Application of PBPK modeling to evaluate data on human exposures to manganese, a toxic but essential metal

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Outline

- Risk assessments with essential elements: An example with Mn

- PBPK model development for Mn
  - Adult rats
  - Basic model structure
  - Key features necessary to describe Mn kinetics
  - Extrapolations across species and life stages
  - Validation of model structure
  - Potential sensitive subpopulation

- Summary of Uses of PBPK modeling with Essential Elements
Manganese

- Essential nutrient with multiple adverse effects noted when levels in diet are inadequate
  - Adequate Intake (AI) for Mn in adult men is 2.3 mg/day with Tolerable Upper Intake Level (UL) 11mg/kg/day
  - Normal tissue Mn concentrations are substantial

- High dose toxicity by various routes with effects in specific portions of central nervous system in humans
  - Inhalation of dust (most frequent)
  - Drinking water/diet (infrequent)
  - Complications of liver disease - humans
  - Intravenous supplementation - humans
Objective of the Mn Research Effort

Develop a common risk assessment strategy for Mn for both oral and inhalation exposures taking into account Mn essentiality as well as Mn toxicity

based on variation in normal $[\text{Mn}_{\text{midbrain}}]$ 

Normal: $[\text{Mn}_{\text{brain}}] = \text{Mn} \pm \sigma [\text{Mn}_{\text{midbrain}}]$ 

Acceptable exposures would lead to an increase in $[\text{Mn}_{\text{midbrain}}]$ of no more than some small percentage of the normal variability.
Goals of PBPK Modeling with Mn

- **Organize** the biological data to provide a quantitative physiological description (i.e., a PBPK model) which describes determinants of Mn tissue dosimetry.

- **Predict** brain dose metrics – tissue concentration, time above a specific tissue concentration, average free tissue Mn – depending on mode(s) of action.

- **Extrapolate** between species using knowledge of the changing physiology and biochemistry from test animals to humans.
Factors that have to be included in a PBPK model for Mn

- Background tissue concentrations
- Exposure by multiple dose routes – oral vs. inhalation
- Different animal species – rats, monkeys, humans
- Dose dependent kinetics and homeostatic control
- Altered control of Mn kinetics during early life
A Rich Data Set for Modeling

Studies with inhaled and dietary Mn conducted at the CIIT/The Hamner:

- Rat fed on different diets (2, 10, 100 ppm Mn)
- $^{54}$Mn tracer kinetic studies
- Single nasal exposure with occluded nostrils
- Short-term 14-day inhaled exposure (0.03 to 3 mg Mn/ m$^3$)
- Long-term 90-day inhalation exposure (0.01 to 3 mg Mn/ m$^3$)
- Gestational and lactational period exposures
- Primate 90-day period inhalation exposure
- Other data in rats from University of Montreal
Dose-dependencies of elimination noted for both dietary and inhalation routes of exposure

Inhalation

Diet

Rat tracer studies from Dorman et al., (2001a and b)
Model development: Dietary uptake and biliary excretion

Adult Rat Mn kinetics – Steady state vs tracer kinetics

Liver – concentrations

Whole Body Tracer Study
Model structure: saturable tissue stores and asymmetric diffusion

\[ \text{Mn}_{\text{total}} = \text{Mn}_{\text{free}} + \text{Mn}_{\text{bound}} \]

\[ B_{\text{max}} = B_f + \text{Mn}_{\text{bound}} \]
Other characteristics as needed

**Olfactory uptake**

- Direct delivery to olfactory tissues
- Differential tissue Mn due to binding maxima and rate constants
- Homeostatic control for oral uptake (**diet**) and biliary excretion (**bile**)
Model development: Uptake versus inhaled Mn

Adult Rat Mn kinetics - Short term inhalation exposure

Liver

Striatum

Tissue Concentration (ug/g)

Days

Inhaled Concentration (mg/m³)

Observed

Predicted
Model development: Tissue dosimetry on longer exposures

Adult Rat Mn kinetics - Long term inhalation exposure with enhanced biliary excretion

Dorman et al. 2004a

Tapin et al. 2006

without (gray line) and with (black line) increased biliary elimination of Mn
Determinants of Mn Distribution

Primary stores are B-Mn

After saturation of binding enhanced elimination, increased free Mn

Tissue Binding, Degradation & Turnover

Membrane Transport with Differential Accumulation

Inhalation & Diet

[Mn] dependent biliary excretion rate constant
Toxicity may involve increased free Mn causing oxidative cycling with increased dopamine quinones in the mid-brain.

After ‘essential’ sites are occupied, free Mn increases disproportionately.
Dose dependencies in free and bound Mn in mid-brain

- $Mn_{total}$
- $Mn_{free}$
- $Mn_{bound}$
Model extrapolation: Rats to monkeys

Extrapolation

- Body weight
- Tissue volumes
- Blood flows
- Biliary excretion
- Scaled parameter
Correlation of Brain Magnetic Resonance Imaging Changes with Pallidal Manganese Concentrations in Rhesus Monkeys Following Subchronic Manganese Inhalation

David C. Dorman,*† Melanie F. Struve,* Brian A. Wong,* Janice A. Dye,† and Ian D. Robertson‡
Model extrapolation: Monkey brain predictions (90-day exposure)

Globus Pallidus

Concentration (µg/g) vs. Days

Globus Pallidus

Concentration (µg/g) vs. Days

Cerebellum

Concentration (µg/g) vs. Days

Cerebellum

Concentration (µg/g) vs. Days

Pituitary

Concentration (µg/g) vs. Days

Pituitary

Concentration (µg/g) vs. Days

Olfactory Bulb

Concentration (µg/g) vs. Days

Olfactory Bulb

Concentration (µg/g) vs. Days

Dorman et al. 2006a and Schroeter et al., 2011.
Dastur (1971) ip:

- 12 monkeys (2.5 kg) injected ip with 200 μCi $^{54}$Mn
- examined whole-body retention

Whole-body retention after ip administration
Furchner (1966) - iv vs. oral:

- 3 monkeys (8.5 kg) injected iv with 0.6 µCi $^{54}$Mn
- 3 monkeys (7 kg) administered $^{54}$Mn orally
- examined whole-body retention
Newland (1987) subcutaneous and inhalation:

- 1 monkey (5 kg), 6-week continuous exposure
  - 200 $\mu$Ci $^{54}$Mn and 400 mg Mn ($\text{MnCl}_2$ soln.) administered subq
- 2 monkeys endotracheally exposed to carrier-free $^{54}$MnCl$_2$ aerosol
- measured fecal activity
PBPK Model Evaluation of Monkey Toxicity Data

- Gwiazda et al. 2007:
  “Adequacy and Consistency of Animal Studies to Evaluate the Neurotoxicity of Chronic Low-Level Manganese Exposure in Humans”
  - Considered all routes of exposure
- Gwiazda et al. used estimated cumulative absorbed dose as the only metric of exposure for comparison
  - Concluded that toxicity was route-dependent, with inhalation being more toxic
- This re-analysis uses more appropriate exposure metrics: PBPK model predicted brain Mn concentrations
  - Cumulative dose (AUC)
  - Average concentration
  - Peak concentration
Eriksson (1987) - subQ Dosing (8g total dose)

Globus pallidus concentration
(CMax = 36)
Van Bogaert and Dallemagne (1946) - oral dosing

Concentration (µg/g) vs Days for Globus pallidus.

Mg Mn/day: 4 7 11 0 18
Eriksson (1987) – subQ Dosing (8g total dose)

Globus pallidus concentration

\( C_{\text{max}} = 36 \ \mu g/g \)
Nishiyama et al. (1977) – MnO₂ inhalation

- two exposure concentrations: 0.7 and 3.0 mg Mn/m³
- 22 hours/day, 7 days/week for 10 months

![Graph showing concentration of Globus pallidus over time for two exposure concentrations: 0.7 and 3.0 mg Mn/m³.](image)
Guilarte (2006) - iv dosing

Globus pallidus concentration at lowest exposure:

4 mg Mn iv dose of MnSO₄ given once/week for 44 weeks

Predicted blood concentrations ranged from 0.01 to 11 ppm vs ~0.1 measured
Monkey Toxicity Data - Study Summary

- **Oral exposure**
  - 2 studies: 1-7 mg/kg day Mn

- **iv injection**
  - 3 studies: 1-5 mg/kg Mn once/week

- **ip injection**
  - 1 study: up to 2 mg/kg Mn every other day

- **sc injection**
  - 6 studies: up to 440 mg/kg Mn injections

- **inhalation**
  - 5 studies: up to 30 mg/m³ Mn
Cumulative Target Tissue Dose during Exposure

\[ EC_{50} = 9287 \]
\[ N = 50 \]
\[ R^2 = 0.21 \]
Average Target Tissue Concentration during Exposure

EC$_{50}$ = 4.8
N = 50
R$^2$ = 0.16
Peak Target Tissue Concentration during Exposure

\[ EC_{50} = 7.3 \]
\[ N = 50 \]
\[ R^2 = 0.53 \]

**Graph Details**

- **Graph Title**: Peak Target Tissue Concentration during Exposure
- **EC\(_{50}\)**: 7.3
- **N**: 50
- **R\(^2\)**: 0.53

**Legend**
- Gupta
- Van Bogaert/Dallemane
- Mella
- Olanow
- Newland and Weiss
- Guilarte
- Suzuki
- Eriksson 1987
- Eriksson 1992a
- Eriksson 1992b
- Neff
- Pentschew
- Bird
- Nishiyama
- Coulston/Griffin
- Ulrich
- Dorman
- Hill function

**Concentration (ug/g)**

**Response**

**Axes**

- Y-axis: Response
- X-axis: Concentration (ug/g)
Dose-Response for Mn Neurotoxicity Evidence from Monkey Studies

Neurotoxicity across studies with different routes and durations correlates with estimated Mn concentrations in the brain target tissue
- Peak concentration provides better correlation than average
- Cumulative dose (AUC) provides a much poorer correlation

Inhalation exposure is associated with less toxicity than IV dosing that produces similar average brain target tissue concentrations
- IV injection produces wide, rapid fluctuations in brain concentration that may enhance toxicity
- Slower inhalation uptake produces lower temporal variation

Predicted brain and blood trough concentrations for the IV studies of Guilarte et al. are consistent with the reported concentrations
- but estimated peak concentrations produced by the IV dosing are greater than the troughs by factors of 2 and 1000, respectively
Cross species extrapolation: Validation of model structure

- PBPK models integrate biological and physiological mechanism to understand Mn disposition across dose-route and animal species.

- Main dose-dependent characteristics of Mn disposition including tissue binding, brain uptake and biliary excretion across species.

- The success in moving from rat to primate guides the extrapolation to humans and use of these models to reduce uncertainties in risk assessments and consider both oral and inhalation Mn exposures.

- Other questions raised about potentially sensitive populations addressed by including different life stages.
Human PBPK model for Mn

- Extrapolation
  - Body weight
  - Tissue volumes
  - Blood flows
  - Biliary excretion
  - Scaled parameter

Extrapolation

Validation with human tracer data
Human Mn model: Whole body retention of Mn tracer

Mahoney and Small (1968): Subject WS
begin 800 mg/day Mn

Mahoney and Small (1968): Subject JM
preloaded with Mn 300mg/kg/day 10 d before the study
Model extrapolation: Across species

Rat striatum: Dorman et al. (2004), Tapin et al. (2006)

Monkey globus pallidus: Dorman et al. (2006a)

Rat striatum: simulation, Nong et al. (2009)

Monkey globus pallidus: simulation

Human globus pallidus: simulation

Schroeter et al., 2011.
Tissue-dose based risk assessment

Human model simulation for continuous exposure to Mn for 2 years
Effect of Dietary Variation on Mn in Blood

Predicted human plasma concentrations for controlled variable dietary intakes

Plasma Mn concentration (µg/L) vs. Days

- Freeland-Graves (1994)
- Simulation
Comparison of Inhalation and Oral Exposure

Predicted human brain and blood concentrations for continuous 200-day inhalation exposure with variable dietary intakes
Summary

- The monkey PBPK model accurately simulated the fast “free” and slow “bound” elimination phases of Mn tracer using multiple exposure routes

- We were able to assess possible ranges of Mn tissue concentrations due to differences in dietary intake (4-5 fold) using the human PBPK model

- Increases in brain Mn concentration levels occur at inhalation exposures between 0.01 and 0.1 mg/m\(^3\) Mn

- These validated PBPK models can be used to identify potential points of departure for a dosimetry-based risk assessment based on changes in brain Mn levels
Mn Developmental Modeling

Whether infants and children should be regarded as a susceptible population for effects of Mn

- Characterizing Mn transfer across placenta and through milk
- Evaluating lifestage differences in Mn pharmacokinetics
- Comparing Mn exposures from inhalation, breast milk, and formula
Extending adult model to perinatal periods: Rat developmental Models

Yoon et al. 2009a and 2009b
Rat Developmental Model makes it possible...

- To predict Mn transfer from mother to fetus/neonate:

- To estimate Mn tissue dosimetry in the target during perinatal period:
Rat Developmental Model makes it possible...

- To describe the changes in Mn kinetics during postnatal development:

- To compare exposures from different sources of Mn:
  - Milk
  - Diet
  - Inhalation

Temporal changes in Mn tissue concentrations during neonatal development

Mn Daily Dose (mg/kg BW/day) in the Pups

Mn Daily Dose (mg/kg BW/day) in the Dam
Findings in Rat Models: Key Processes to Describe Mn Kinetics during Perinatal Period

- Incorporation of Mn transfer processes from the dam to offspring: Keeping maternal homeostasis while ensuring adequate Mn to the offspring

- Changes in physiological processes responsible for Mn homeostatic mechanism
  - Enhanced Mn uptake in gut in neonates
  - Tissue binding characteristics - reflecting increased Mn needs in brain during fetal and postnatal development
  - Apparent low, but inducible biliary excretion in neonates
Developing human gestation and lactation models

Features of human model based on successful rat description, human tissue Mn observations, and the species differences in key processes
Placental and lactational transfer of Mn

Yoon et al., 2011

Placenta

Milk
Fetal and neonatal brain Mn

Fetus

Role of placenta ensuring adequate Mn supply for normal development while preventing over-exposure

Infants and children

Neonatal specific homeostatic control to retain adequate Mn in response to low Mn in human milk by enhanced uptake and limited biliary excretion

Yoon et al., 2011
Model extrapolation: Across life stages

Yoon et al., 2011
Estimation of Mn Daily Doses from Various Sources: Comparison among Adults, Infants, and Children

Daily systemically available dose to the adult, infant (6 months), and child (3 years) were compared among milk, dietary, and inhaled doses on the selected day. Inhalation at 0.01 mg/m³ of Mn was simulated.
Data from wide variety of studies on the dosimetry and toxicity for manganese (Mn) to understand the factors that regulate disposition of this essential element in the body were organized with PBPK models.

PBPK modeling approach was applied to understand the role of dose route, form of Mn, and homeostatic control processes in regulating tissue Mn concentrations under various exposure conditions.

The current PBPK model described well both the background tissue Mn levels without environmental exposure as well as the increase after high dose inhalation exposure.
Conclusion

- This understanding of the processes was used to develop generic methods for risk assessments for Mn which is also applicable for other compounds that have significant endogenous background concentrations.

- PBPK predicted brain dosimetry can be used to evaluate impact of environmental exposure on target tissue dose by comparing brain dosimetry at proposed inhalation guideline with variation due to dietary exposure.

- Tissue dose in the target brain region estimated from PBPK model can be used to support environmental guidelines instead of current approaches that simply apply uncertainty factors to BMDL.
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