational exposure (8 h/day, 5 days/week) for 1 year in a 77.7 kg man with a dietary intake of 3 mg Mn/day.

μg Mn/m^3 respirable dust	MMAD = 3, GSD = 3.4, head 54.2%, lungs 14.3%	MMAD = 6, GSD = 3.4, head 69.8%, lungs 13.5%	MMAD = 9, GSD = 3.4, head 78.8%, lungs 13.1%	MMAD = 3, GSD = 1.5, head 54.9%, lungs 23.5%	MMAD = 6, GSD = 1.5, head 81.3%, lungs 15.1%	MMAD = 9, GSD = 1.5, head 91.3%, lungs 7.3%
0	0.39					
21	0.45	0.46	0.46	0.45	0.46	0.47
46	0.50	0.52	0.52	0.51	0.53	0.53
65	0.53	0.55	0.56	0.55	0.57	0.57
104	0.59	0.61	0.63	0.61	0.63	0.63
163	0.65	0.68	0.70	0.68	0.71	0.70
179	0.67	0.70	0.71	0.70	0.72	0.72
201	0.69	0.72	0.73	0.72	0.74	0.74
243	0.72	0.75	0.77	0.75	0.78	0.78
244	0.72	0.75	0.77	0.75	0.78	0.78
265	0.74	0.77	0.79	0.77	0.80	0.80
314	0.77	0.80	0.82	0.80	0.83	0.83
319	0.77	0.81	0.83	0.81	0.84	0.84
468	0.85	0.89	0.92	0.89	0.93	0.93
499	0.87	0.91	0.93	0.91	0.95	0.95
530	0.88	0.93	0.95	0.93	0.97	0.96
613	0.92	0.97	0.99	0.97	1.01	1.01
634	0.93	0.98	1.00	0.98	1.02	1.02
796	0.99	1.05	1.08	1.05	1.10	1.10
907	1.04	1.10	1.14	1.10	1.16	1.15
1201	1.14	1.23	1.27	1.23	1.30	1.29
1317	1.19	1.27	1.33	1.28	1.36	1.35

MMAD: mass media aerosol diameter.
GSD: geometric standard deviation.
Head: percentage of Mn deposited in the head (nasal cavities and brain) as estimated by the MPPD model.
Lungs: percentage of Mn deposited in the lungs (tracheobronchial and pulmonary regions) as estimated by the MPPD model.

conducted in monkeys. Gwiazda et al. (2007) had conducted a review of subchronic to chronic rodent and nonhuman primate studies to determine whether a consistent dose-response relationship existed among different studies. The goal of the Gwiazda et al. (2007) review was to determine whether animal studies could be used to evaluate the neurotoxicity of chronic low-level manganese exposures in humans.

Since the completion of the Gwiazda et al. (2007) review and with the development of recent PBPK models (Nong et al., 2009; Schroeter et al., 2011; Yoon et al., 2011), it is now possible to estimate the target tissue concentrations that would be associated with exposures to manganese in rat or monkey studies. Schroeter et al. (2012) focused on applying PBPK modeling approaches to a subset of the monkey studies identified by Gwiazda et al. (2007). Unlike rats, monkeys develop regionally selective increases in brain manganese concentrations (Dorman et al., 2006a, 2006b; Newland et al., 1987, 1989; Eriksson et al., 1992; Aschner and Aschner, 2005) and behavioral effects similar to those reported for manganese affected humans (Olanow et al., 1996). Using a PBPK model for Rhesus monkeys, Schroeter et al. (2012) simulated exposure scenarios from each monkey study reported by Gwiazda et al. (2007) and predicted the corresponding concentrations in the brain for various dose routes, exposure concentrations and durations. The observed responses were categorized using a clinical scoring system for dose-response analysis. We also compared results from this earlier analysis in monkeys to the current analyses relying on occupational studies.

2.6. Statistical analysis approach

A no-statistical-significance-of-trend (NOSTASOT) approach was used to estimate the NOAEL in target tissue (Tukey et al., 1985), using the data that indicated whether each individual had an adverse neurological test response combined with the PBPK model estimated Mn target tissue concentrations. The data separated into two groups (exposed to respirable Mn dust or unexposed), and the exposed group was sorted lowest to highest by the PBPK model predicted concentration of Mn in the globus pallidus. A Fisher's exact test was used to compare the combined incidence rate of abnormal scores for eye-hand coordination or the hand steadiness test between the exposed and unexposed groups. If there was a significant difference in incidence of an abnormal score between the exposed and unexposed group, the test was repeated after removing the subject(s) from the exposed group with the highest concentration of Mn in the globus pallidus. This process continued until a Fisher's exact test comparing the prevalence rate of abnormal neurological test response in the remaining exposed group to the prevalence rate in the unexposed group was not statistically significant. Removing the highest exposed individuals until a point was reached of no statistical significance allowed for the identification of a target tissue concentration NOAEL.

A second approach using the Tukey et al. (1985) NOSTASOT to derive a NOAEL used the Cochran-Armitage trend test (Armitage, 1955; Cochran, 1954). As with the method described above using the Fisher's exact test, this approach involves repeated application of a test for a significant dose-response trend grouping only by the PBPK model predicted concentration of Mn in the globus pallidus. If there is a significant difference in the complete data set, the test is repeated after removing the highest dose group. This procedure is repeated until the trend test is no longer statistically significant.

3. Results

3.1. Relevant epidemiological data

Twenty-one occupational studies with Mn exposure were initially identified (Bast-Pettersen et al., 2004; Bouchard et al., 2007a, 2007b; Deschamps et al., 2001; Fittro et al., 1992; Gibbs et al., 1999; Health Canada, 2010; Lee et al., 2003; Lees-Haley et al., 2006; Lucchini et al., 1995, 1997, 1999; Mergler et al., 1994; Myers et al., 2003a, 2003b, 2003c; Roels et al., 1987a, 1987b, 1992, 1999; Young et al., 2005). Emphasis was placed on occupational studies because these studies have provided the basis for current regulatory recommendations regarding inhalation exposure to Mn compounds (USEPA, 1998). Fourteen were selected for detailed review (Bast-Pettersen et al., 2004; Bouchard et al., 2007a, 2007b; Deschamps et al., 2001; Gibbs et al., 1999; Lucchini et al., 1999; Mergler et al., 1994; Myers et al., 2003a, 2003b, 2003c;

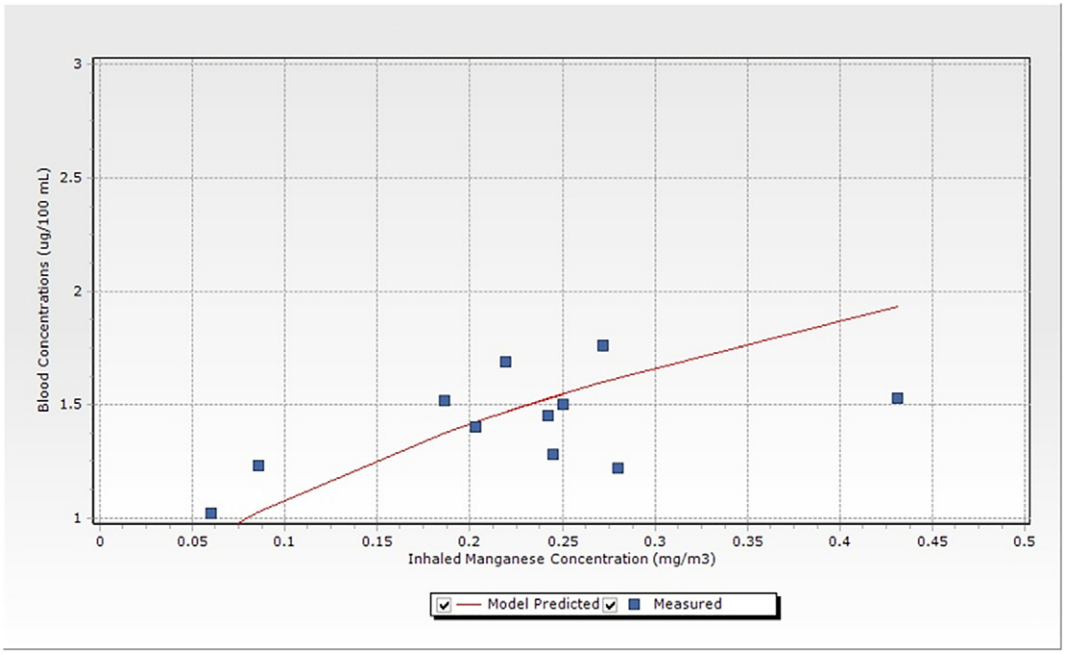

Fig. 1. Data from Roels et al. (1987a)—respirable Mn vs. blood concentrations at start of work week.

Roels et al., 1987a, 1987b, 1992, 1999; Young et al., 2005) that reported having the data necessary for quantitative exposure estimates and objective measures of neurological effects. Full individual exposure and response data were available for three of these studies (Bast-Pettersen et al., 2004; Roels et al., 1992, 1999[3]) and partial exposure and response data were available in seven studies (Bouchard et al., 2007a, 2007b; Gibbs et al., 1999[3]; Mergler et al., 1994; Myers et al., 2003a, 2003b; Young et al., 2005). Published studies provided data that were used in verifying the PBPK model and/or estimating Mn target tissue levels (Lucchini et al., 1999; Myers et al., 2003a; Roels et al., 1987a).

3.2. Exposure to manganese from ambient air and the diet

The estimated background air mean Mn concentrations and 95% confidence limits (CLs) for each country and respirable PM size (<10 μm) are 0.013 μg/m^3 (CL 0.012–0.013 μg/m^3) in Canada and 0.014 μg/m^3 (CL 0.013–0.016 μg/m^3) in the US (see Supplemental information). The estimated respirable ambient air concentration of Mn used in our PBPK models ranged from 0.01–0.02 μg/m^3.

For occupational exposure modeling, the dietary exposure was 3 mg Mn per day based on average dietary intakes of Mn for Canadian and US populations (see Supplemental information). This value could be an underestimate of the mean dietary intake of adults in both the US and Canada as values of Mn from the dietary data reported in NHANES ranged up to >20 mg/day from diet alone (CDC, 2012) and to values >60 mg/day when supplements were also considered (Supplemental Table S4).

3.3. Verification of the human PBPK Mn model

The Mn PBPK models were developed using tissue and whole body concentration data, but due to their physiological structure, the models also can predict blood concentrations. To demonstrate that the simulated Mn blood concentrations from our PBPK model were consistent with those measured in occupational studies, available blood concentrations (Roels et al., 1987a) were compared to blood Mn concentrations simulated with the adult human PBPK model (Schroeter et al., 2011). Since specific information on particle size distribution for this cohort was not available, the median particle size and GSD from a later study (Roels et al., 1992) were applied and exposure was simulated for 8 h/day, 5 days/week. As occupational exposure is not continuous and "steady state" would not be achieved, the model was run until the pattern of blood concentrations observed reached a constant, recurrent pattern, referred to as "periodicity". Estimates of the blood concentration at the beginning and end of the work week were determined for comparison to the published data (Roels et al., 1987a).

The results of the PBPK modeling were similar in trends with the available Mn blood concentrations (Roels et al., 1987a), whether it was assumed that the blood samples were drawn from the workers at the beginning or the end of the work week (Figs. 1, 2). The model was applied without incorporation of available information on variability of physiological parameters, and thus does not reflect individual variability observed from individual blood measurements. However, the model simulation is consistent with the available measured Mn blood concentrations, particularly for individuals with lower exposures, a point that is critical for the evaluation of ambient exposures.

The PBPK model-predicted results from another study (Myers et al., 2003c) are shown along with the measured blood data (Figs. 3, 4). The simulations were compared with the blood draws at either the start or the end of the work week.

The data from a study measuring Mn blood concentrations in a group of ferroalloy workers (Lucchini et al., 1999) was also considered for comparison with the PBPK model. However, there were both internal (Lucchini et al., 1999) and external (Health Canada, 2010) inconsistencies related to estimating the fraction of inhaled Mn in the respirable range. In the publication (Lucchini et al., 1999), the authors reported that the respiratory fraction of Mn (as MnO_2 and Mn_3O_4) ranged from 40 to 60%. However, comparison of the values reported in the publication for total Mn dust (5–1490 μg/m^3; geometric mean 54.25 μg/m^3; arithmetic mean 175.59 μg/m^3) to respirable dust (1–670 μg/m^3; geometric mean 17.18 μg/m^3; arithmetic mean 67.08 μg/m^3) suggests a lower respirable fraction, 20–40%. Examination of individual data by Health Canada (2010), presenting the range, median, and mean concentrations across all job categories for total and respirable dust, suggests that only a limited number of "intermittent" samples of respirable Mn were taken. Health Canada (2010) relied upon these data to estimate the average respirable concentrations (ARE) over the duration of the

[3] Roels et al. (1992, 1999) and Gibbs et al. (1999) data were supplemented by raw data from authors.

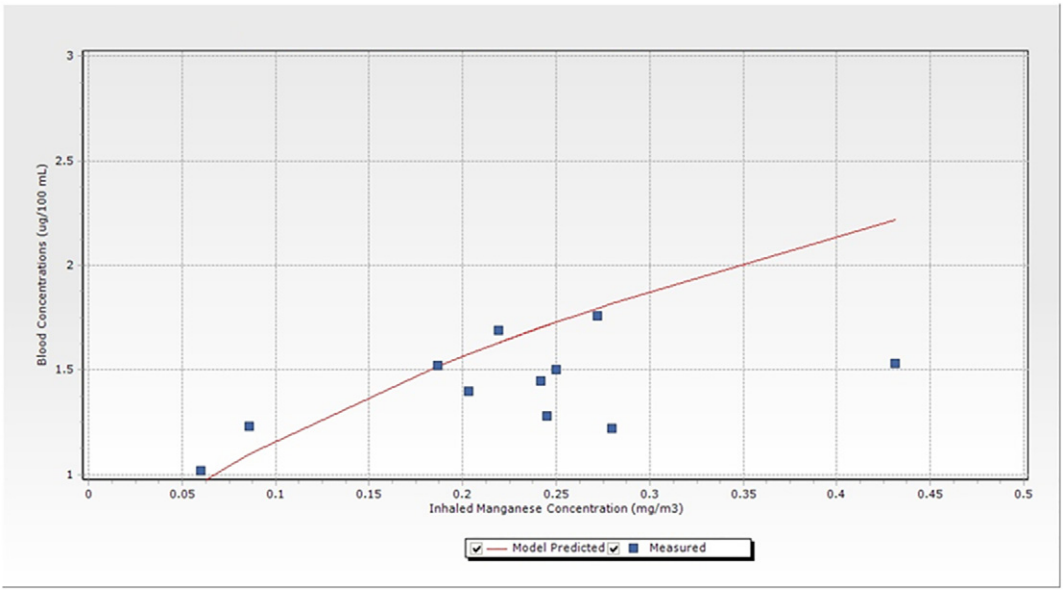

Fig. 2. Data from Roels et al. (1987a)—respirable Mn vs. blood concentrations at end of work week.

work history for each subject and over a shorter 5 year period (ARE5). Comparing the Health Canada (2010) estimates of ARE (1.21 to 285.16 μg/m^3, arithmetic mean of 70.92) or ARE5 (1.1 to 145.43 μg/m^3, arithmetic mean of 41.79) to the total dust concentrations reported by Lucchini et al. (1999) suggested that the respirable fraction of Mn could range from approximately 10–25% of the total concentration.

The estimates of the respirable fraction of Mn have a significant effect on predicted Mn blood concentrations from the Lucchini et al. (1999) study (Fig. 5). Using 20–60% as the respirable fraction gives a better match for expected blood Mn concentrations associated with total Mn dust concentrations of <0.2 mg/m^3. For total Mn dust concentrations >0.2 mg/m^3, however, model results are more consistent with a 20% respirable fraction. This outcome illustrates how different assumptions made for the respirable fraction affects model results for Mn in target tissue. Thus, the use of dose metrics from this study (Lucchini et al., 1999) for the evaluation of Mn target tissue concentrations associated with potential adverse effects carries a high degree of uncertainty.

Since a strong agreement between model estimates and measured blood Mn concentrations is found in two of the three available occupational studies that report blood concentrations (Myers et al., 2003a; Roels et al., 1987a), the confidence in the model using data from these studies is higher. While the data necessary to validate the target tissue concentration of concern (i.e. increases of Mn in selected regions of the brain) are not available, the ability to simulate the available Mn blood concentrations measured in workers, as well as the available

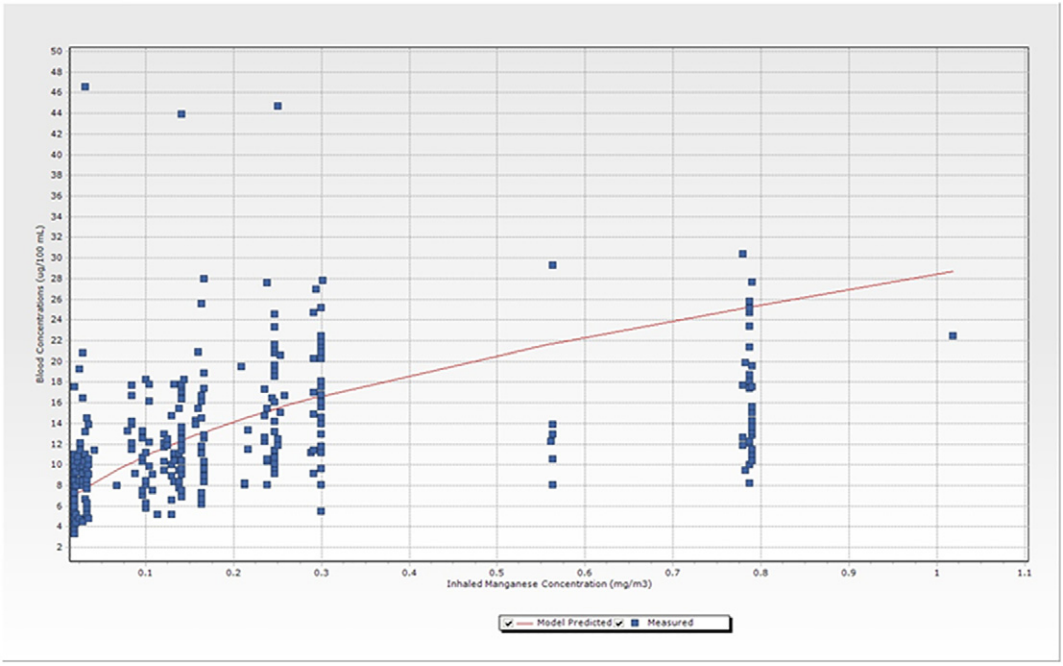

Fig. 3. Data from Myers et al. (2003a)—respirable Mn vs. modeled blood concentration at start of work week.

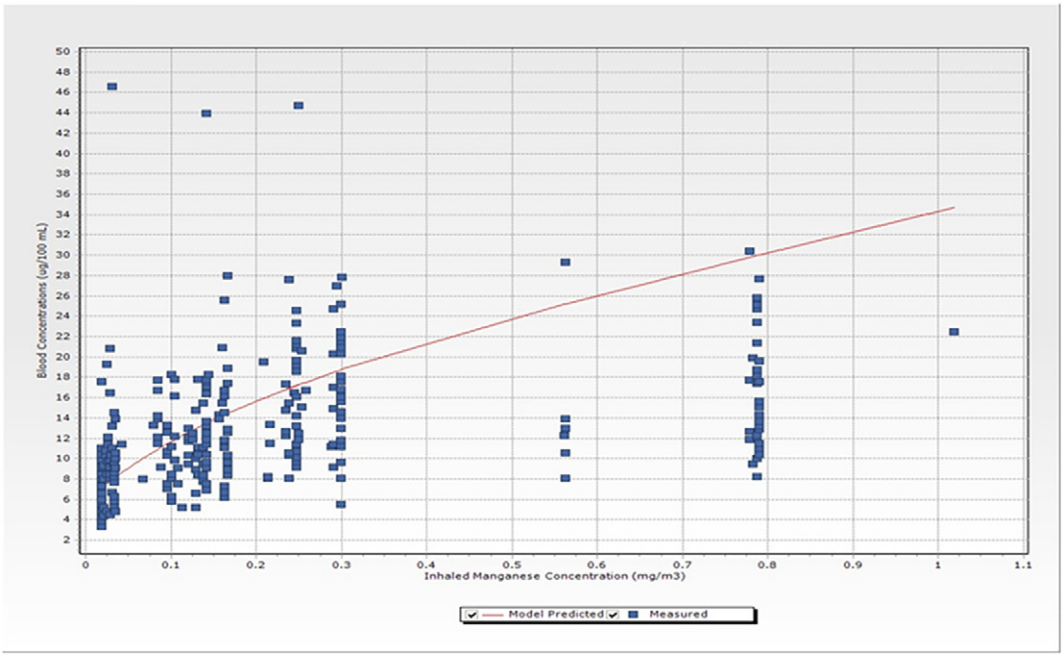

Fig. 4. Data from Myers et al. (2003a)—respirable Mn vs. modeled blood concentrations at end of work week.

data from human tracer studies, provides additional confidence in the estimates provided by the PBPK model. Further confidence comes from the ability of the primate model to accurately predict target tissue concentrations over an even greater range of dose-routes, including intravenous, intraperitoneal, and subcutaneous, in addition to inhalation and oral (Schroeter et al., 2012).

3.4. Application of the human PBPK Mn model

Based on the changes in specific, subclinical neurological effects associated with Mn air concentrations reported in several occupational studies, the increase of Mn in the globus pallidus of the brain can be viewed as the relevant target tissue for neurological effects (Guilarte, 2010; Health Canada, 2010). Thus, the PBPK model can be used to derive a "target tissue NOAEL" by applying the model to simulate the concentration of Mn expected in the globus pallidus following exposures at the highest air concentrations reported to have no effect on neurological function.

Exposure information and individual dichotomized data (response/no response) were available for each subject in a study of workers exposed to Mn oxide dust in a dry alkaline battery factory (Roels et al., 1992). This study evaluated the prevalence of neuropsychological symptoms and neurofunctional performance, thus allowing several types of analyses to be conducted to estimate a potential Mn target tissue NOAEL. The PBPK model was applied using data from this study (Roels et al., 1992), simulating the available respirable air concentrations for each individual and assuming 8 h/day, 5 day/week inhalation exposure. The target tissue concentrations (i.e. μg Mn/g tissue in the

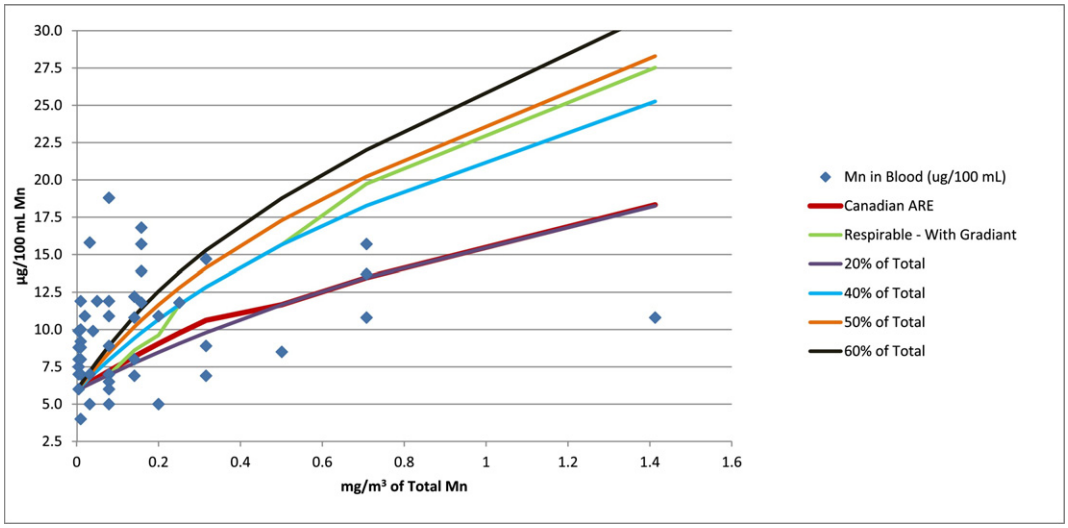

Fig. 5. Data from Lucchini et al. (1999)—impact of respirable fraction of Mn on blood concentration estimates.

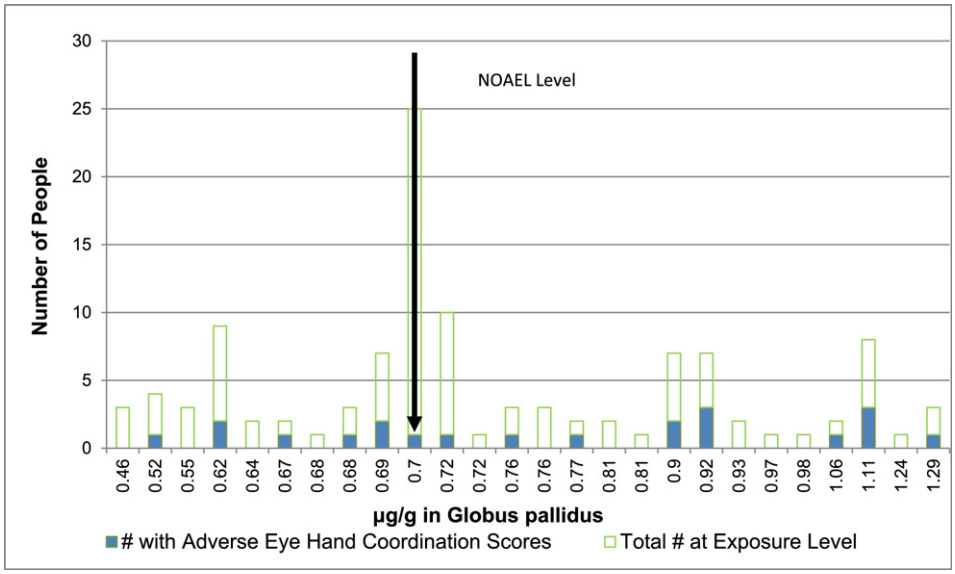

Fig. 6. Data from Roels et al. (1992)—individuals with adverse eye-hand coordination scores vs. globus pallidus Mn concentrations at end of work week.

globus pallidus) were estimated for each individual and used to determine a potential Mn target tissue NOAEL.

No-statistical-significance-of-trend (NOSTASOT) approach with a Fisher's exact test predicts a target tissue (Mn concentration in the globus pallidus) NOAEL of 0.7 µg/g using the prevalence rate of abnormal eye-hand coordination, (Fig. 6) and a target tissue NOAEL of 0.9 µg/g using the adverse effect endpoint of abnormal hand steadiness (Fig. 7). Applying the NOSTASOT statistical approach using trend tests to the data from the Roels et al. (1992) study, using eye-hand coordination data grouped into the same seven exposure groups (Table 2) determined by ATSDR (2012), yields a NOAEL corresponding to the exposure group with respirable continuous Mn concentrations of 100–199 µg/m^3 consistent with the ATSDR (2012) BMDL of 142 µg/m^3, with a corresponding PBPK modeled target Mn tissue concentration of 0.7 µg/g. These two different statistical evaluations provided similar results.

Other epidemiological studies identified through literature searching reported no statistically significant abnormal neurofunctional test results following Mn exposure in occupational cohorts, even in the highest exposure groups (Myers et al., 2003a; Gibbs et al., 1999). One study (Myers et al., 2003a) evaluated nervous system effects in a large cohort of South African mineworkers exposed to concentrations of Mn in the workplace. Only total dust Mn concentrations were reported, so it was assumed that the respirable fraction of the total dust Mn also changed as the concentration increased. The range of total dust Mn (digitized from Fig. 3 of Myers et al., 2003a) was 58 µg/m^3–5088 µg/m^3. Due to the shallow rise in the blood levels associated with the rise in the total dust, it appeared that the percentage of respirable dust declined with increasing total dust. Three different fractions were assumed for converting the total dust to respirable dust. For values of 58–<500 µg/m^3 of total dust, the assumed percentage of respirable dust was 33%. For values of 500–<1000 µg/m^3 of total dust, the assumed percentage of respirable dust was 25% and for concentrations of total Mn dust of ≥1000 µg/m^3, the respirable fraction was assumed to be 20% of the total dust. These assumptions result in a range of respirable concentrations of Mn dust of 19–1018 µg/m^3. Since this study did not report any neurological effects associated with any

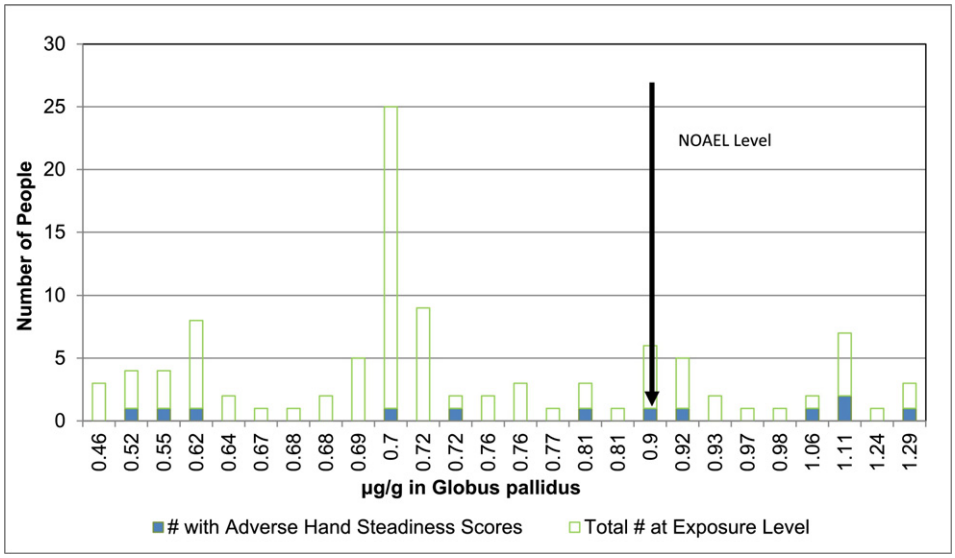

Fig. 7. Data from Roels et al. (1992)—individuals with adverse hand steadiness scores vs. globus pallidus concentrations of Mn at end of work week.

Table 2
Grouped prevalence data for abnormal eye-hand coordination scores in workers exposed to respirable manganese.

Group	Range of respirable manganese exposure concentrations (μg Mn/m^3)	Average respirable manganese exposure concentrations (μg Mn/m^3)	# of workers with abnormal eye-hand coordination scores	Total number of workers in exposure group
1	Control	0	5	101
2	1.0–99	33	1	7
3	**100–199**	**174**	**6**	**39**
4	200–299	224	4	28
5	300–399	307	2	3
6	400–499	451	4	9
7	>500 (523–650)	564	4	6

Source: ATSDR, 2012, based on Roels et al., 1992. The $BMDL_{10}$ used in the MRL derivation is 142 μg/m^3 (ATSDR, 2012).
Bolded data are those corresponding to the NOAEL.

exposure groups, the highest respirable concentration could be considered a NOAEL. Based on these assumptions, the PBPK model was run resulting in estimates of approximately 1 μg/g tissue in the globus pallidus, which could be considered the NOAEL. Similarly, a study in which a population of US workers exposed to low levels of Mn dust were evaluated for subclinical neurological effects (Gibbs et al., 1999) reported no significant effects on neurofunctional performance at monthly average respirable Mn air concentrations as high as 328 μg/m^3. Therefore, the highest air concentration reported for this study could be considered a NOAEL and the PBPK model, assuming an occupational exposure scenario (8 h/day, 5 days/week), provides an estimate of a Mn target tissue NOAEL of approximately 0.8 μg/g.

In conducting the PBPK modeling simulations of the occupational exposures, it was assumed that there was no significant contribution to internal dose resulting from the ingestion of non-respirable particulates.

3.5. Comparison with results from analyses in nonhuman primates

The results from the Schroeter et al. (2012) analysis suggested a clear dose-response behavior of effects versus peak and cumulative dose area under the curve (AUC) in the globus pallidus region of the brain. Using a CatReg analysis, a 10% extra risk for a mild response, which would be comparable to subclinical effects such as those measured in human occupational studies, occurred at a peak concentration of 0.8 μg/g manganese in the globus pallidus estimated using the PBPK model. This is consistent with the target tissue NOAELs 0.7 μg/g Mn (eye-hand coordination) to 0.9 μg/g Mn (hand steadiness scores) estimated in the current analysis.

3.6. Estimation of target tissue levels based on ambient air monitoring

Evaluation of the available air monitoring data from both Canada and the US (see Supplemental information) indicate that the 95% upper confidence limit (UCL) on the mean ambient respirable Mn air concentrations are approximately 0.01–0.02 μg/m^3. The concentration of Mn in the globus pallidus associated with this ambient air concentration was estimated using the median diameter of Mn-containing particles based on the relationship between the amount of Mn measured in filters with a 2.5 μm cut-point and a cut-point between 2.5 and 10 μm. Values of 1.5 and 2.5 μm were used for the median particle size diameters with a standard deviation of 1.5.

The PBPK model was applied assuming continuous exposure concentrations of 0.01–0.02 μg/m^3 for 10 years to ensure that the target tissue concentrations achieved steady state. The modeling results indicated that this was achieved within 100 days for dietary exposure alone. The model estimated a target tissue concentration of Mn of 0.4 μg/g with only dietary exposure. For occupational exposure, comparable patterns of tissue concentration (considering that occupational exposure is not continuous) were achieved within 200 days. In addition, in order to determine the ambient air concentrations that would be needed to result in target tissue concentrations similar to those reported for the target tissue NOAEL from occupational studies, the PBPK model was applied assuming continuous exposure, increasing the concentration of respirable Mn until a target tissue concentration of approximately 1 μg/g globus pallidus was achieved (Table 3, Fig. 8). This would be an exposure to respirable dust of at least 150 μg/m^3.

3.7. Estimation of margins of safety based on target tissue concentrations

To develop a MOS, the continuous ambient air concentration associated with target tissue NOAELs from Roels et al. (1992) (0.7 μg/g Mn for eye-hand coordination; 0.9 μg/g Mn for hand steadiness scores) was compared to the 95% UCL on the mean on the ambient air concentration (0.01 to 0.02 μg/m^3). Based on the results of PBPK modeling, continuous exposure to an ambient air concentration of 50 μg/m^3 would be associated with a target tissue concentration of approximately 0.7 μg/g Mn (Table 3), while continuous exposure to an ambient air concentration of 120 μg/m^3 would be associated with a target tissue concentration of approximately 0.9 μg/g Mn. Based on these results, the MOS would be approximately 2500–5000 (calculated as 50 μg/m^3 divided by 0.02 μg/m^3 or 0.01 μg/m^3). For the target NOAEL based on results from neuropsychological testing to measure hand steadiness, the MOS would be approximately 6000–12,000 (calculated as 120 μg/m^3 divided by 0.02 μg/m^3 or 0.01 μg/m^3).

In all of the current regulatory assessments, application of adjustment factors have been applied to "convert" an occupational air concentration to an ambient air concentration and then safety factors have been applied. These approaches implicitly assume that any effect is linearly related to the external air concentration. However, the results of this analysis demonstrate that target tissue concentrations are not linearly associated with external air concentrations due to homeostatic

Table 3
Ambient respirable Mn concentrations vs. PBPK estimates of Mn concentration in globus pallidus.

μg/m^3 respirable dust	Total μg/g in globus pallidus
0.01	0.39
0.1	0.39
1	0.41
10	0.50
20	0.57
30	0.63
40	0.67
50	0.71
60	0.74
70	0.77
80	0.80
90	0.83
100	0.85
110	0.87
120	0.89
130	0.91
140	0.93
150	0.95

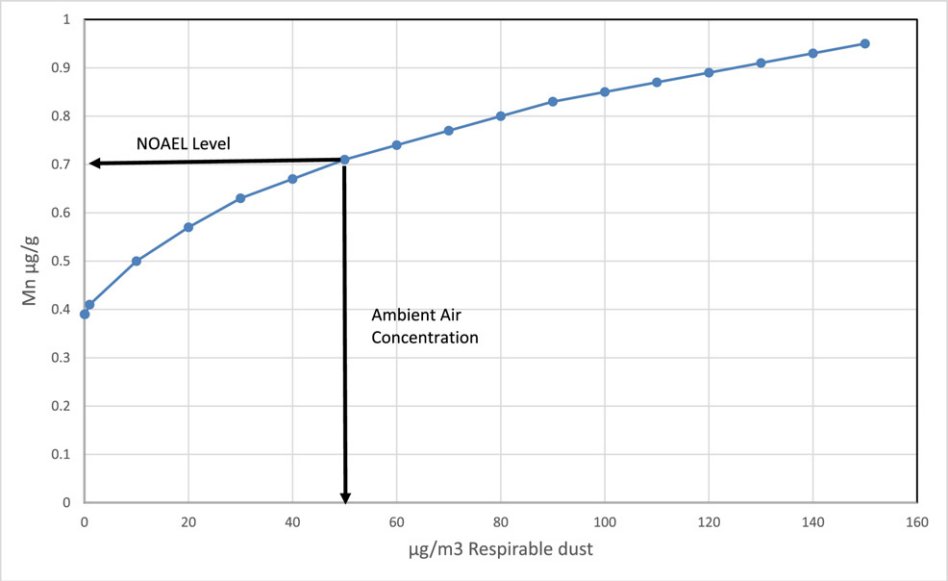

Fig. 8. Predicted total Mn (µg/g) in the globus pallidus from exposure to varying amounts of respirable Mn dust.

mechanisms that maintain target tissue levels within a certain range of exposure. With the application of the PBPK model, Table 4 demonstrates that decreasing an ambient air concentration by safety factors of 10 does not result in corresponding decreases in the target tissue concentrations.

4. Discussion

Current regulatory assessments for Mn rely on occupational studies to provide a point of departure (POD), which would be adjusted by safety factors to derive an acceptable Mn ambient air concentration. However, an approach that relies on the application of safety factors is only biologically reasonable if reductions in acceptable air Mn concentrations are associated with reductions in the concentration of Mn in target tissues. Incorporating PBPK modeling into the safety assessment for Mn allows consideration of available quantitative data, rather than applying default uncertainty or safety factors for this essential element.

Application of the PBPK model to estimate Mn concentrations in target tissues following occupational inhalation exposure demonstrated that there will not necessarily be linear decreases in target tissue Mn with decreasing Mn air concentrations. When the external Mn air concentration is below the concentration that would result in an increase in target tissue concentrations above background Mn, these air concentrations would not be associated with health effects. Decreases in concentrations assumed with the use of additional safety factors would not result in lower target tissue Mn concentrations (Table 4).

One uncertainty noted for the current analysis is the potential contribution from ingestion of Mn resulting from the non-respirable fraction of Mn in the occupational studies. The current analysis only incorporates the contribution from inhalation of respirable particles. The concentrations and particle sizes reported for the available occupational studies suggest there could be non-respirable Mn inhaled and removed from the respiratory tract via mucociliary processes and then swallowed, resulting in the potential for swallowed particles to contribute to target tissue concentrations. Inclusion of this additional contribution to estimated target tissue concentrations would result in an increase in the target tissue concentration associated with the NOAEL, so would only increase the MOS. Using information from Roels et al. (1992), inhalation exposure of workers to 1 Mn mg/m^3 total dust (80% is non-respirable) would result in approximately 8 mg Mn available for potential transfer to the gastrointestinal tract (assuming 10 m^3 of air inhaled for an 8 h workday). PBPK model-predicted concentrations in the globus pallidus following exposure to various dietary levels (Fig. 9a) indicate that the contribution due to swallowing the non-respirable fraction of inhaled particles could increase the target tissue concentration by approximately a factor of 2. The contribution from diet of 8 mg/day only results in approximately 0.65 µg/g Mn in striatum/globus pallidus which, if considered, could potentially increase the NOAEL from 0.7 µg/g Mn to approximately 1.4 µg/g Mn or a factor of 2.

In addition, while the current study does not directly assess potentially sensitive subpopulations (e.g. susceptibility in fetal or neonatal children), Yoon et al. (2011) developed a PBPK model to integrate the available information on Mn kinetics during gestation and lactation in

Table 4
Target tissue concentrations of Mn associated with Mn ambient air concentrations derived from applying adjustment factors to NOAEL values.

Study	NOAEL concentration of Mn in the target tissue, globus pallidus (µg/g)	Ambient air concentration (µg Mn/m^3)	After applying adjustment factors of:					
			10		100		1000	
			Ambient air concentration (µg/m^3)	Mn conc. in the globus pallidus (µg/g)	Ambient air concentration (µg/m^3)	Mn conc. in the globus pallidus (µg/g)	Ambient air concentration (µg/m^3)	Mn conc. in the globus pallidus (µg/g)
Roels et al. (1992)	0.7	47	4.7	0.5	0.47	0.4	0.047	0.4
	0.9	124	12.4	0.5	1.24	0.4	0.124	0.4
Myers et al. (2003a)	1	157	15.7	0.6	1.57	0.4	0.157	0.4
Gibbs et al. (1999)	0.8	80	8	0.5	0.8	0.4	0.08	0.4

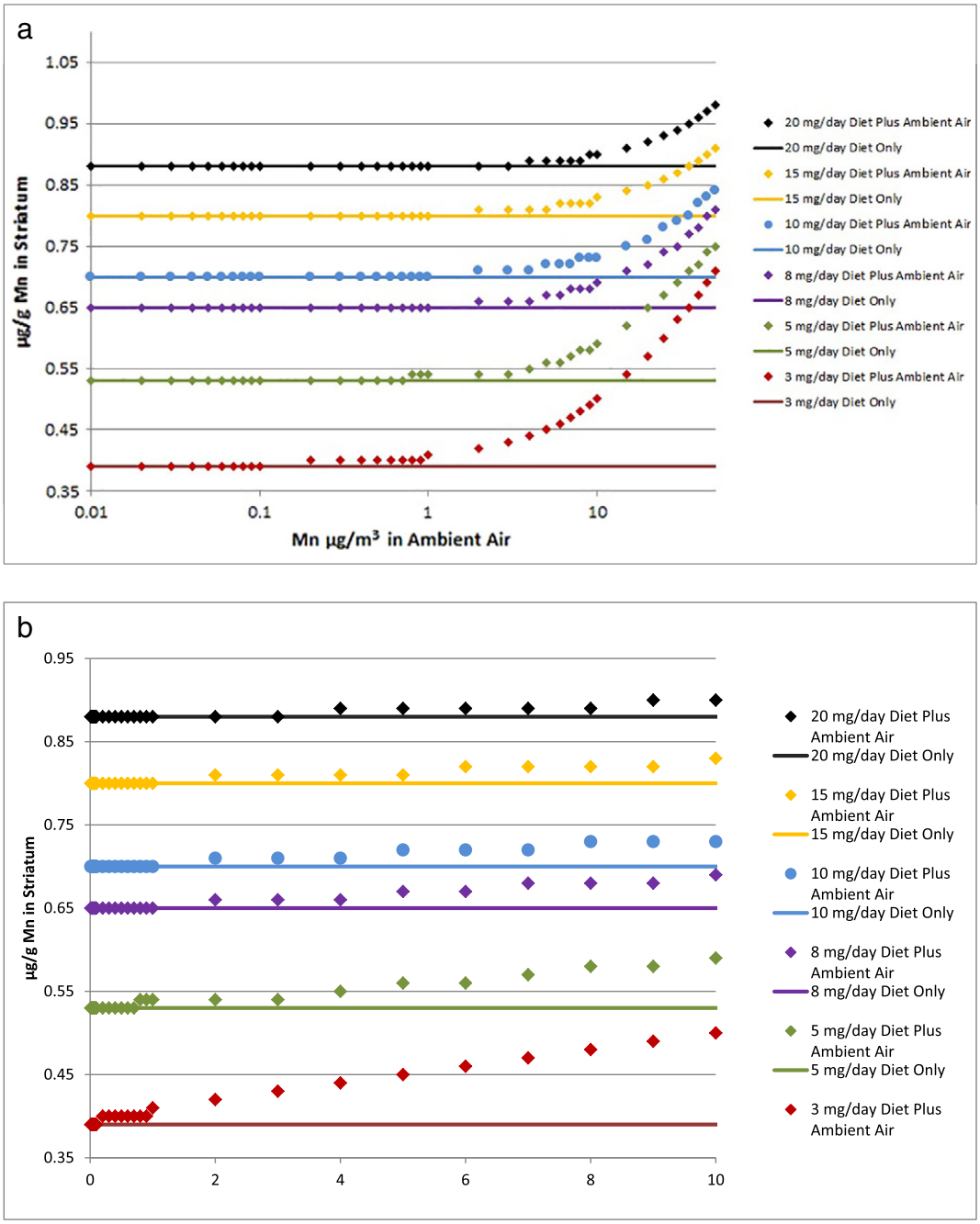

Fig. 9. a. Model-predicted concentration of Mn (μg/g) in the globus pallidus (striatum) from exposure to various daily dietary Mn levels, or dietary Mn daily intake plus various ambient air levels of respirable Mn. Figure is based on a 70 kg human with exposure for 10 years at the indicated dietary intake. b. Model-predicted concentration of Mn (μg/g) in the globus pallidus (striatum) from exposure to various daily dietary Mn levels, or dietary daily intake plus low ambient air levels of respirable Mn. Figure is based on a 70 kg human with exposure for 10 years at the indicated dietary intake.

an attempt to describe homeostatic controls of Mn during human development when challenged with overexposure to Mn. Mn plays a central role in normal prenatal and neonatal development and its deficiency may cause developmental deficits, such as abnormal brain function (Yoon et al., 2011). These life-stage PBPK models showed that adult males achieved a higher daily dose-to-target tissue than fetal, neonatal, pregnant, and non-pregnant female life-stages (Yoon et al., 2011). Based on these pharmacokinetic results, early life stages would be expected to have lower target tissue concentrations, therefore, no sensitive subpopulation adjustment would be necessary when basing risk assessments on occupational studies of adult males exposed to Mn (Taylor et al., 2012).

The potential variability in dietary intake of Mn on target tissue concentrations also needs to be considered when assessing the effects of inhaled Mn. A sensitivity analysis was conducted to determine the parameters that have the most significant impact on the estimation of Mn tissue concentrations in the globus pallidus when the human PBPK model was developed (Schroeter et al., 2011). At low Mn concentrations, the parameters having the largest impact were the influx and efflux diffusion rate constants into the brain, changes in dietary absorption and biliary excretion. Model predictions became less sensitive to dietary absorption and biliary excretion with increasing Mn inhalation exposure concentrations, because brain Mn levels were driven more by inhalation than diet. This observation demonstrates the importance

of any assumptions regarding dietary intake/absorption and biliary excretion in the estimation of changes in target tissue concentrations based on the contribution of inhalation exposure, as well as setting a target tissue NOAEL in occupational studies.

An average dietary intake of 3 mg/day Mn (slightly higher than the "adequate intake" level Aschner and Aschner, 2005), was applied in the human PBPK model for the current study. However, data from both the US (CDC, 2010, 2012) and Canada (Health Canada, 2011) indicate that average dietary intake of Mn varies from 2–>6 mg/day in adults depending on the age range, with maximum intake up to 60–75 mg/day for individuals taking dietary supplements. These dietary intake values can significantly change the baseline target tissue concentration (Fig. 9a), particularly when considering the low Mn concentrations anticipated in ambient air (Fig. 9b). Analyses reported by Schroeter et al. (2011) demonstrated that PBPK model predictions became less sensitive to dietary absorption and biliary excretion with increasing Mn inhalation exposure concentrations, because brain Mn levels were driven more by inhalation than diet at higher air concentrations. Thus, dietary intake, rather than Mn air concentration, may be a dominant factor in determining target tissue concentration following exposure to low air concentrations, and thus, a likely determining factor in human variability of background Mn tissue concentrations.

Multiple occupational studies were evaluated using the human PBPK model for Mn to estimate tissue concentrations from corresponding air concentrations to derive the air concentrations associated with NOAELs. Two studies were negative for health outcomes (Gibbs et al., 1999; Myers et al., 2003a), so the highest Mn air concentrations from these studies were used. All of the estimates of Mn target tissue concentrations were 0.7–1.0 μg/g with corresponding air concentrations based on a typical workday of 200 μg/m^3–1000 μg/m^3. The PBPK model was also used to estimate the continuous inhalation exposure expected to cause these Mn target tissue concentrations. The modeled air concentrations of 50 μg/m^3–>150 μg/m^3 are compared to measured ambient Mn concentrations for Canada and the US to derive a MOS. The estimated MOS of 2500–12,000 suggest how much of an increase in ambient concentrations would be required to produce target tissue concentrations associated with the NOAEL for subclinical neurological effects.

The PBPK model for Mn may also be used to estimate inhaled concentrations required to cause increases in target tissues above background. This point of inflection falls in the ambient air range from 1 to 10 μg Mn/m^3 (Table 3). The model may also be used to estimate the target tissue concentration and corresponding air concentration corresponding to a NOAEL. Our simulations show that tissue concentration at the point of inflection is substantially below target tissue Mn associated with changes in subclinical neurological test results in occupational exposures (Roels et al., 1992). For instance, a recent study in welders indicated that plasma Mn (P-Mn) does not increase until the respirable Mn levels reach 10 μg/m^3 (Hoet et al., 2012). This observation, combined with the results of the current analysis, suggest that a Mn TLV-TWA larger than the currently recommended value of 20 μg/m^3 for respirable dust (ACGIH, 2012) could be relied upon and still be health protective.

In summary, the results of studies on Mn pharmacokinetics demonstrate that homeostatic mechanisms regulate the levels of Mn in the body from inhalation. Increases in target tissues above background occur only when humans (regardless of their age or gender) are exposed to air Mn concentrations far higher than those historically or currently measured in Canada or the US. These findings suggest that regulatory air Mn guidelines are extremely conservative and that the application of standard safety factors may not represent alterations in tissue delivery of Mn due to the nonlinearities in biological processes.

The current human PBPK models (Schroeter et al., 2011; Yoon et al., 2011) are calibrated for adults or infants during gestation and lactation, and have not been extended to specifically address potential differences in children. Therefore, development of a model with pharmacokinetic values specific to children would be of value in addressing the need for an uncertainty factor to extrapolate from adults to children. However, it is expected that children will display similar pharmacokinetics to adults—with significant background tissue levels of Mn that change very little with exposures until reaching 10 μg/m^3. Questions regarding the potential for Mn to affect brain development and cognitive function might be addressed by extension of the model during gestation and lactation to evaluate potential changes in the cerebral cortex as the target tissue. Nonetheless, the globus pallidus is the most sensitive brain region regarding Mn accumulation, and is thus likely an appropriate surrogate for tissue level changes in the frontal cortex (Dorman et al., 2006a, 2006b). While the results of PBPK modeling have indicated critical parameters in estimating Mn target tissue concentrations, a Monte Carlo analysis for selected parameters in the PBPK model, in particular dietary variability among children and adults, would help refine the model estimates and more clearly evaluate the contribution of such factors as dietary exposure versus inhalation exposure in the low exposure regions.

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Conflict of interest statement

The authors declare that there are no conflicts of interest and have read and understood the Conflict of Interest Policy from for the Journal.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Appendix A. Supplementary data

Information on the determination of the amount of Mn consumed by an individual in their diet and through the use of supplements is presented as supplementary data. Additionally, the determination of the ambient air concentration of Mn inhaled by an individual is also presented as supplementary material. This information is published online. Supplementary data associated with this article can be found in the online version, at 10.1016/j.taap.2017.02.015.

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