Outline of Talks

- Background on Mn PBPK Models and Applications to Evaluate Safe Exposures
  - Harvey Clewell, Ramboll and Miyoung Yoon, ToxStrategies
- Sensitivity Analysis for the Published Adult Human Mn Model
  - Cynthia Van Landingham, Ramboll
- PBPK Modeling of Bioavailability of Mn in Diet and Drinking Water
  - Miyoung Yoon, ToxStrategies
- PBPK Modeling for Mn with Rapid Association – Dissociation in Tissues
  - Mel Andersen, Andersen ToxConsulting

Application of PBPK modeling to evaluate safe exposures to Manganese: an essential toxic metal

Harvey Clewell, Ramboll

and

Miyoung Yoon, ToxStrategies

Overview

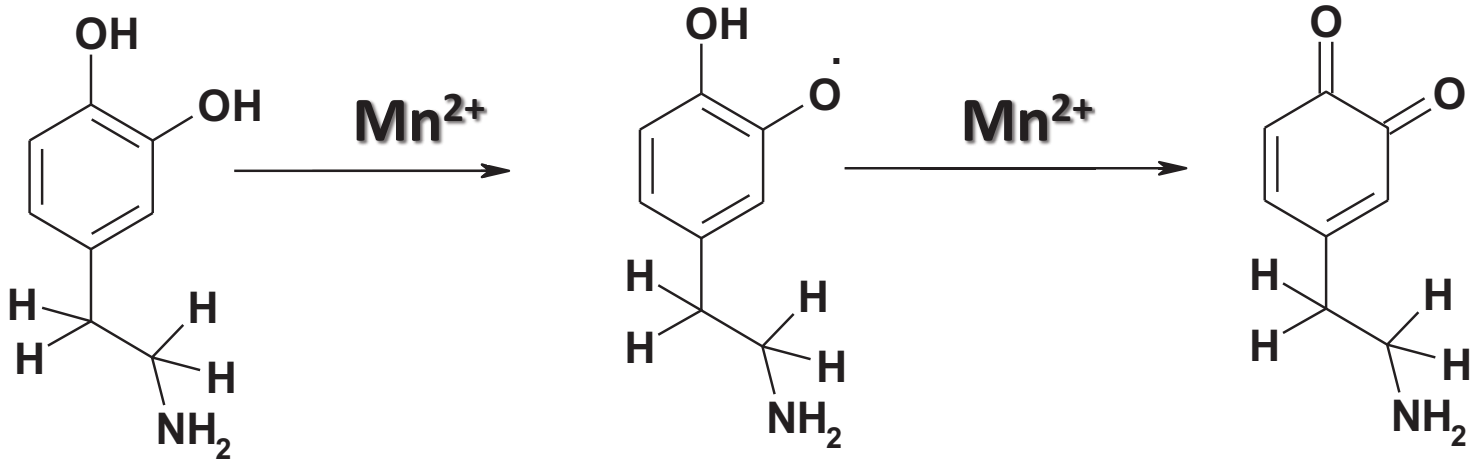
Objective – development of PBPK models for Mn to evaluate safe exposure to this toxic but essential metal

- **PBPK model development for Mn**
  - Development of basic model structure with adult rat data
  - Extrapolation across species: rat → monkey → human
  - Validation of model
  - Prediction of brain target tissue Mn levels from environmental exposure to Mn
- **PBPK Modeling of early life**
  - Characterizing Mn transfer across placenta and through milk
  - Evaluating lifestage differences in Mn pharmacokinetics
  - Comparing Mn exposures from inhalation, breast milk, and formula
- **Potential applications of PBPK models in risk assessments**

Mn risk assessment concerns driving the development of a PBPK model

- **Implications of essentiality of Mn / homeostatic control**
- **Potential differences between inhalation and oral exposure**
- **Different forms of Mn / rates of dissolution**
- **Possible early life sensitivity**
  - Gestation
  - Lactation
  - Childhood
- **Human interindividual variability**

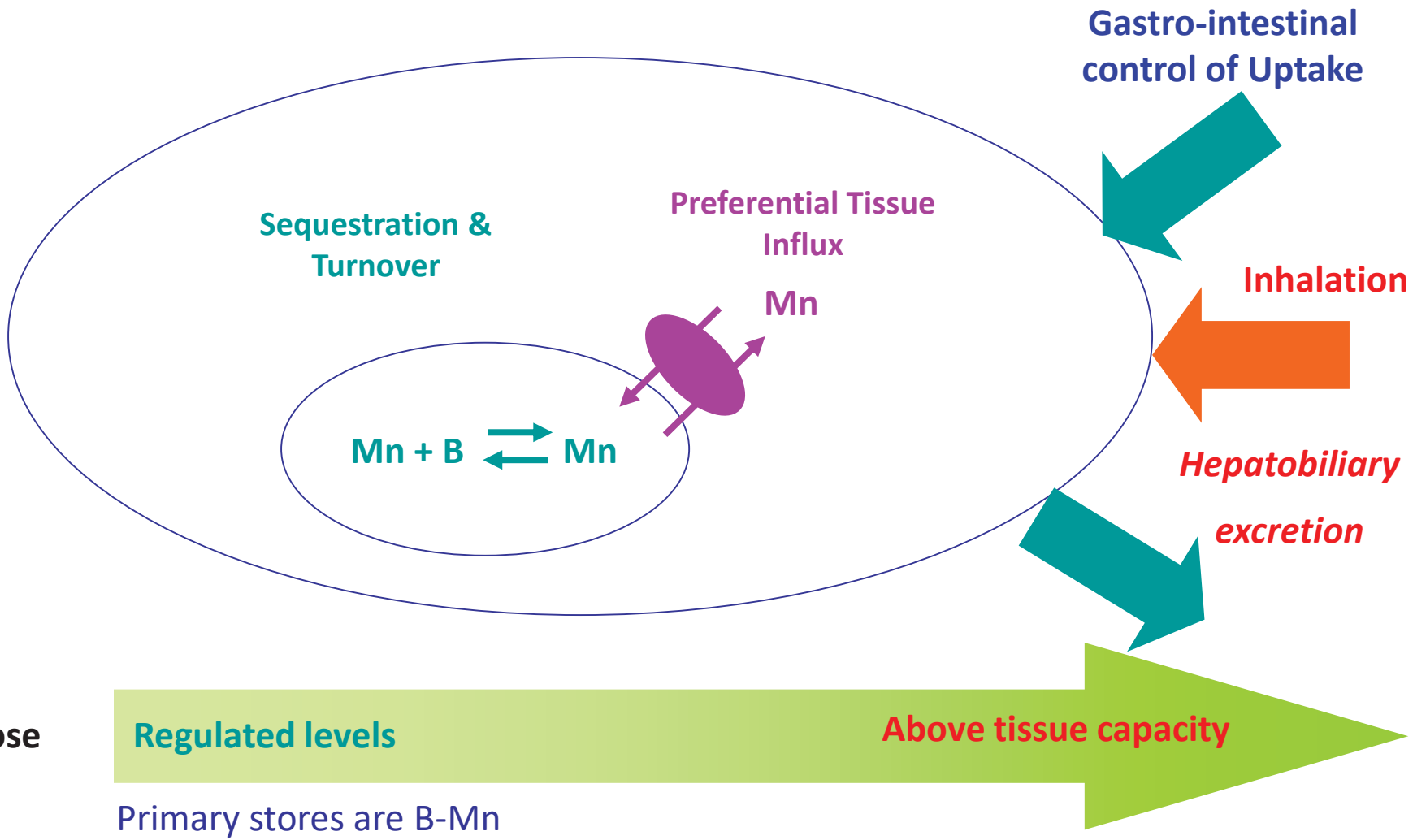
Mode of action



- 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

Determinants of Mn homeostasis:

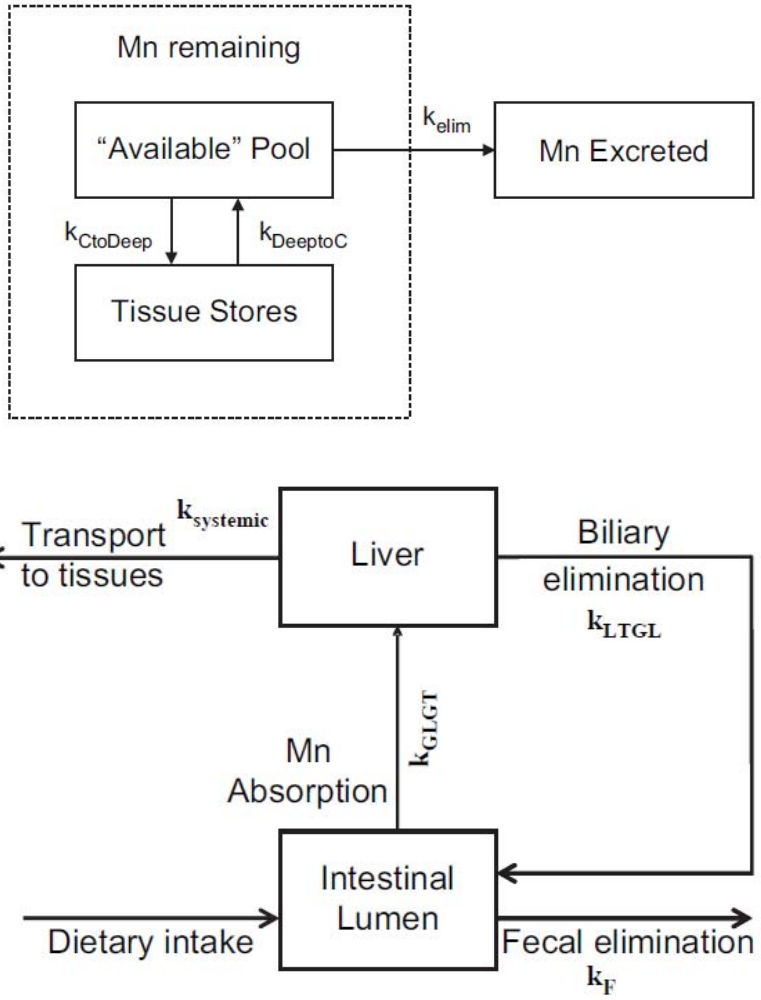


A Rich Data Set for Modeling

Studies with inhaled and dietary Mn conducted at the CIIT/The Hamner under a Clean Air Act test rule (Smith et al 2017):

- 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

In the beginning…
a series of modelling studies



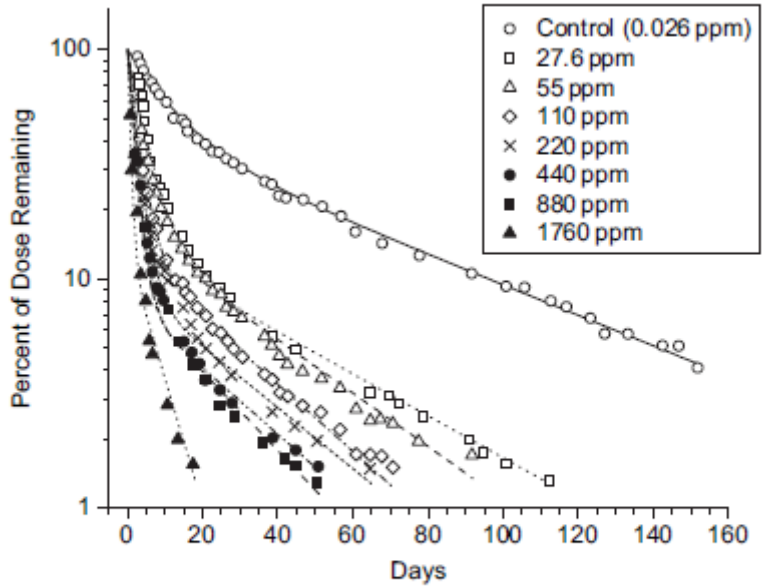
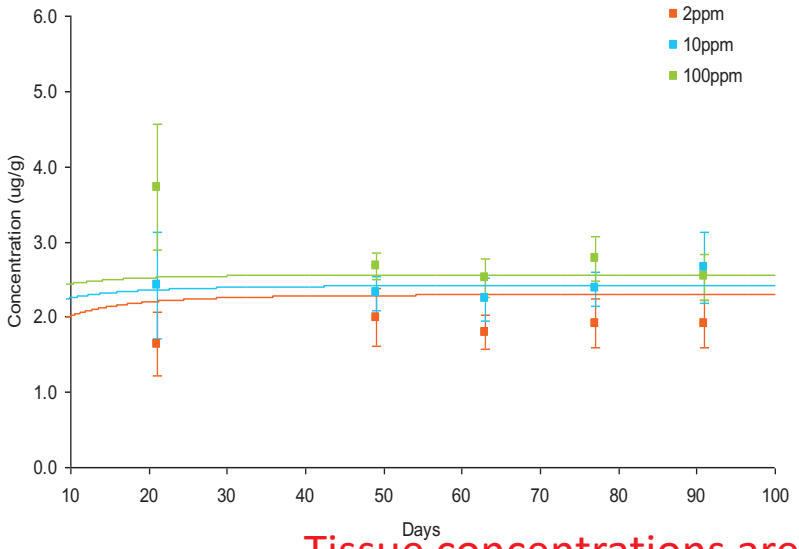


FIG. 7. Modeled (lines) and observed (symbols) kinetics of ^{54}Mn (administered iv) elimination in mice following dietary exposure to Mn. Data are from Britton and Cotzias (1966).

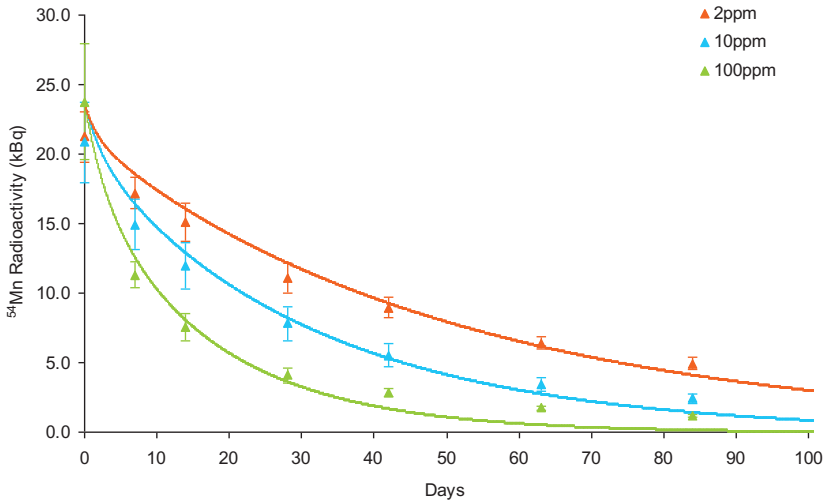
Dose-dependent dietary uptake and biliary excretion

Adult Rat Mn kinetics – Steady state vs tracer kinetics

Liver – concentrations



Whole Body Tracer Study

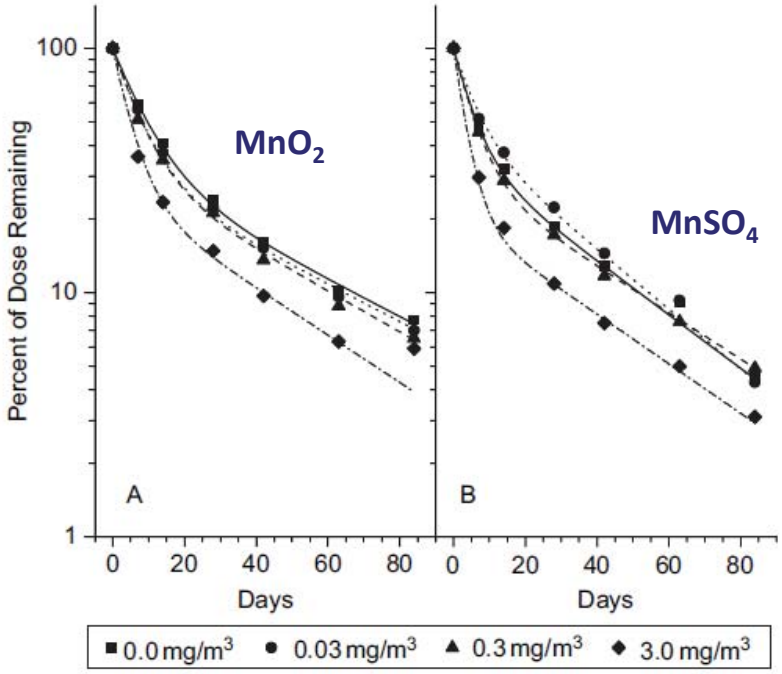


Tissue concentrations are maintained at relatively constant levels.

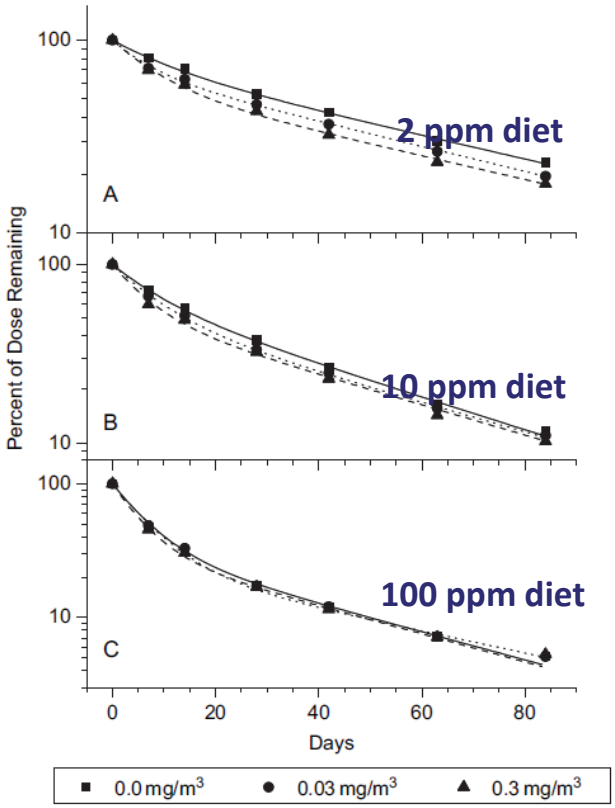
Homeostatic control occurs at the level of **dietary uptake in the gut** and **biliary excretion** at **steady-state**

Dose-dependent elimination for both dietary and inhalation routes of exposure

Inhalation

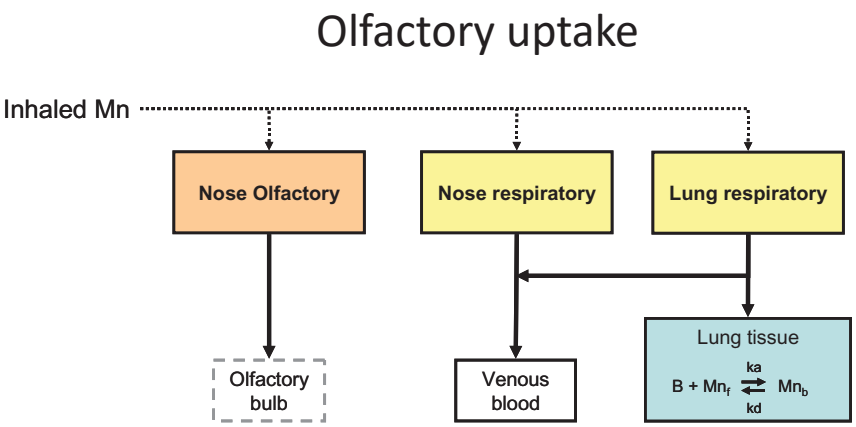


Diet

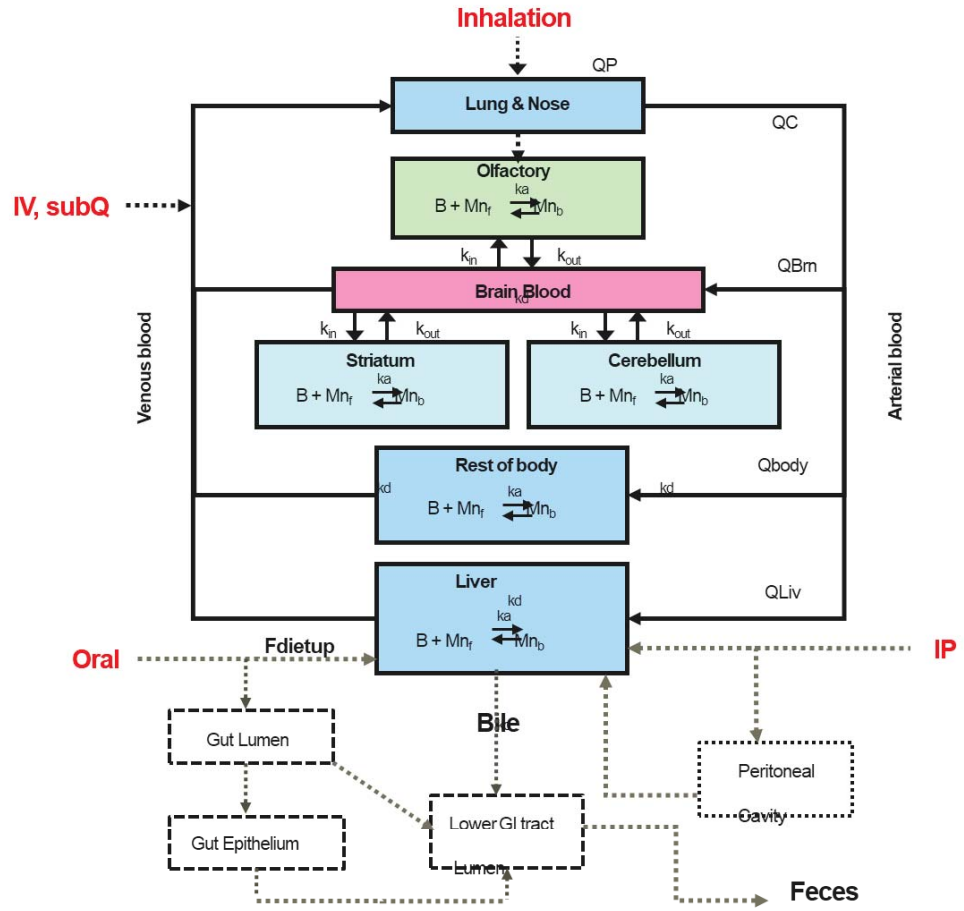


Rat tracer studies from Dorman et al., (2001a and b)

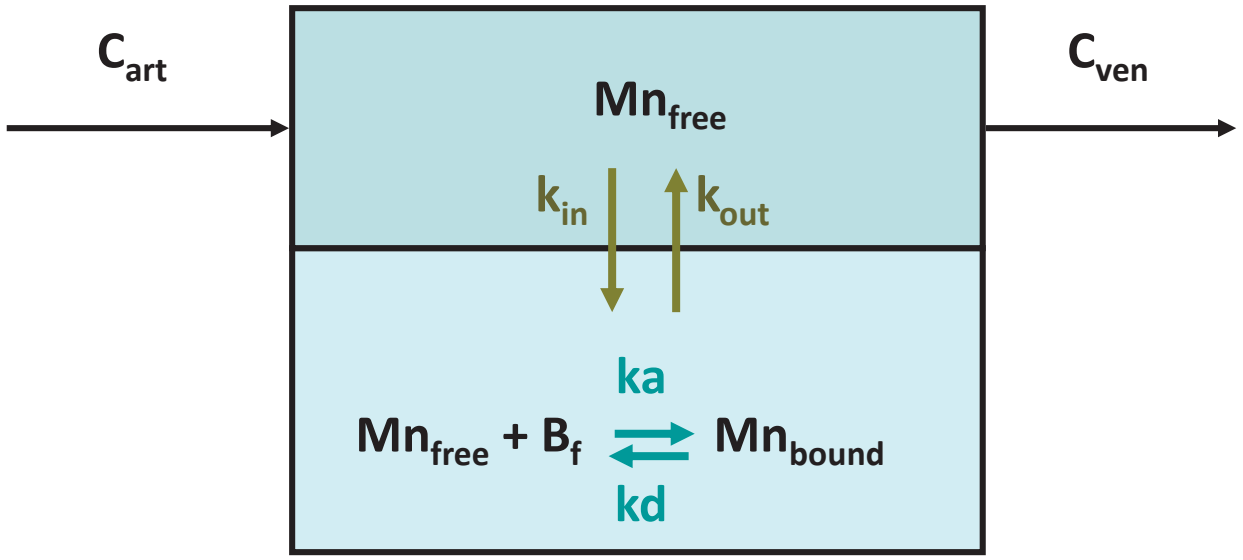
Other model characteristics as needed



- 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**)



Key model structure: Saturable tissue storage and asymmetric diffusion

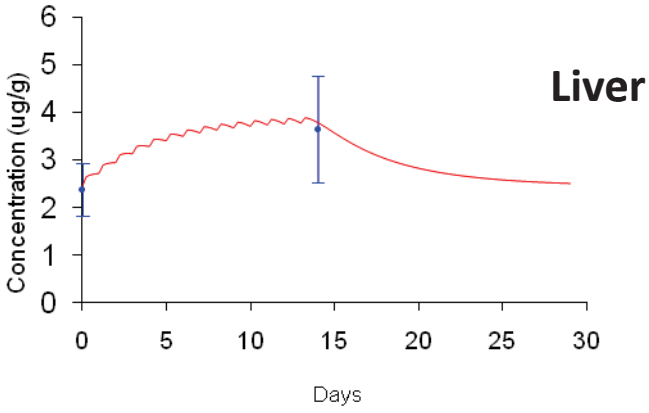


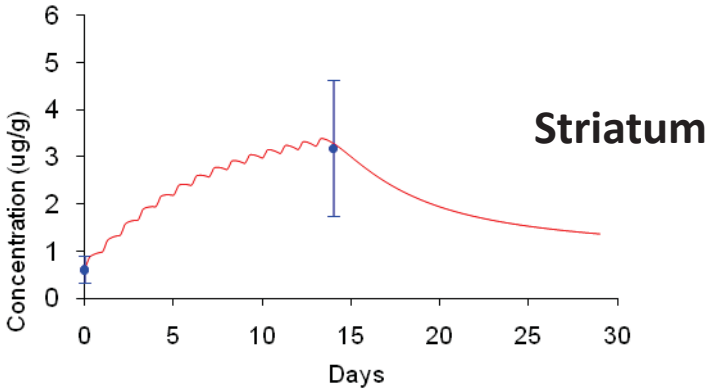
$$Mn_{total} = Mn_{free} + Mn_{bound}$$

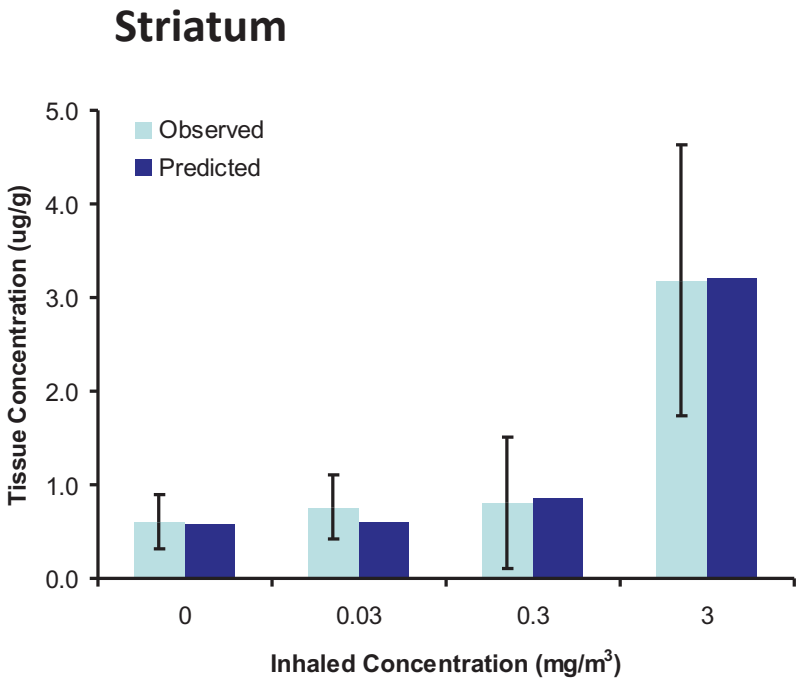
$$B_{max} = B_f + Mn_{bound}$$

Dose-dependent uptake of inhaled Mn

Adult Rat Mn kinetics - Short term inhalation exposure





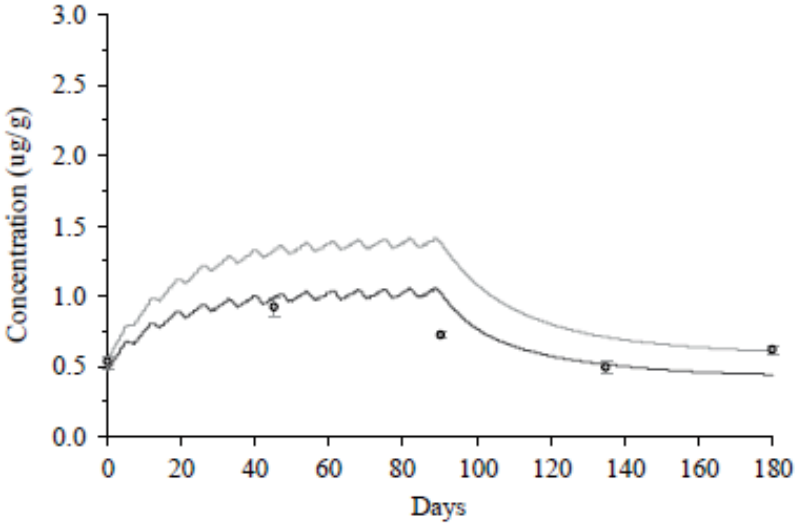


Studies demonstrate dose-dependent transition from controlled tissue Mn levels at low exposures to accumulation of tissue Mn at higher exposures

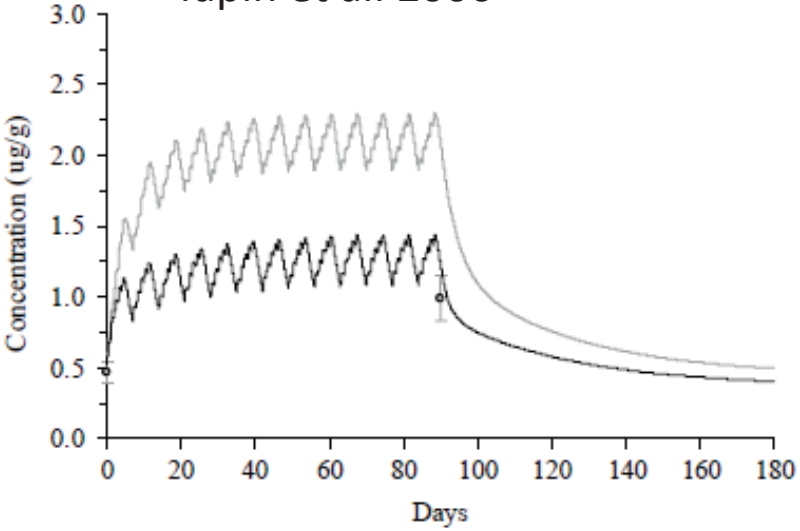
Model development:
Tissue dosimetry on longer exposures

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

Dorman et al. 2004a



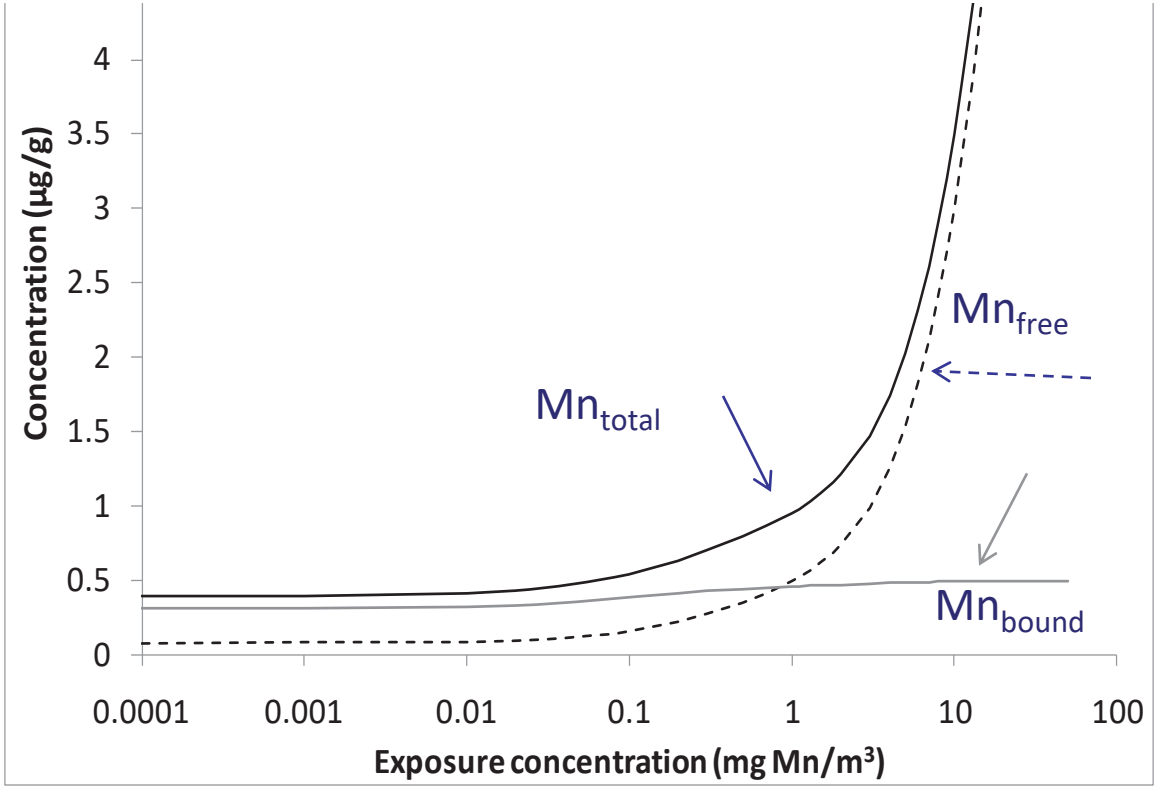
Tapin et al. 2006



without (gray line) and with (black line) induction of biliary elimination of Mn

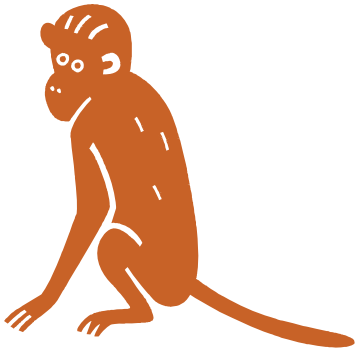
Incorporation of **dose-dependent induction of Mn biliary elimination**

Dose dependency of free vs. bound Mn in mid-brain



Model extrapolation: Rats to monkeys

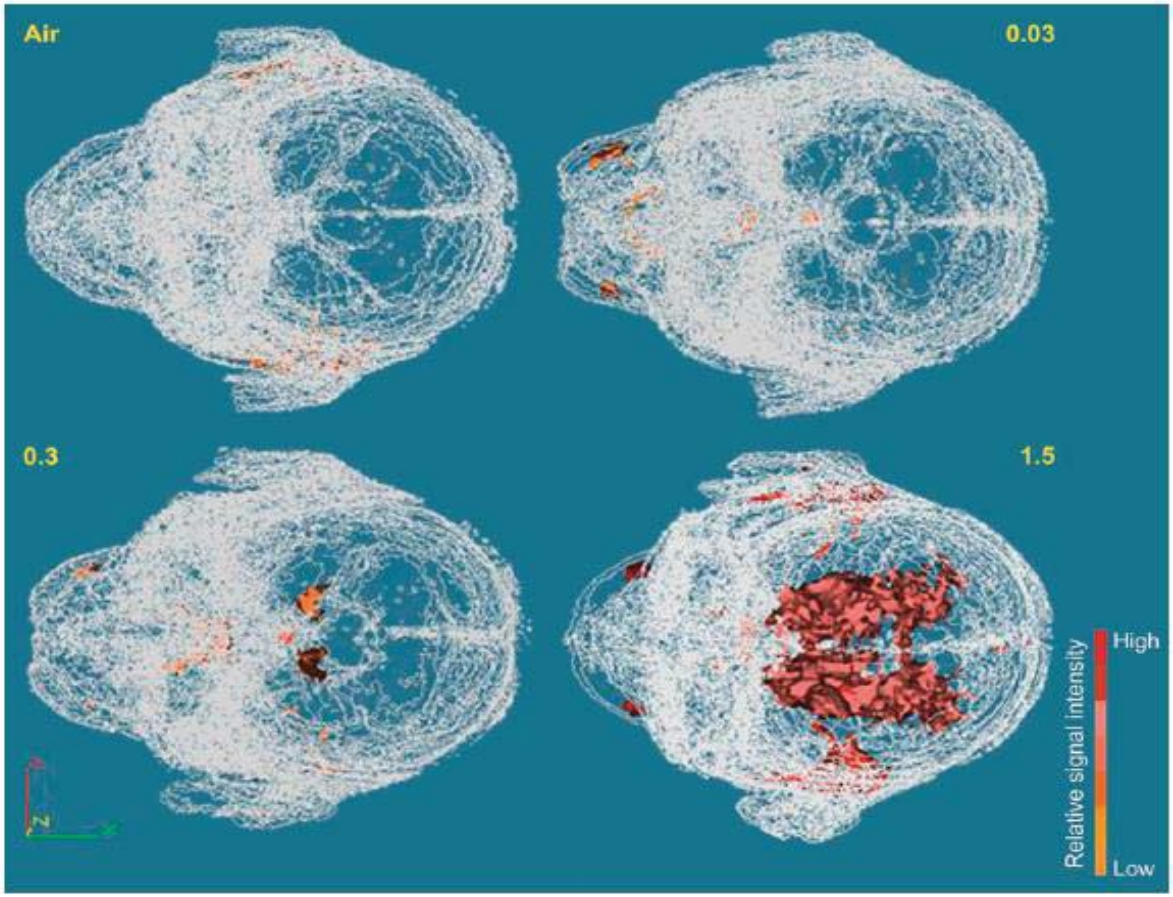
Extrapolation



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

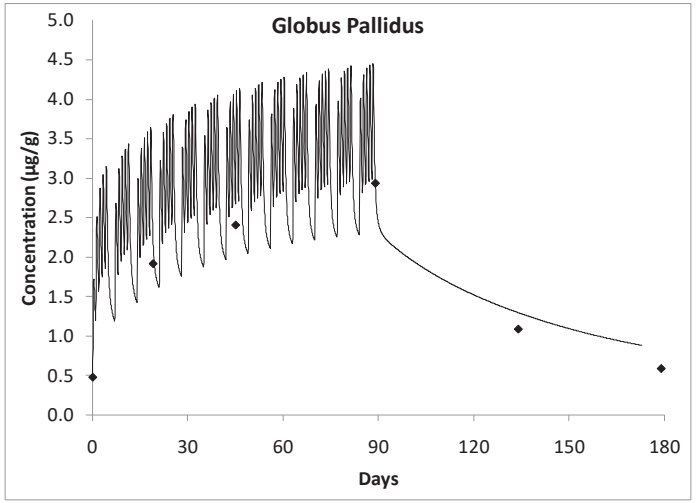
Correlation of Brain Magnetic Resonance Imaging Changes with Pallidal Manganese Concentrations in Rhesus Monkeys Following Subchronic Manganese Inhalation

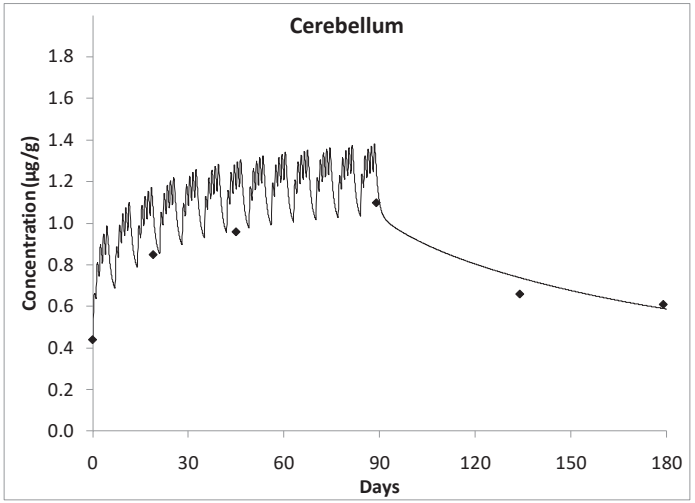
David C. Dorman,*,[1] Melanie F. Struve,* Brian A. Wong,* Janice A. Dye,† and Ian D. Robertson‡

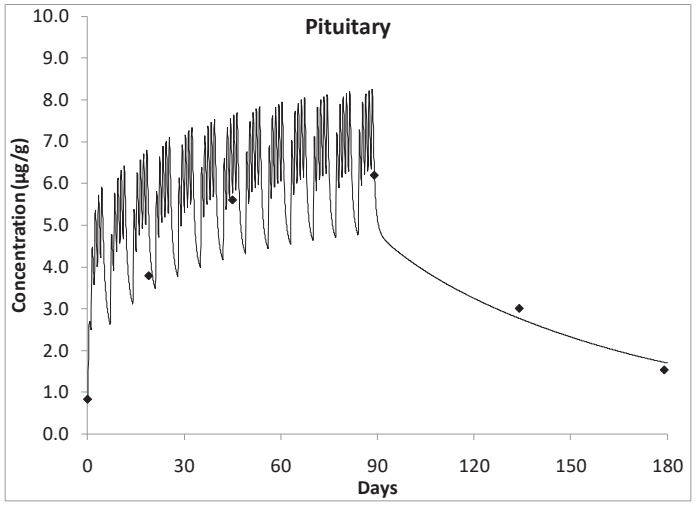


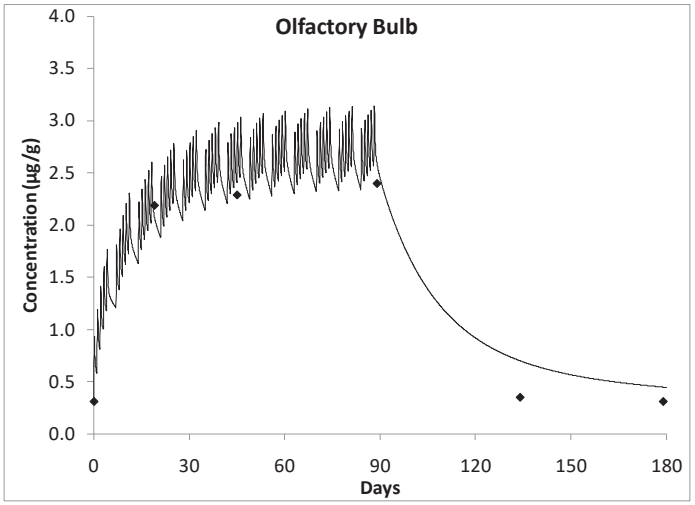
Also have additional monkey data using ip, iv, oral, and inhalation dosing

Model extrapolation from Rat to Monkey: Monkey brain predictions (90-day exposure)



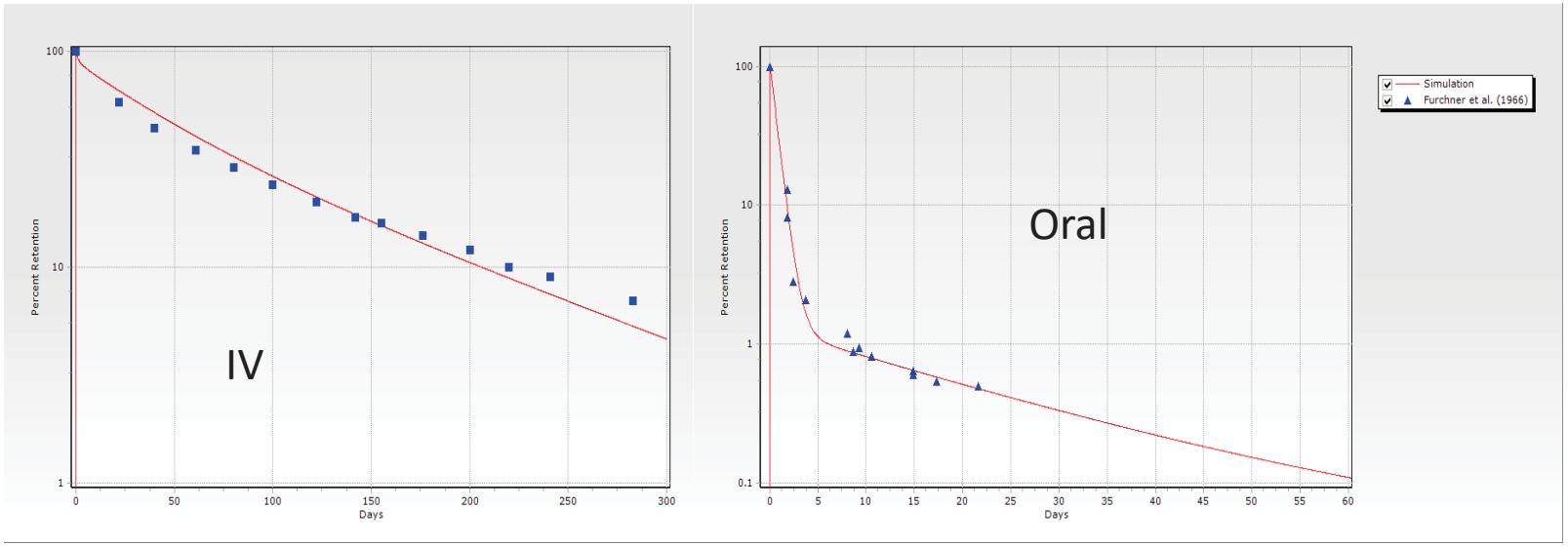


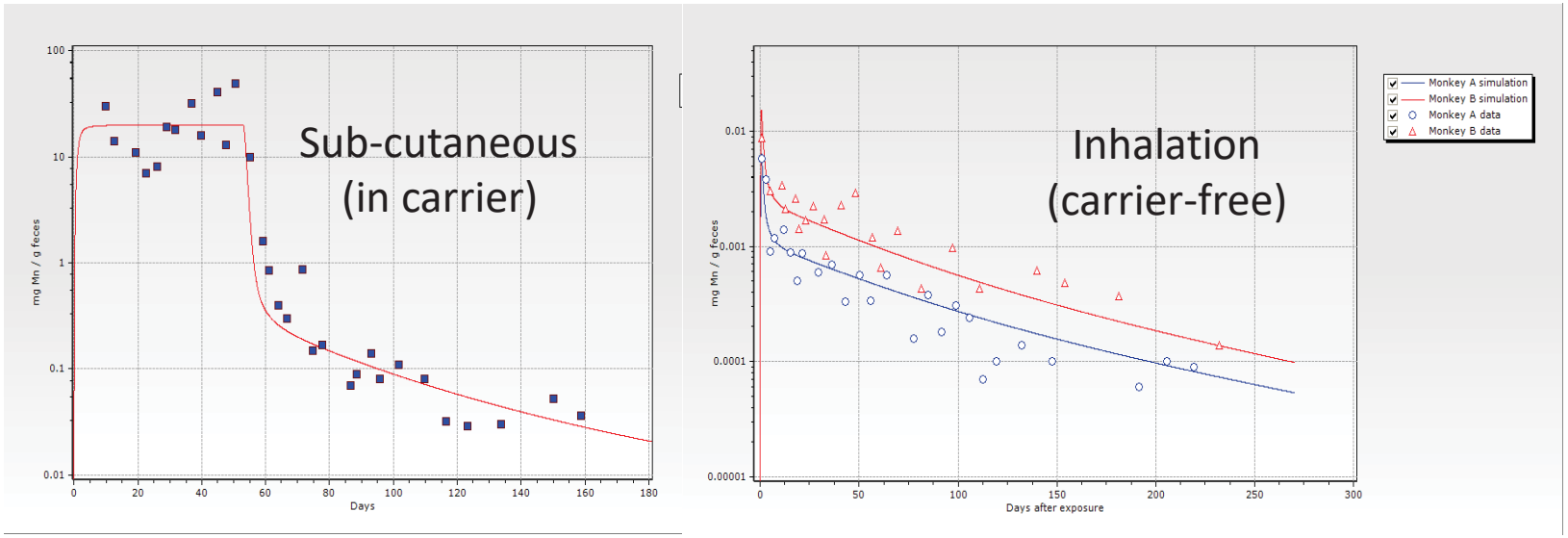




Dorman et al. 2006a and Schroeter et al., 2011.

Modeling of Different Exposure Routes





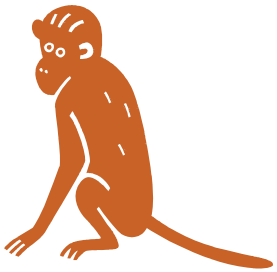
Cross species extrapolation: Validation of model structure

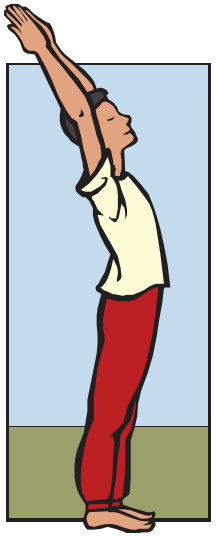
- PBPK models integrate biological and physiological mechanism to understand Mn disposition across dose-route and animal species.
- Main dose-dependent characteristics of Mn disposition including tissue binding, brain uptake and biliary excretion across species
- The success in moving from rat to primate 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

Extrapolation

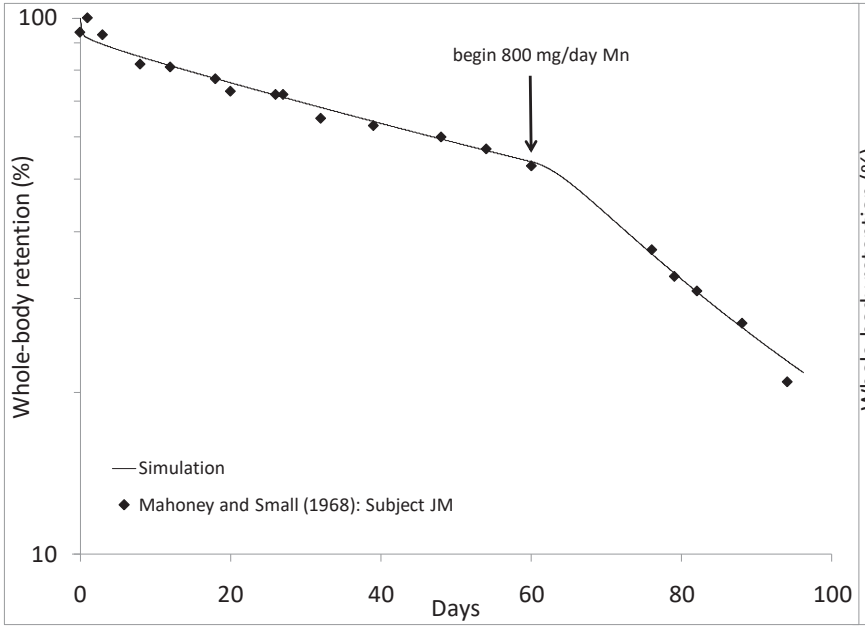


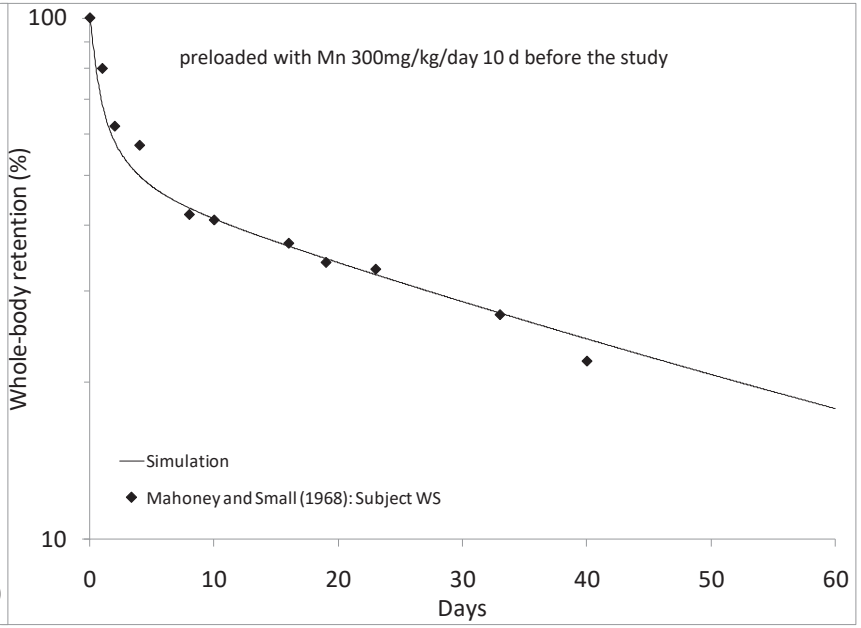


- Body weight
- Tissue volumes
- Blood flows
- Biliary excretion
- Scaled parameter

+ Validation with human tracer data

Human Mn model: Whole body retention of Mn tracer

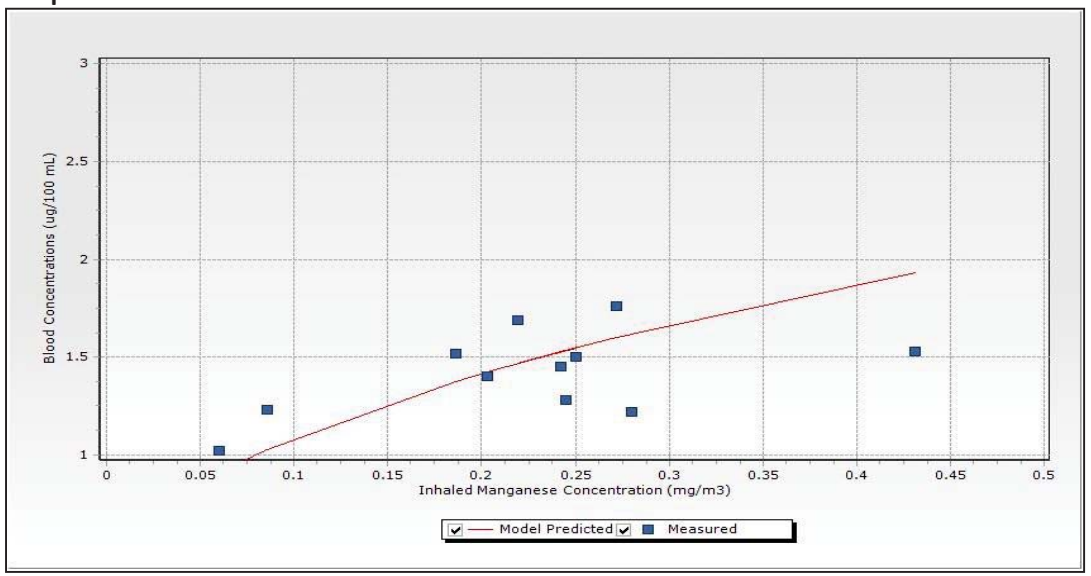




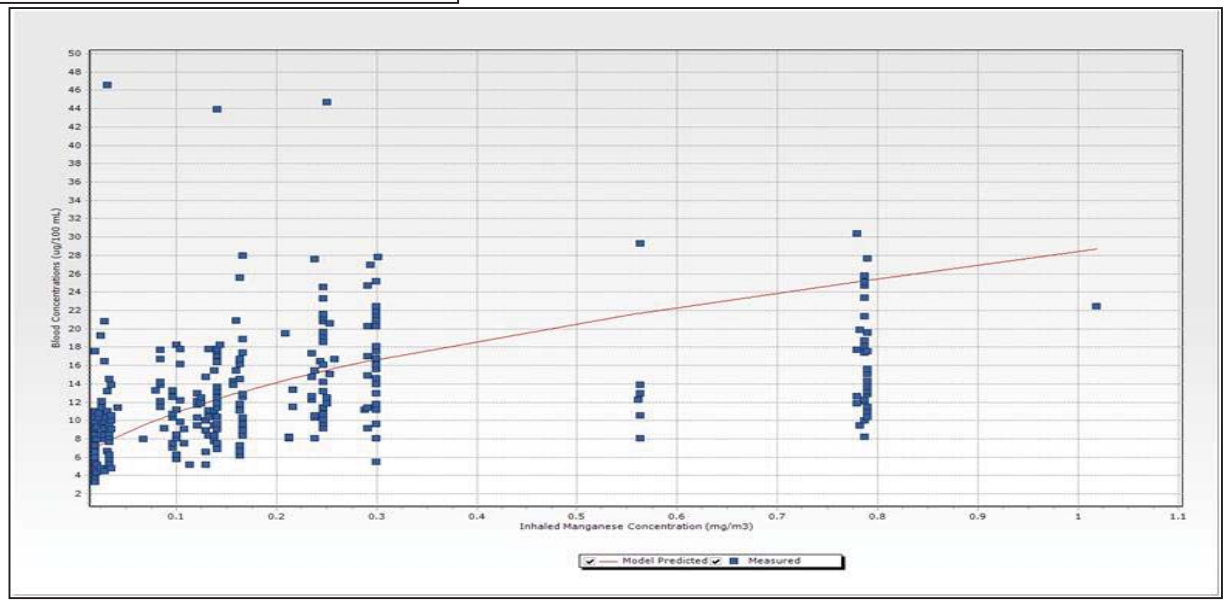
Mahoney and Small (1968)

Effect of Inhaled Manganese on Blood Concentration

Roels et al. (1987)
Respirable Mn vs. Blood Concentrations at Start of Work Week

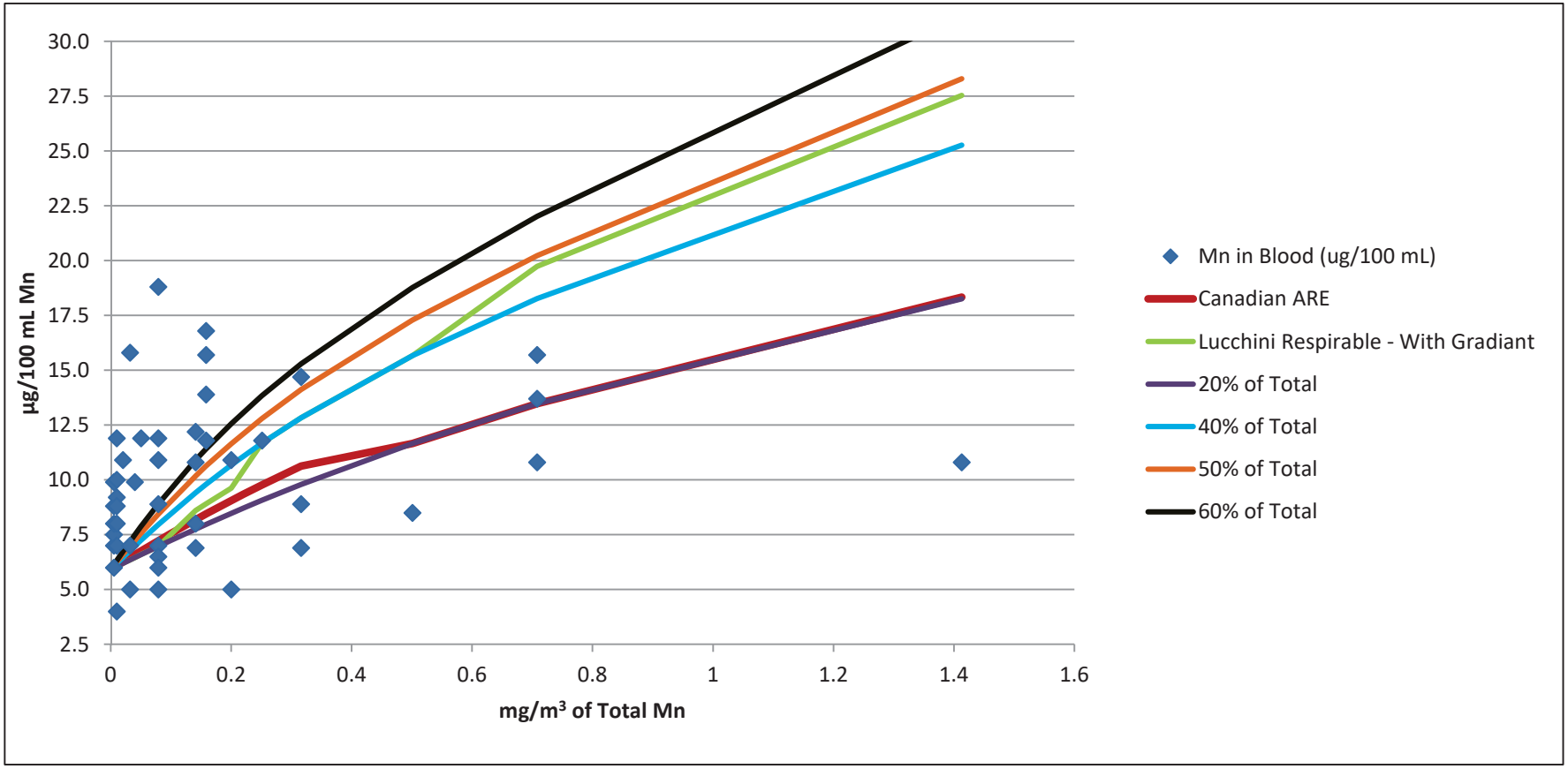


Myers et al. (2003)
Respirable Mn vs. Blood Concentration at Start of Work Week

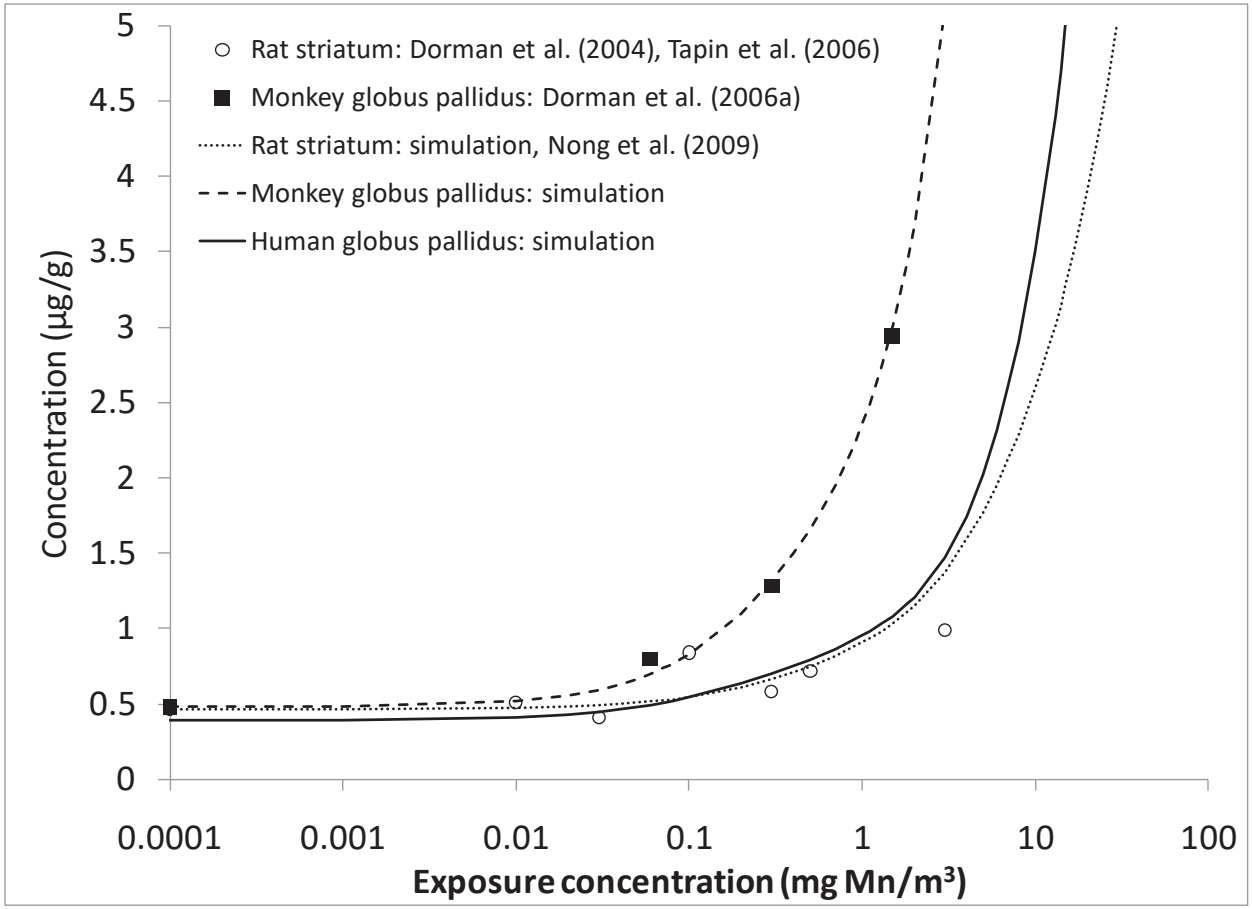


Impact of Respirable Fraction of Mn on Blood Concentration Estimates

Lucchini et al. 1999

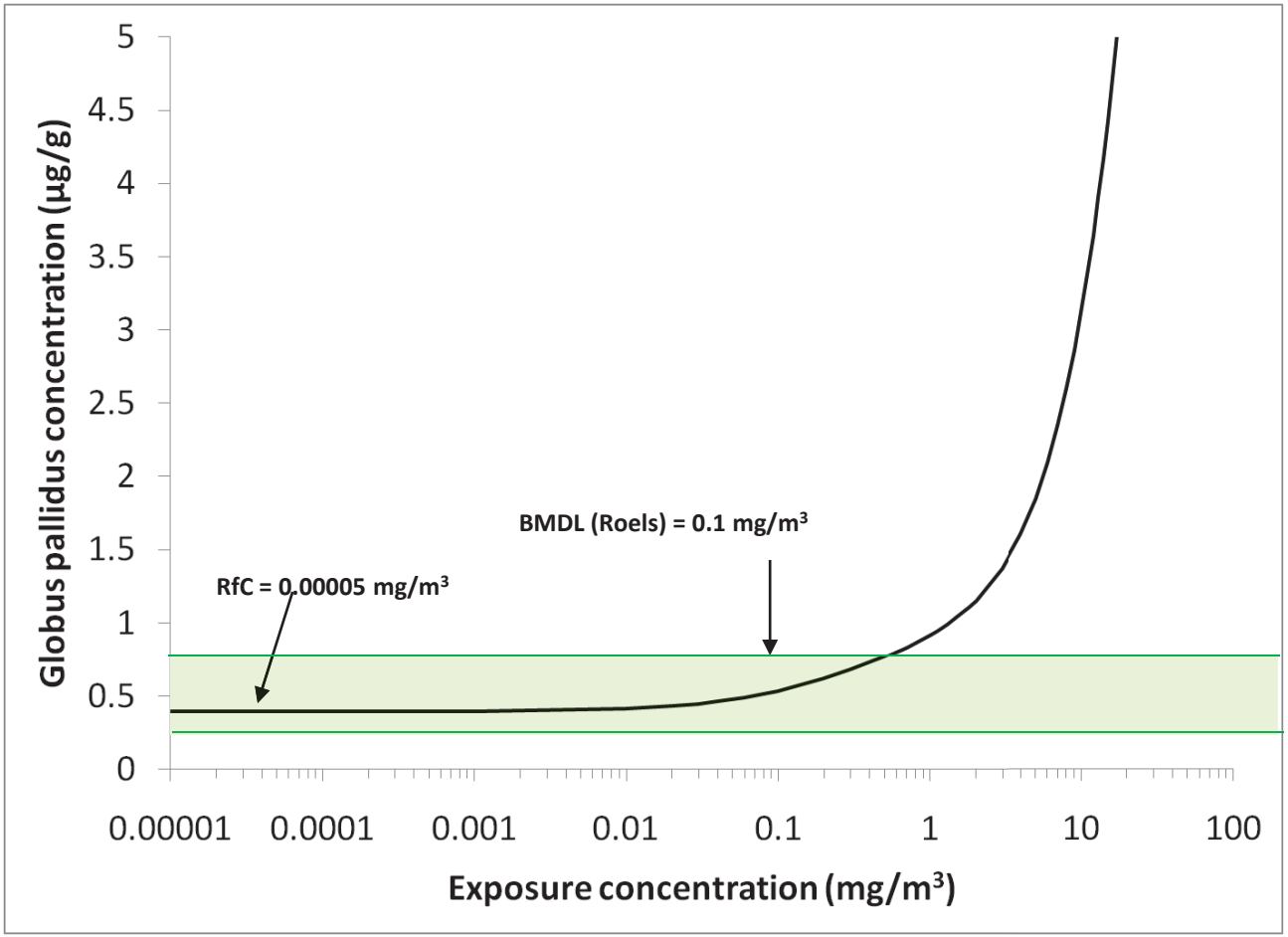


Model extrapolation: Across species



Schroeter et al., 2011.

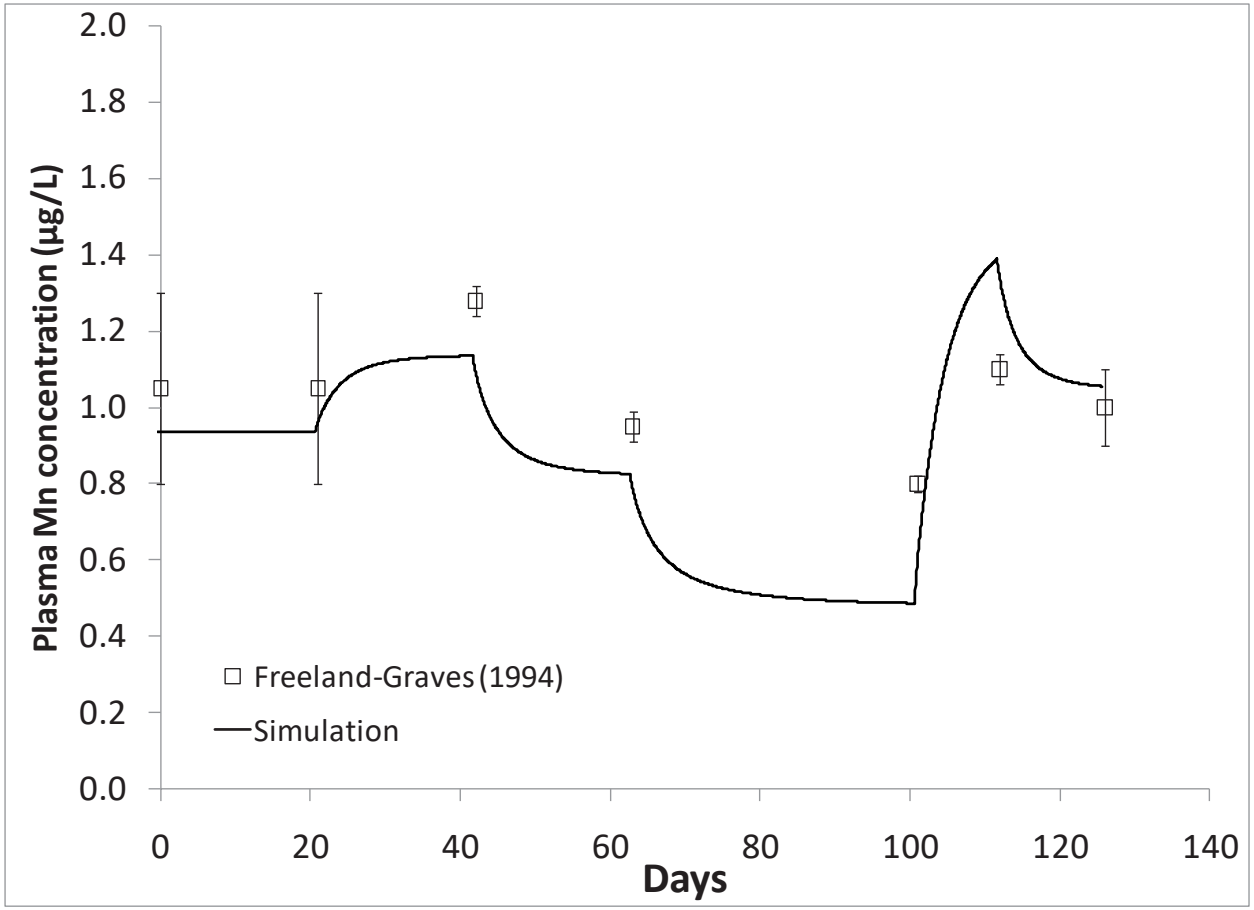
Tissue-dose based risk assessment



Human model simulation for continuous exposure to Mn for 2 years

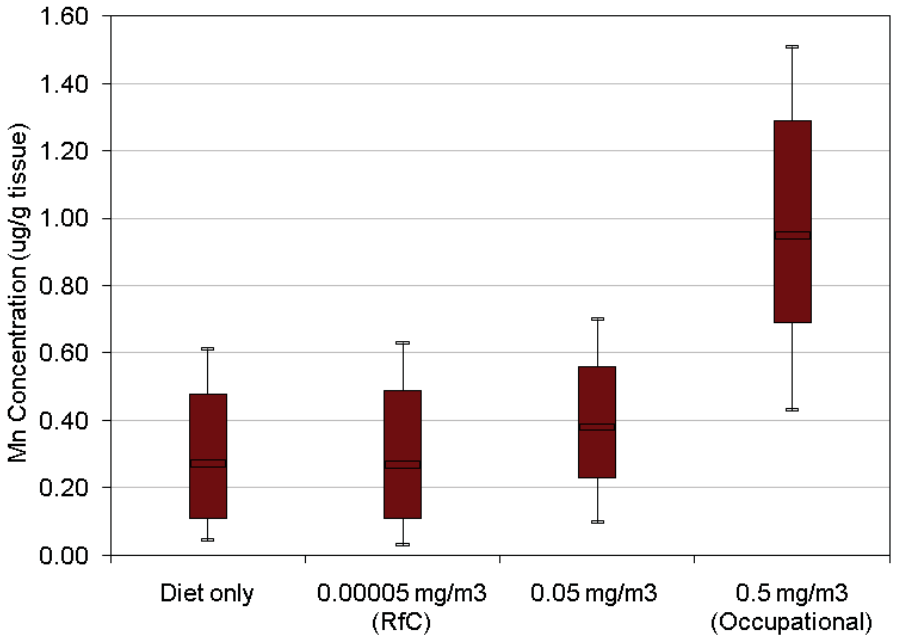
Effect of Dietary Variation on Mn in Blood

Predicted human plasma concentrations
for controlled variable dietary intakes

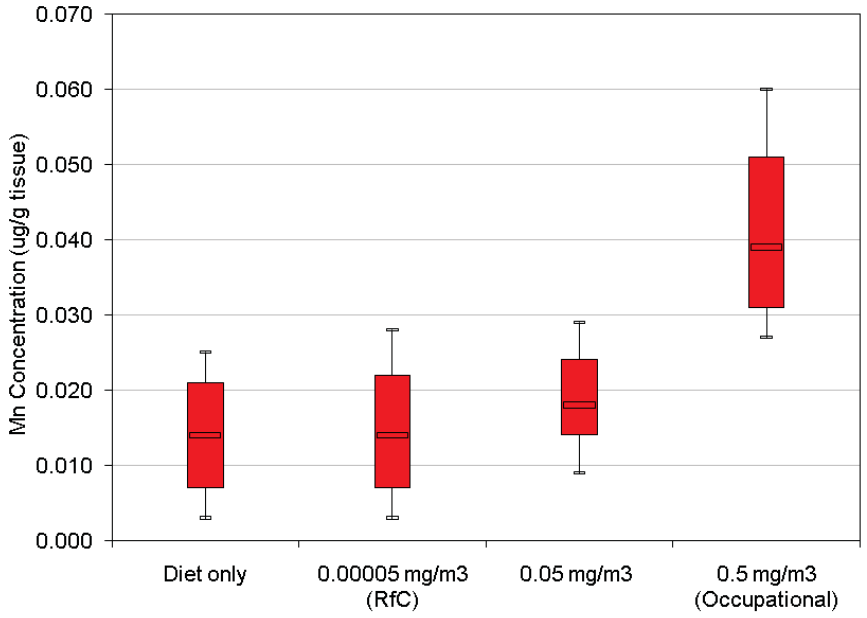


Comparison of Inhalation and Oral Exposure

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



Brain

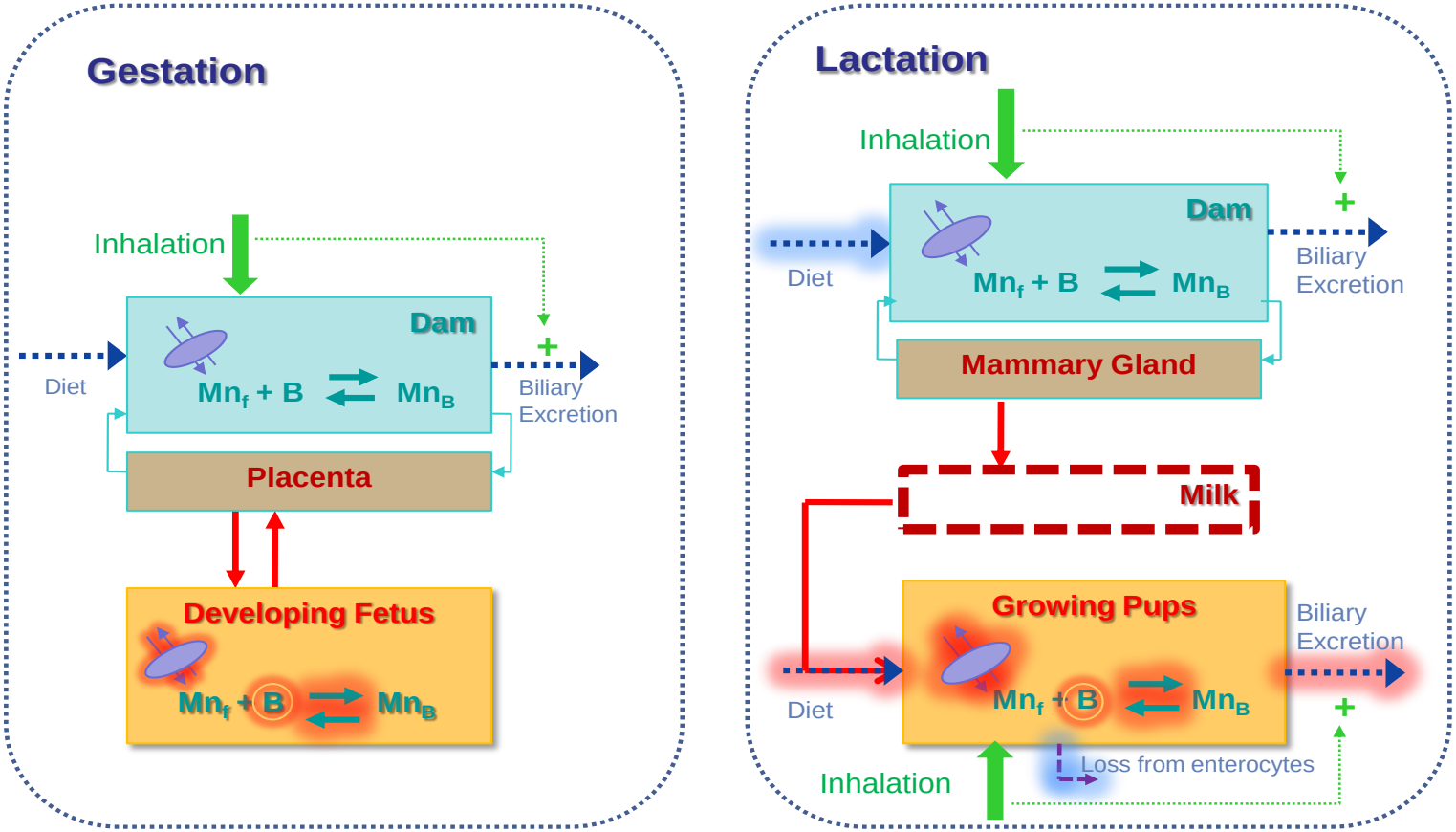


Blood

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

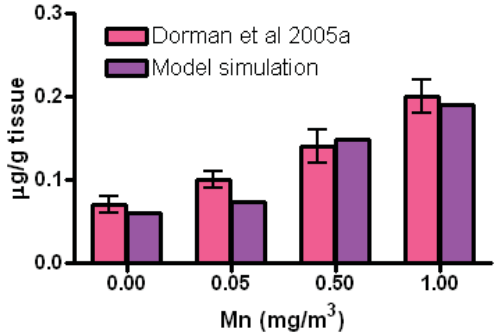


Yoon et al. 2009a and 2009b

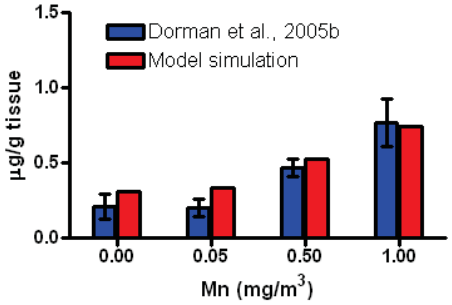
Rat Developmental Model makes it possible…

To predict Mn transfer from mother to fetus/neonate:

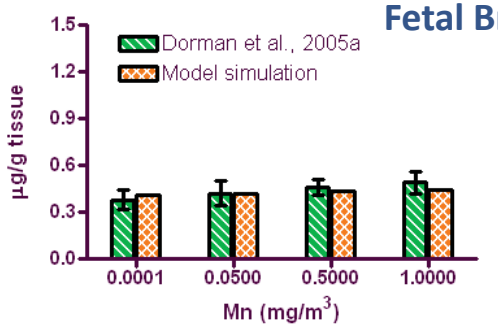
Placental Mn

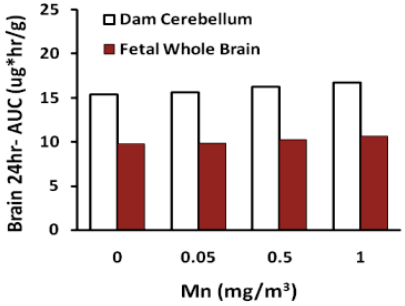


Milk Mn



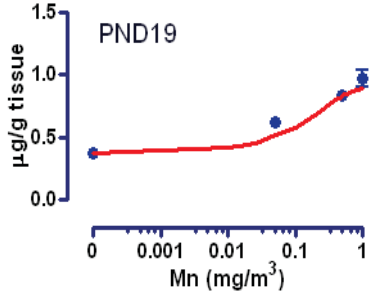
Fetal Brain Concentration & AUC

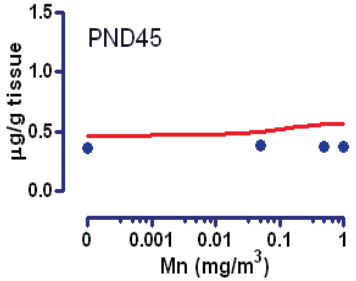


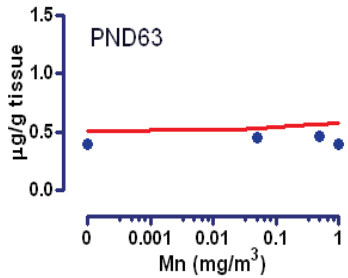


To estimate Mn tissue dosimetry in the target during perinatal period:

Neonatal Striatum Concentration & AUC with Mn inhalation exposures



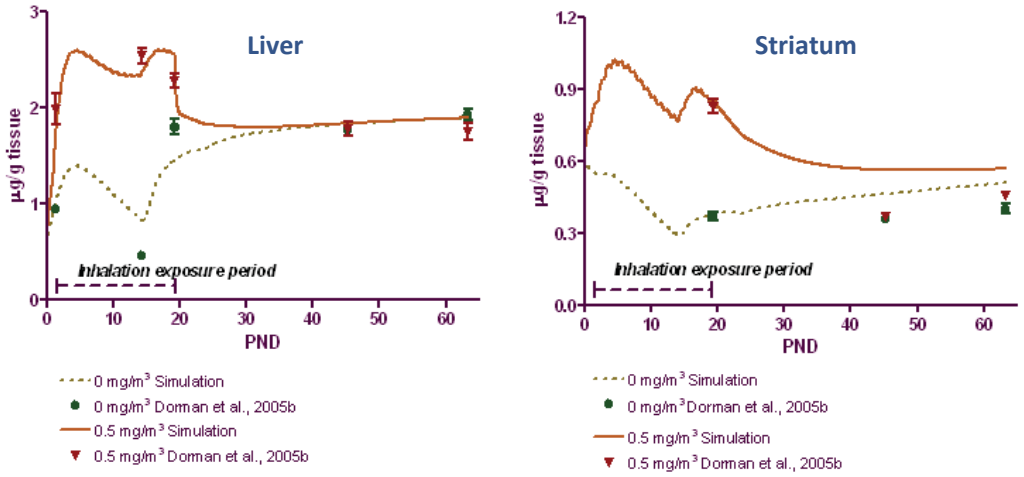




Rat Developmental Model makes it possible…

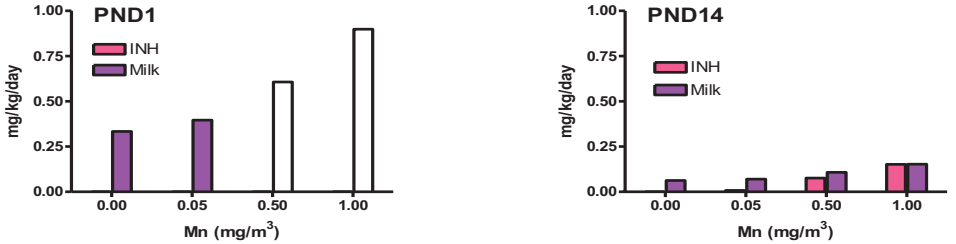
- To describe the changes in Mn kinetics during postnatal development:

Temporal changes in Mn tissue concentrations during neonatal development

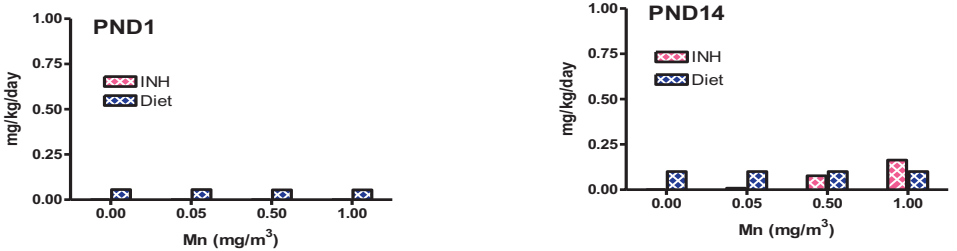


- To compare exposures from different sources of Mn:
  - Milk
  - Diet
  - Inhalation

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



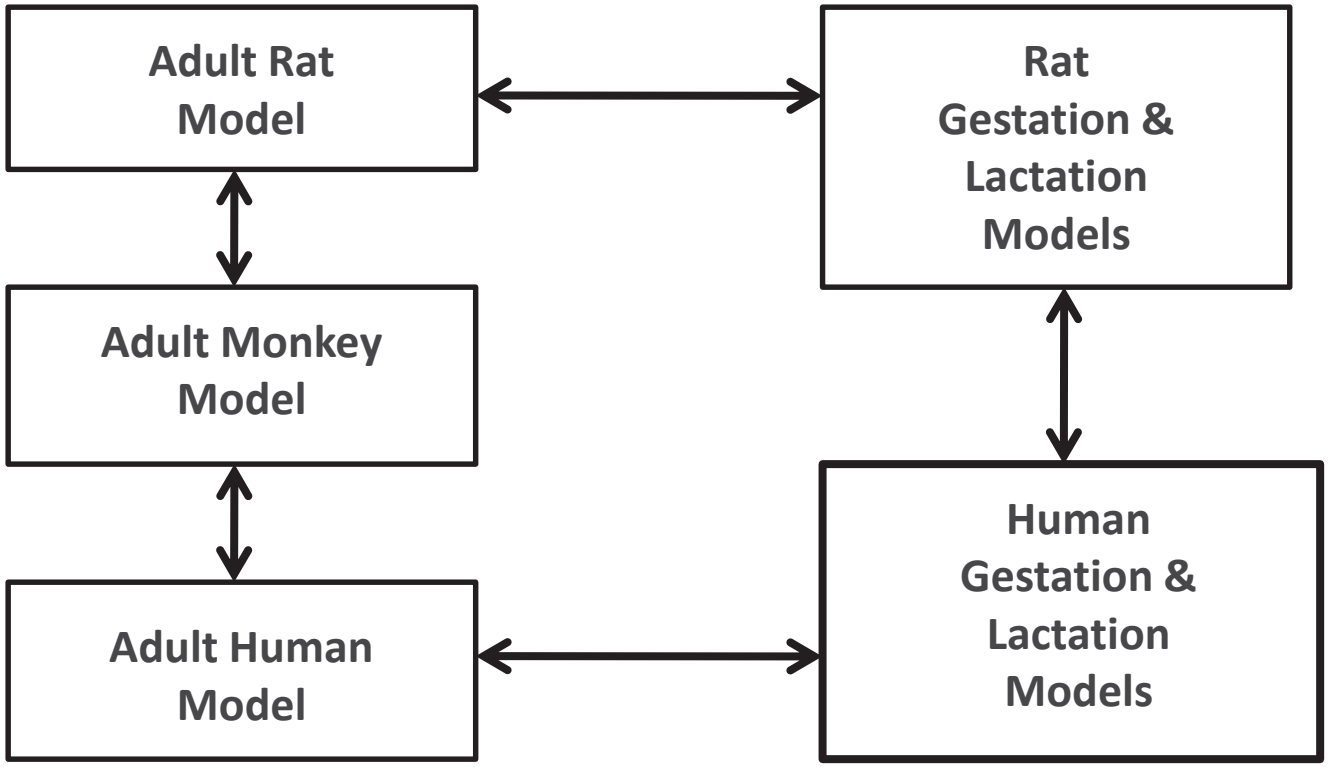
Mn Daily Dose (mg/kg BW/day) in the Dam



Findings in Rat Models: Key Processes to Describe Mn Kinetics during Perinatal Period

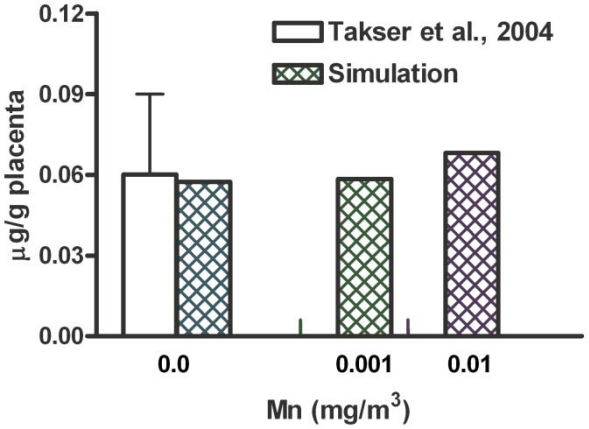
- Incorporation of Mn transfer processes from the dam to offspring: Keeping maternal homeostasis while ensuring adequate Mn to the offspring
- Changes in physiological processes responsible for Mn homeostatic mechanism
  - Enhanced Mn uptake in gut in neonates
  - Tissue binding characteristics – reflecting 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

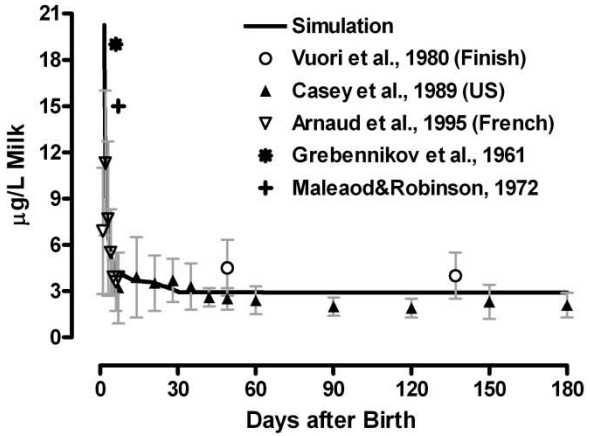
Placental and lactational transfer of Mn

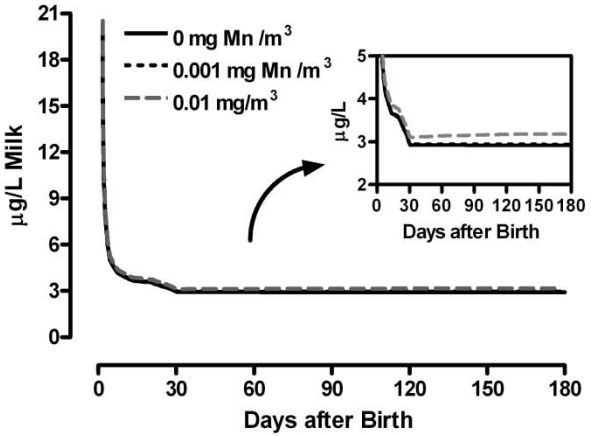


Placenta

Yoon et al., 2011

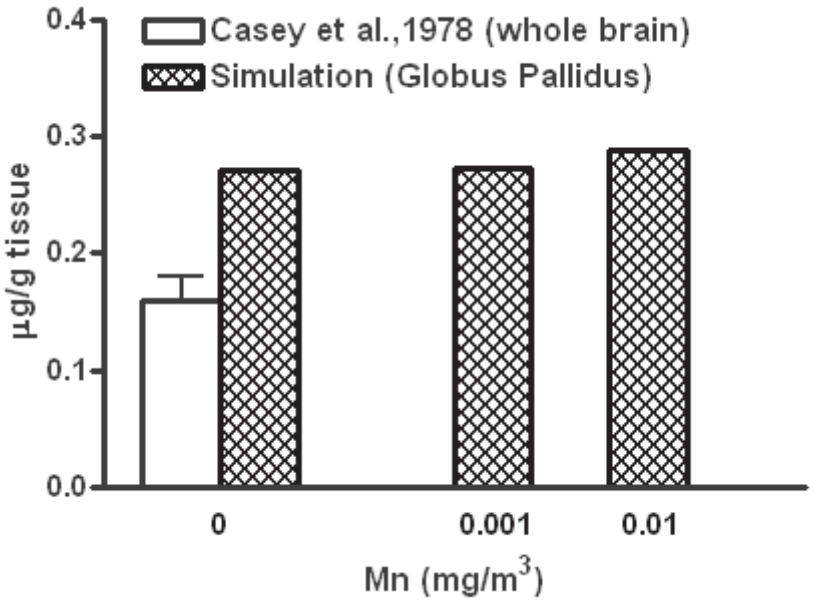
Milk





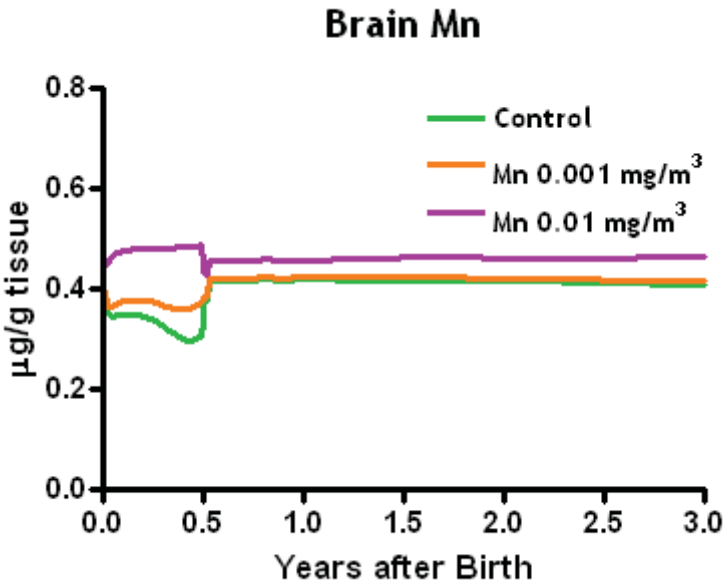
Fetal and neonatal brain Mn

Fetus



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

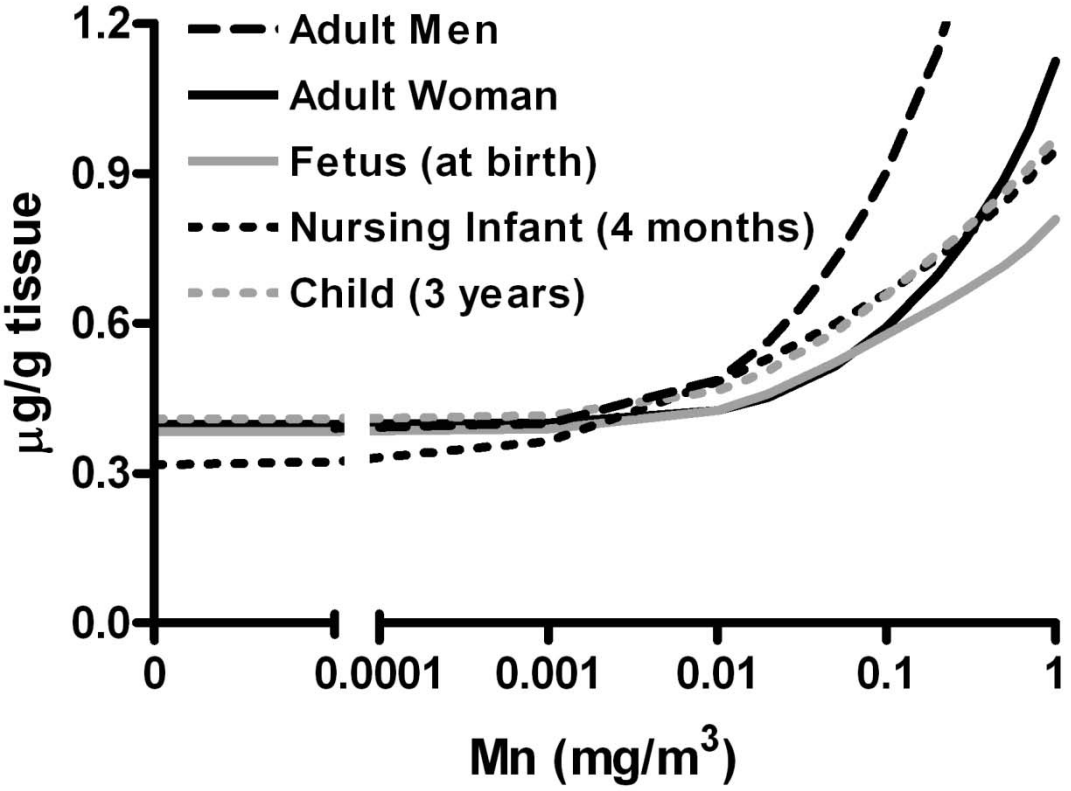
Infants and children



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

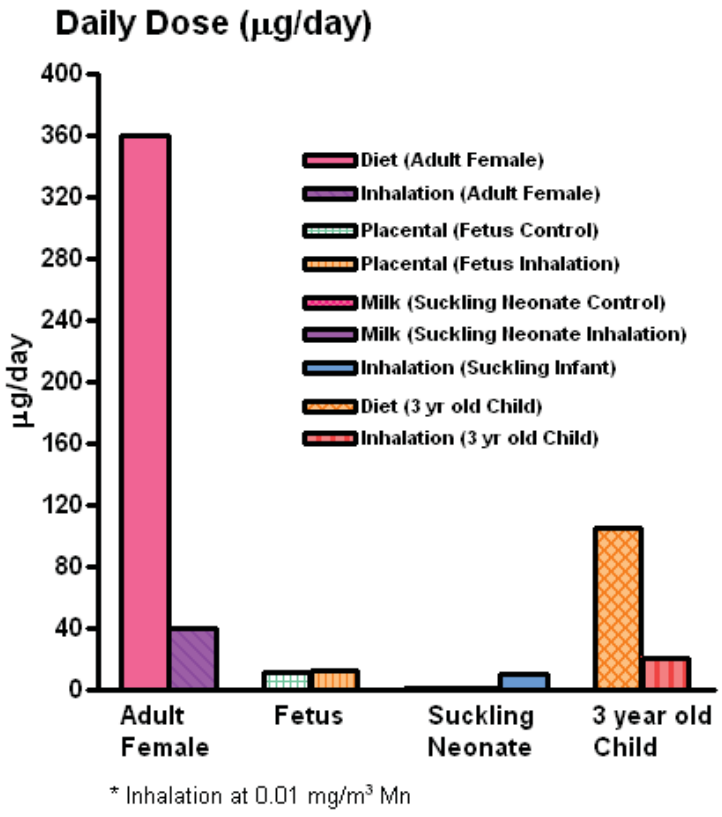
Yoon et al., 2011

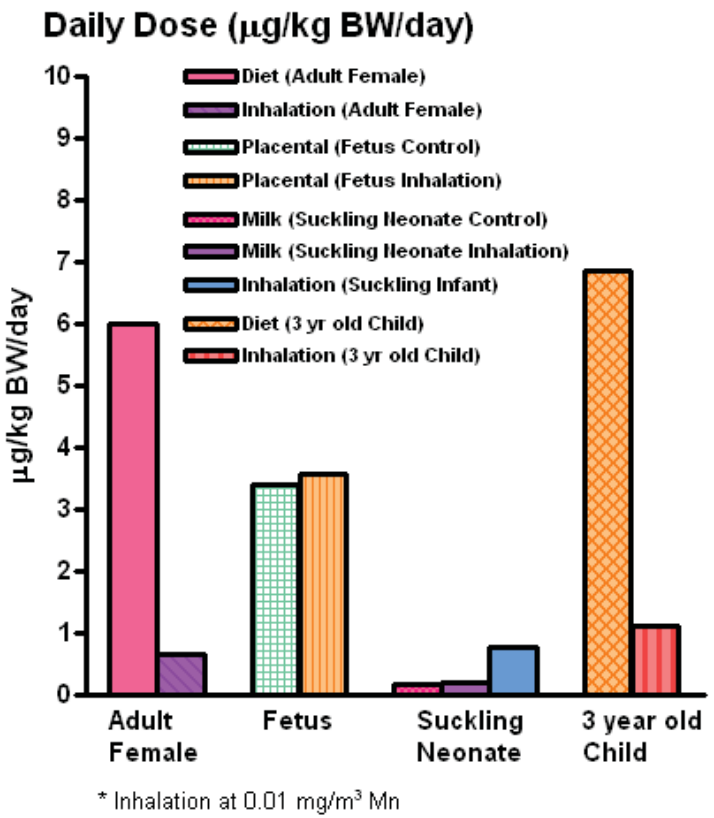
Model extrapolation: Across life stages



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 (6months), and child (3 years) were compared among milk, dietary, and inhaled doses on the selected day.
Inhalation at 0.01 mg/m^3 of Mn was simulated.

Summary: PBPK Modeling of Manganese

- **This suite of published PBPK models was peer reviewed by a Technical Advisory Panel set up under the EPA test rule**

In-Life TAP Members

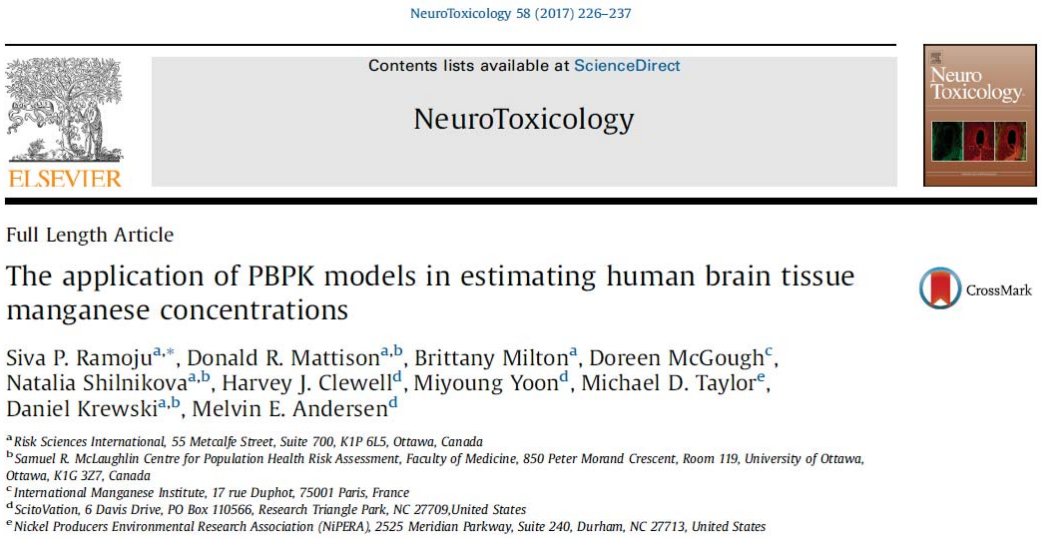
- Gunter Oberdorster (Chair)
- Fred Hochberg
- Joseph Rodricks
- Karl Keene
- Michael Ashchner

Modeling TAP Members

- Daniel Krewski (Chair)
- Jeff Fisher
- Michele Medinsky
- Lynn Haber

Summary: PBPK Modeling of Manganese

- These validated PBPK models can be used to identify potential points of departure for a risk assessment based on changes in target tissue (brain) Mn levels



NeuroToxicology 58 (2017) 226–237

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

The application of PBPK models in estimating human brain tissue manganese concentrations

Siva P. Ramoju[a,*], Donald R. Mattison[a,b], Brittany Milton[a], Doreen McGough[c], Natalia Shilnikova[a,b], Harvey J. Clewell[d], Miyoung Yoon[d], Michael D. Taylor[e], Daniel Krewski[a,b], Melvin E. Andersen[d]

[a] Risk Sciences International, 55 Metcalfe Street, Suite 700, K1P 6L5, Ottawa, Canada
[b] Samuel R. McLaughlin Centre for Population Health Risk Assessment, Faculty of Medicine, 850 Peter Morand Crescent, Room 119, University of Ottawa, Ottawa, K1G 3Z7, Canada
[c] International Manganese Institute, 17 rue Duphot, 75001 Paris, France
[d] ScitoVation, 6 Davis Drive, PO Box 110566, Research Triangle Park, NC 27709,United States
[e] Nickel Producers Environmental Research Association (NiPERA), 2525 Meridian Parkway, Suite 240, Durham, NC 27713, United States

- The models can also be used to evaluate the appropriateness of generic uncertainty factors or to calculate chemical-specific (data-derived) adjustment factors

Potential applications of PBPK modeling in a risk assessment for Mn

POD determination:

- **Extrapolation from occupational studies to environmental exposures**
  - **Different compounds / particle size distributions ***

Evaluation of uncertainty factors / DDEFs:

- **Early life exposure**
- **Different forms of Mn / effect of dissolution rate ***
- **Human interindividual variability**

Evaluation of differences in epi studies

- **Particle size distribution, Mn species ***

*** (using MPPD model for deposition)**

Acknowledgements

- Mel Andersen
- David Dorman
- Robinan Gentry
- Athena Keene
- Andy Nong
- Harry Roels
- Jeffry Schroeter
- Mike Taylor
- Cynthia Van Landingham

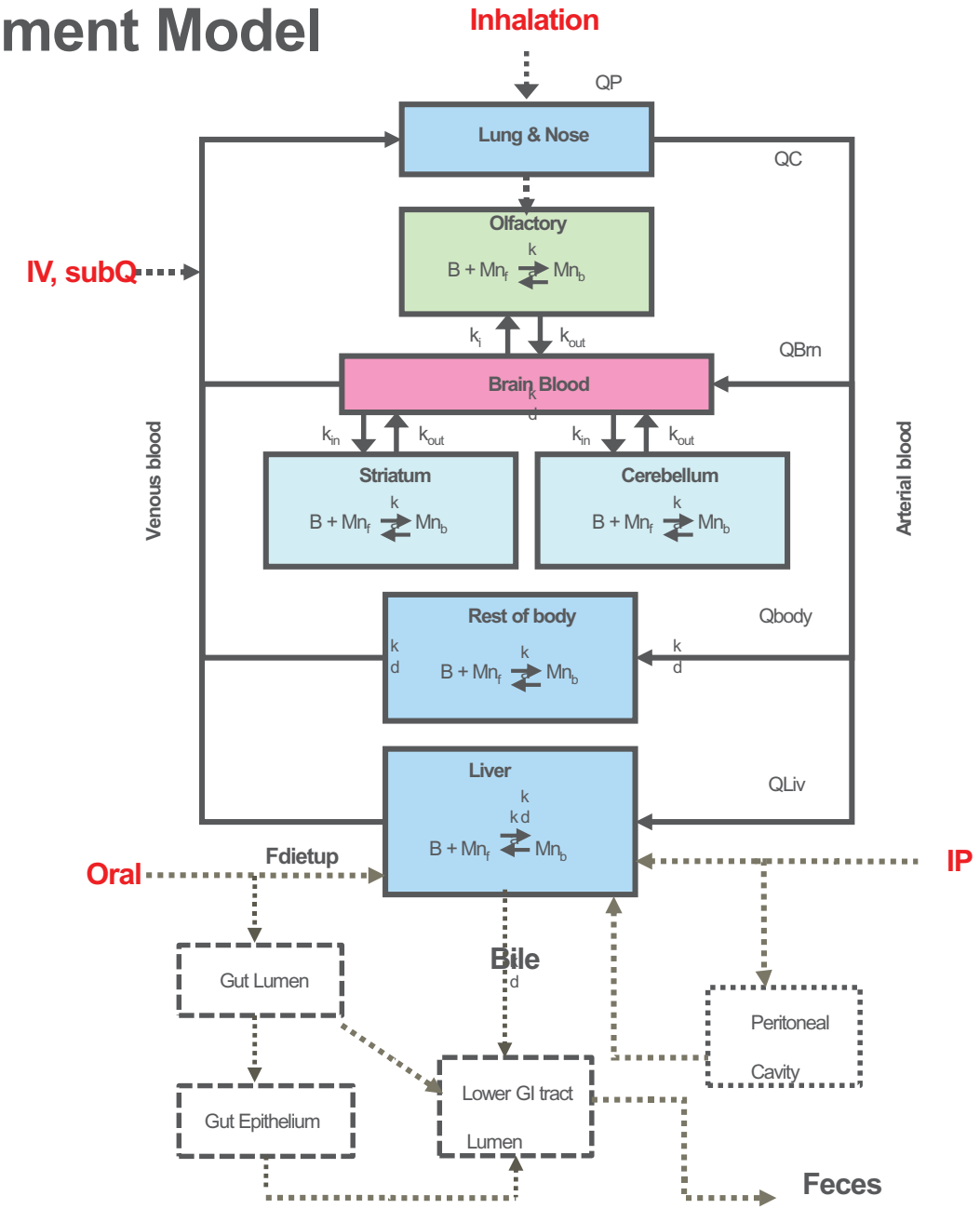
- Funding:
  - Afton Chemical Company
  - U. Ottawa

SENSITIVITY ANALYSIS

PUBLISHED ADULT HUMAN MANGANESE MODEL

RAMBOLL

Mn Risk Assessment Model



RAMBOLL

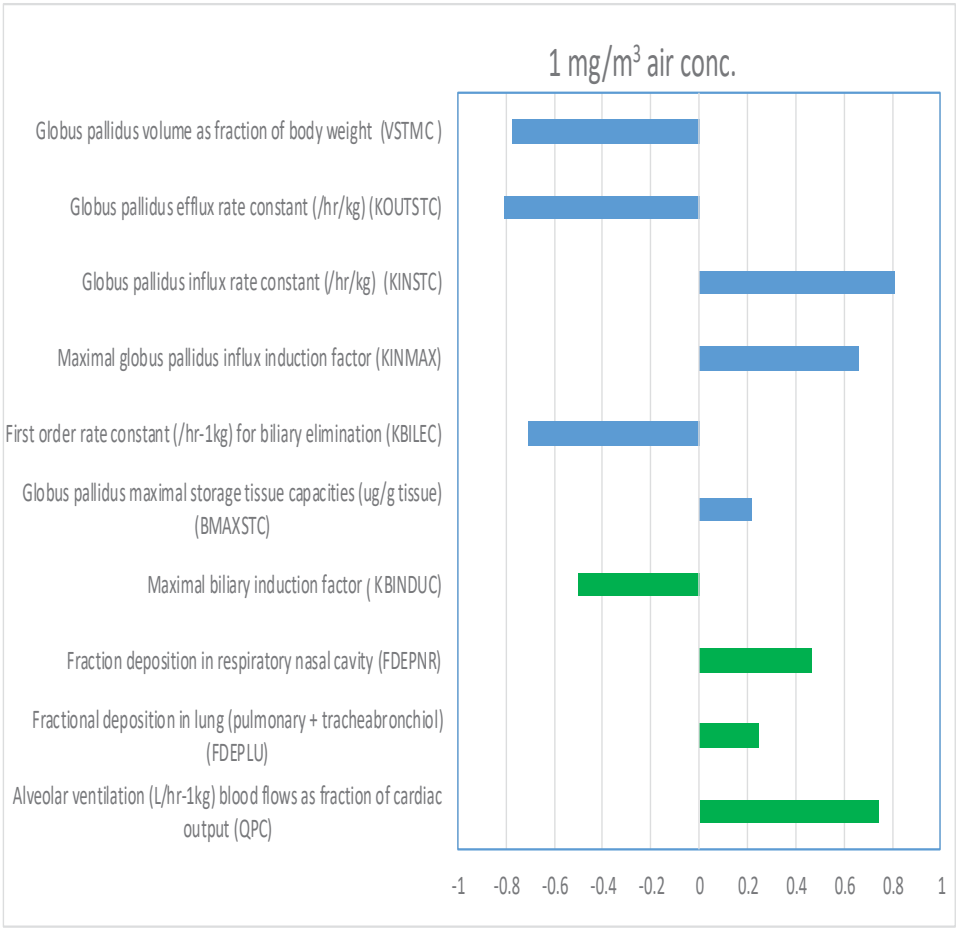
METHODS

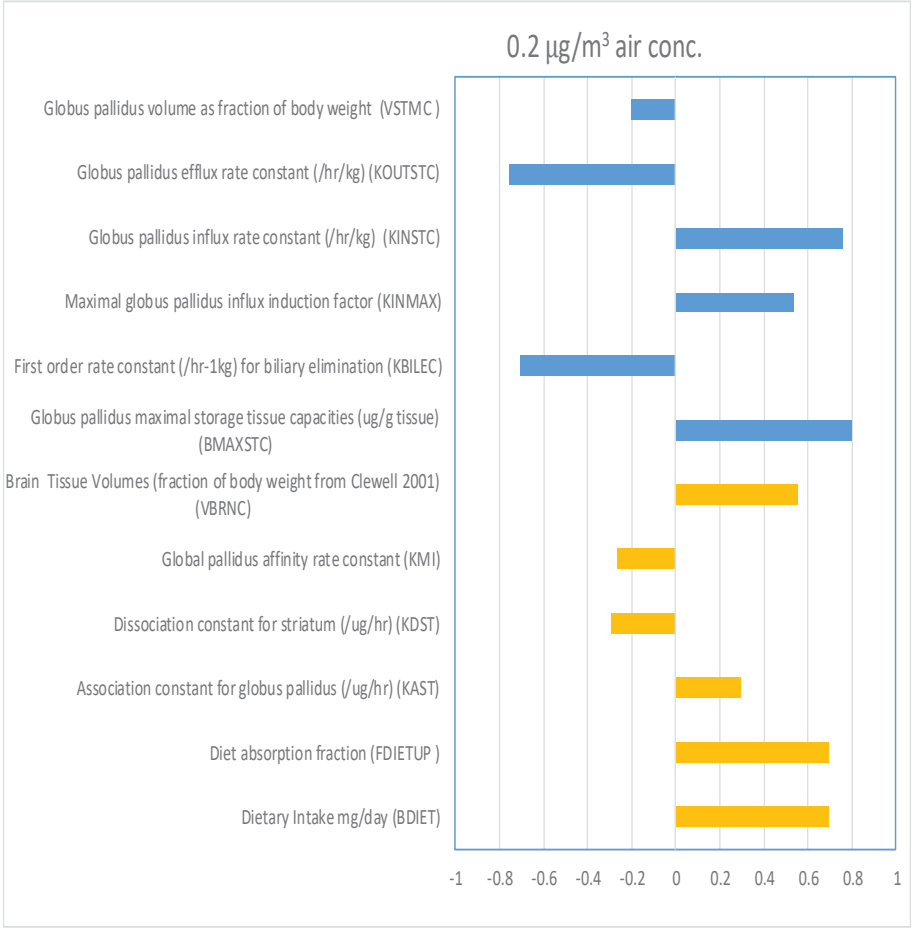
- Sensitivity Analysis in acslXtreme
- Each parameter analysed separately
- Dietary intake varied around 3 mg/day
- Air concentrations were fixed at values of 1, 0.2 and 0.0002 mg/m^3
- Target tissues concentrations were estimated in blood, the globus pallidus and the olfactory bulb
- Two different starting combinations of fractional deposition in lung, respiratory nasal cavity and head were used corresponding to:
  - MnO_2 - density of 5 g/cm^2, MMAD of 6, GSD of 3.4
  - $MnSO_4$ - density of 2.95 g/cm^2, MMAD of 2, GSD of 1.5
- Analyses show little difference in sensitivity based on differences in chemical structure
- Using the MnO_2 additional values were run to find the air concentration where changes occurred

RAMBOLL

EFFECT OF AIR CONCENTRATION ON PARAMETER SENSITIVITY

Sensitivity of the Predicted Total Mn Concentration in the Globus Pallidus to Model Parameters





RAMBOLL

Sensitive at all conc.

at 1 mg/m^3 only

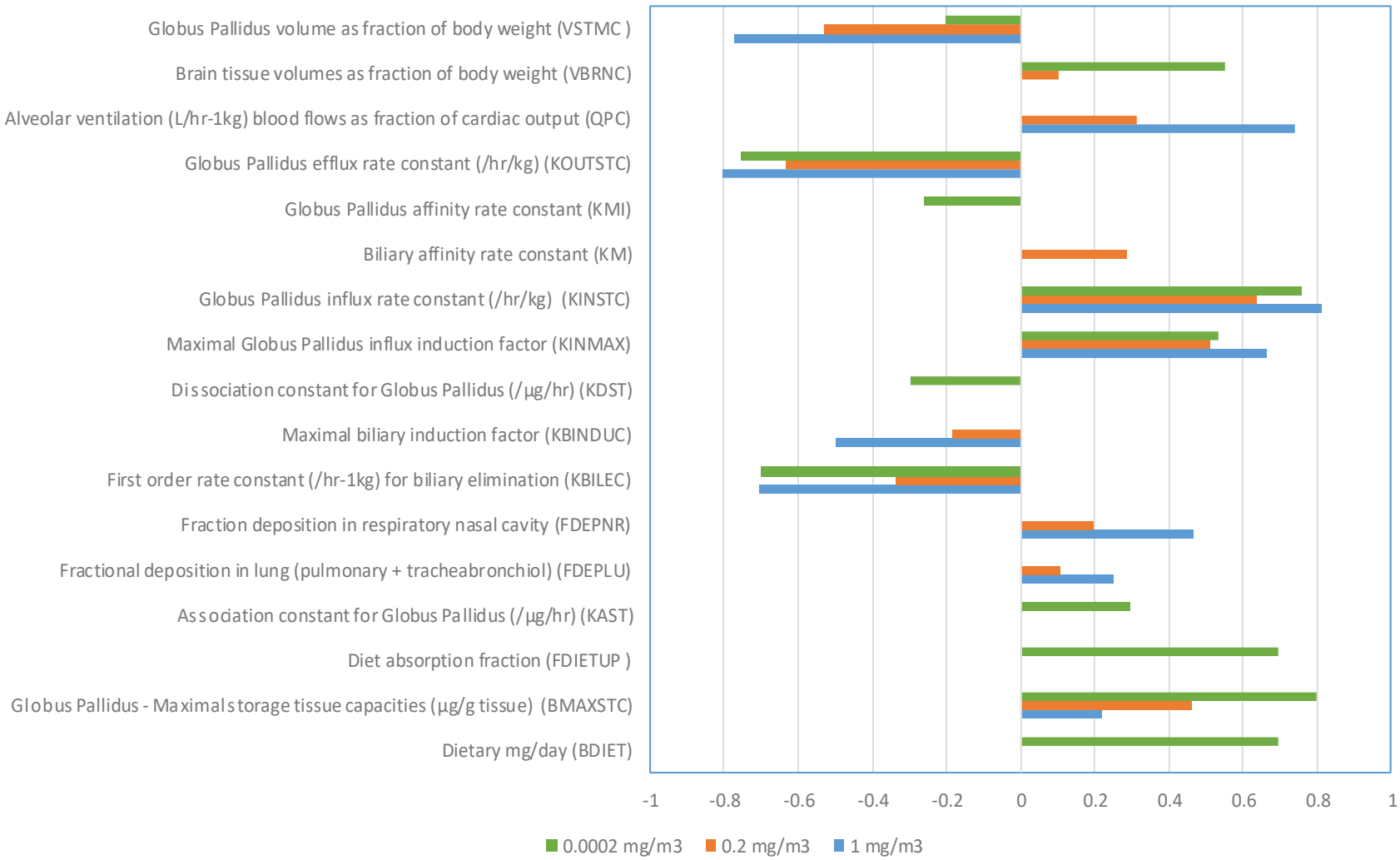
at 0.2 µg/m^3 only

EFFECT OF STARTING DEPOSITION FRACTIONS ON PARAMETER SENSITIVITY

Parameter	MnSO4			MnO2		
	1 mg/m^3	0.2 mg/m^3	0.0002 mg/m^3	1 mg/m^3	0.2 mg/m^3	0.0002 mg/m^3
Dietary mg/day (BDIET)			0.70			0.71
Globus Pallidus - Maximal storage tissue capacities (µg/g tissue) (BMAXSTC)	0.22	0.46	0.80	0.19	0.44	0.80
Diet absorption fraction (FDIETUP)			0.70			0.71
Association constant for Globus Pallidus (/µg/hr) (KAST)			0.29			0.30
Fractional deposition in lung (pulmonary + tracheabronchiol) (FDEPLU)	0.25	0.11		0.13		
Fraction deposition in respiratory nasal cavity (FDEPNR)	0.46	0.20		0.62	0.27	
First order rate constant (/hr-1kg) for biliary elimination (KBILEC)	-0.71	-0.34	-0.70	-0.74	-0.35	-0.71
Maximal biliary induction factor (KBINDUC)	-0.50	-0.18		-0.53	-0.20	
Dissociation constant for Globus Pallidus (/µg/hr) (KDST)			-0.30			-0.30
Maximal Globus Pallidus influx induction factor (KINMAX)	0.66	0.51	0.53	0.68	0.52	0.53
Globus Pallidus influx rate constant (/hr/kg) (KINSTC)	0.81	0.64	0.76	0.83	0.65	0.76
Biliary affinity rate constant (KM)		0.29			0.28	
Globus Pallidus affinity rate constant (KMI)			-0.26			-0.26
Globus Pallidus efflux rate constant (/hr/kg) (KOUTSTC)	-0.81	-0.63	-0.76	-0.83	-0.64	-0.76
Alveolar ventilation (L/hr-1kg) blood flows as fraction of cardiac output (QPC)	0.74	0.31		0.78	0.34	
Brain tissue volumes as fraction of body weight (VBRNC)		0.10	0.55			0.56
Globus Pallidus volume as fraction of body weight (VSTMC)	-0.77	-0.53	-0.20	-0.80	-0.55	-0.20

RAMBOLL

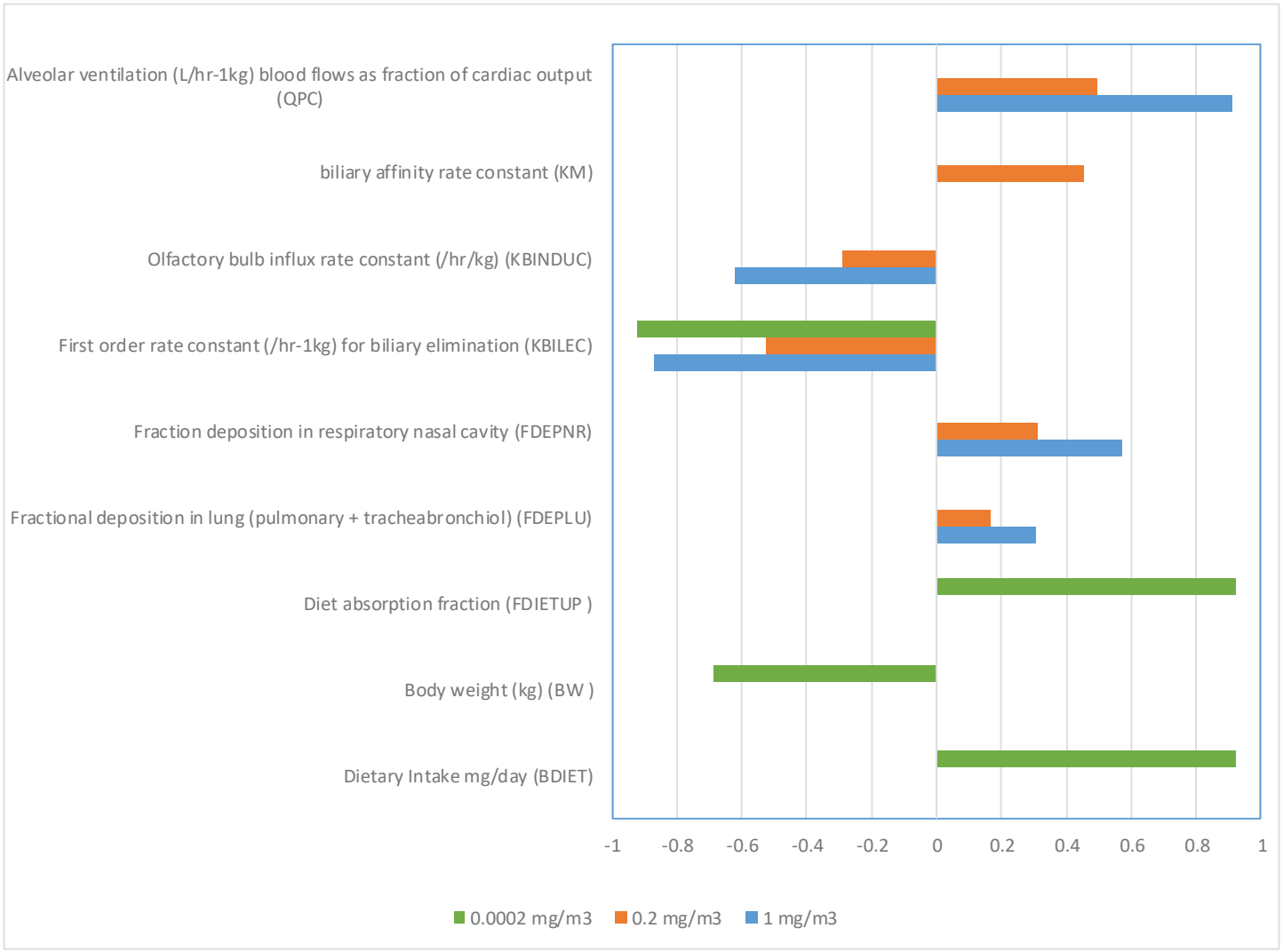
Sensitivity of the Predicted Concentration of Total Mn in the Globus Pallidus to Model Parameters at Multiple Air Concentrations Starting with $MnS0_4$ Deposition Fractions



RAMBOLL

Sensitivity of the Predicted Concentration of Free Mn in the Globus Pallidus to Model Parameters at Multiple Air Concentrations Starting with $MnS0_4$ Deposition Fractions

Sensitivity of the Predicted Arterial Concentration of Mn to Model Parameters at Multiple Air Concentrations



ToxStrategies

New work on manganese PBPK models

Miyoung Yoon, ToxStrategies

November 16, 2018
Meeting at EPA, RTP, NC

Background

- Bioavailability of manganese (Mn) following drinking water ingestion remains a major data gap in understanding Mn kinetics.
- The uncertainty related to whether there is enhanced absorption of Mn from drinking water raises concerns for dosimetry based risk assessments using current PBPK models.
- Infants and early age children are regarded as high-risk groups for potential health effects of drinking water Mn exposure.

Overview of the on-going research on drinking water Mn

- Rat drinking water study (Foster et al., 2015)
- Modeling of drinking water Mn exposure in the adult rat and human (Song et al., 2018)
- Modeling of drinking water exposure in infants and children (in progress)

Drinking water Mn exposure study in rats

OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 145(2), 2015, 244–251
doi: 10.1093/toxsci/kfv047
Advance Access Publication Date: February 26, 2015
Research Article

Pharmacokinetic Evaluation of the Equivalency of Gavage, Dietary, and Drinking Water Exposure to Manganese in F344 Rats

Melanie L. Foster*, Thomas B. Bartnikas†, Laura C. Johnson*, Carolina Herrera†, Michael A. Pettiglio†, Athena M. Keene‡, Michael D. Taylor§, and David C. Dorman*,1

n=8 rats/group

- Group 1: 10 ppm Mn control diet
- Group 2: Diet 200 ppm + 0 ppm in water
- Group 3: Diet 10 ppm + Mn/kg/day in water

Note that Group 2 and 3 were given the same total Mn dose equivalent to the 200 ppm Mn diet.

No apparent difference in Mn bioavailability between diet vs. drinking water in adult rats

PBPK modeling of drinking water Mn exposure in adults

- Describing similar bioavailability of Mn between diet vs. drinking water in the adult rat and human PBPK models – total ingested amount deriving Mn homeostasis

Toxicology and Applied Pharmacology 359 (2018) 70–81

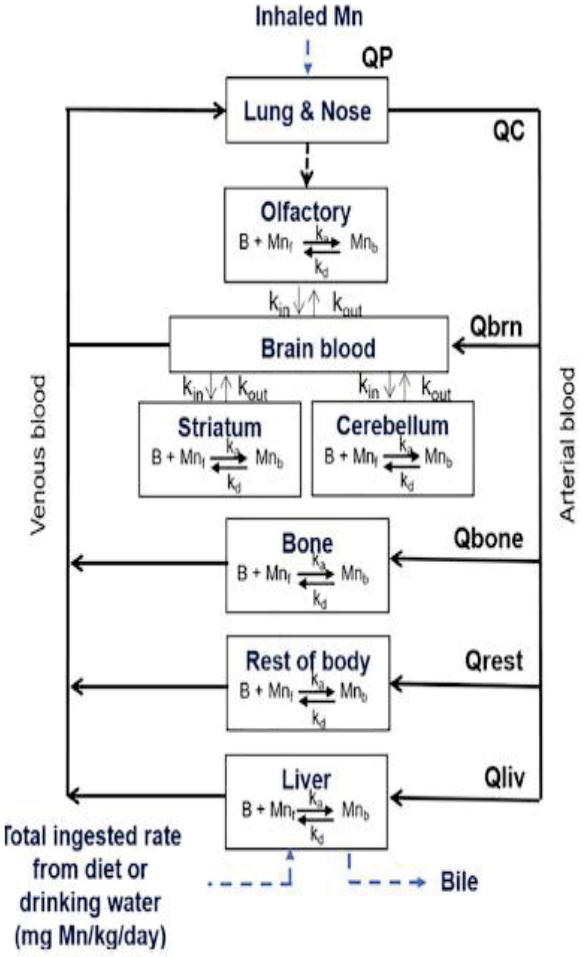
Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

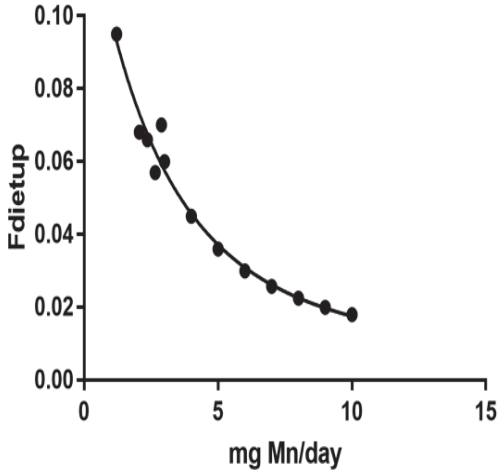
Physiologically-based pharmacokinetic modeling suggests similar bioavailability of Mn from diet and drinking water

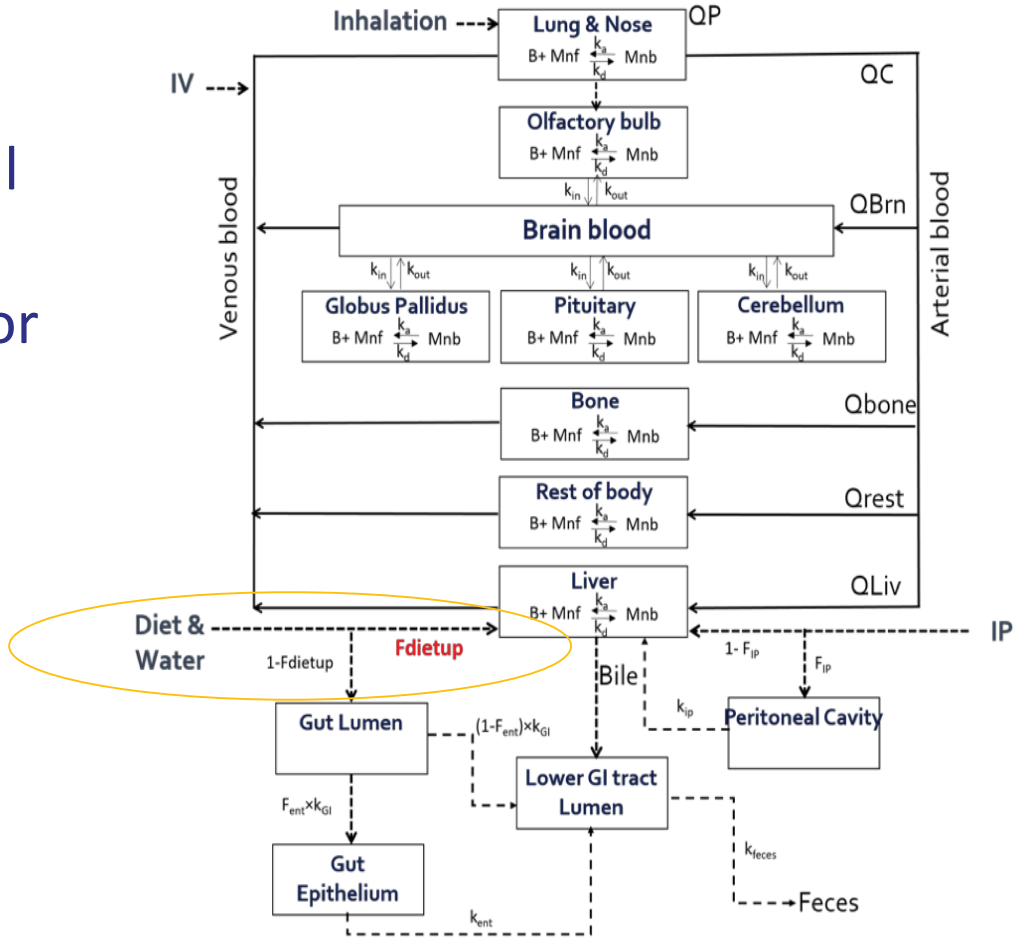
Gina Song[a], Cynthia B. Van Landingham[b], P. Robinan Gentry[b], Michael D. Taylor[c], Athena M. Keene[d], Melvin E. Andersen[a], Harvey J. Clewell[a], Miyoung Yoon[a,e,*]



Incorporation of variability in dietary and drinking water Mn uptake in the adult human PBPK model

Implemented a ingestion dose-dependent fractional uptake in the gut for a normal Mn intake range for human (1 to 10 mg/day).





Drinking water exposure in early age humans: Work-in-progress

- Drinking water exposure in addition to other exposure routes in infants and young children, who are at a higher risk due to higher water consumption rates.

Model conversion from acslX to R
Yoon et al., 2011

Updating the 2011 model with drinking water exposure
age-specific daily Mn intake range, capability to input water Mn concentrations, age-specific exposure factors (water consumption rates)

Simulation of various scenarios of early age Mn exposure in humans
EPA case study on Mn as water contaminant (Brown and Foos, 2009)

Publication targeted in early 2019

Physiologically Based Pharmacokinetic Modeling for Manganese with rapid association – dissociation in tissues

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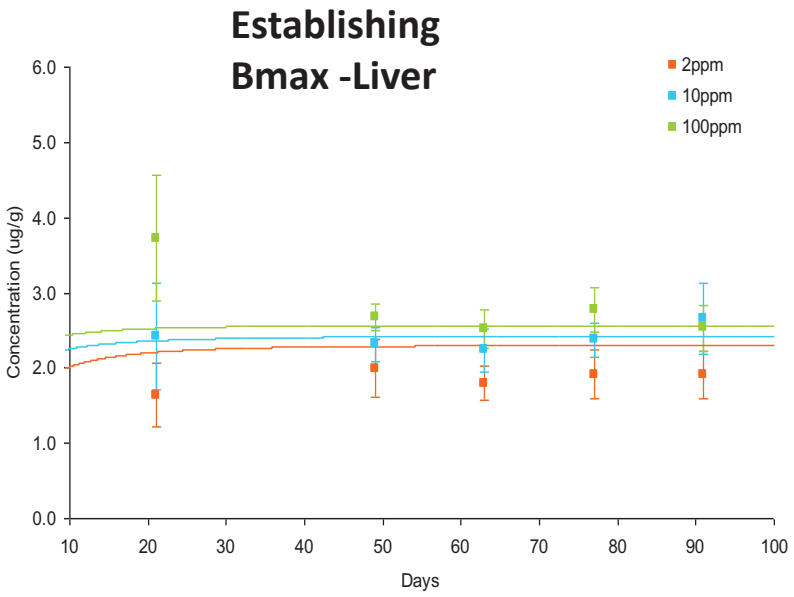
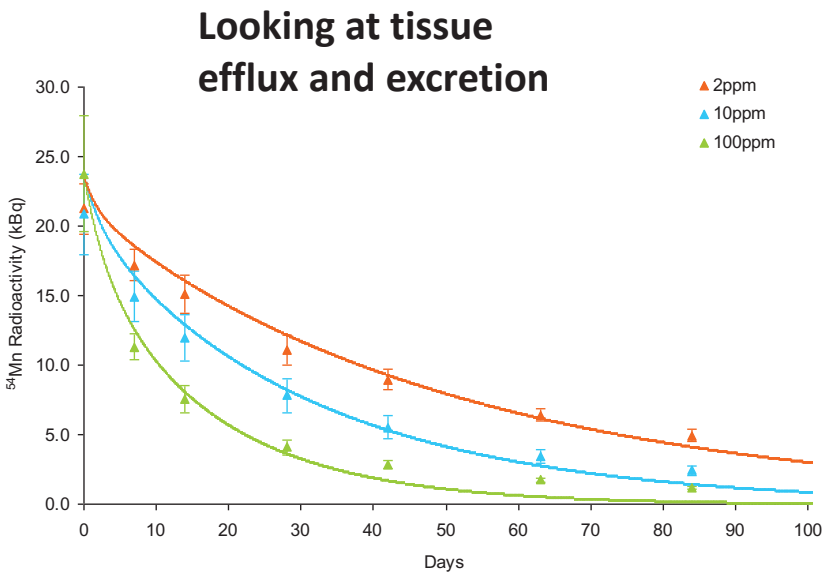
Background

- Multi-dose-route, multi-species pharmacokinetic modeling conducted at CIIT/The Hamner was consistent with dose-dependent kinetic behaviors and captured data from a broad array of studies.

- At lower intakes free [Mn] was controlled by tissue binding and limited biliary excretion; at higher intakes tissue stores saturate, biliary excretion is induced and free tissue Mn increases disproportionately

Tissue concentrations and tracer methods assessed tissue binding capacity and movement of Mn throughout body

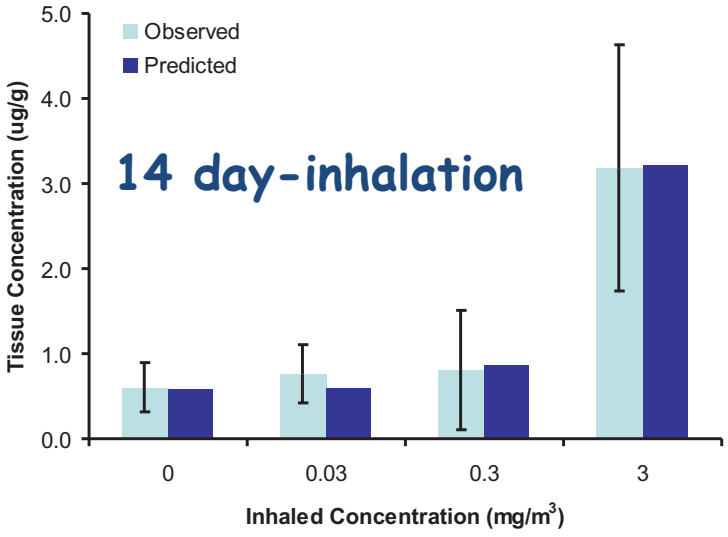


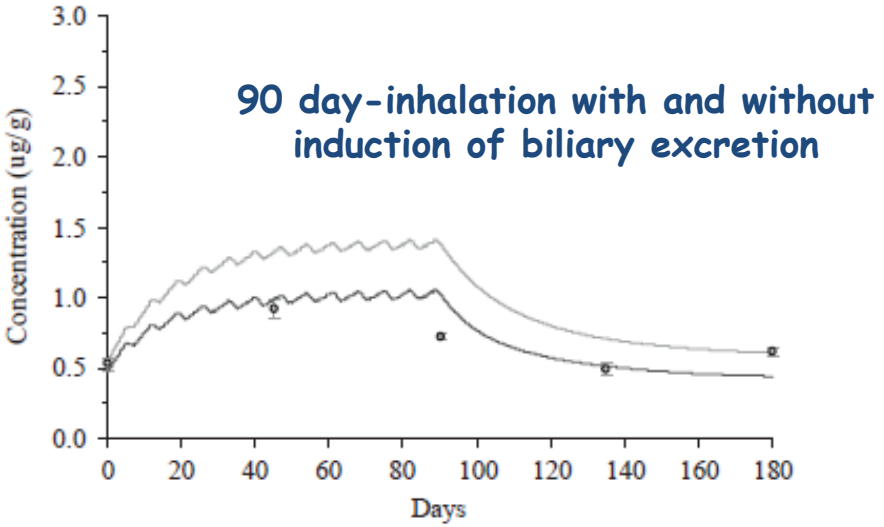


Then added inhalation exposures:

Adult Rat Mn kinetics - Inhalation exposure

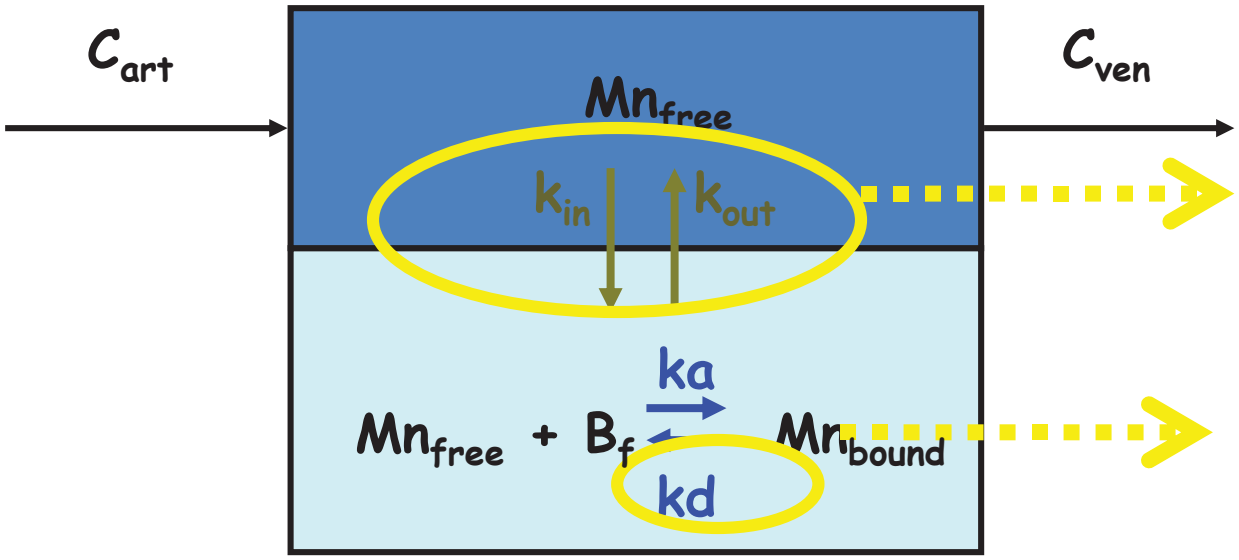
Striatum (Dorman et al. 2004)





- *Comparison of end of 14-day exposure brain Mn with that following 90 days – not much difference!*
- Incorporation of **dose-dependent induction of Mn biliary elimination**

Key determinants in tissue Mn: Saturable tissue storage (Nong et al., 2009)



Relatively **Rapid**

Relatively **Slow**

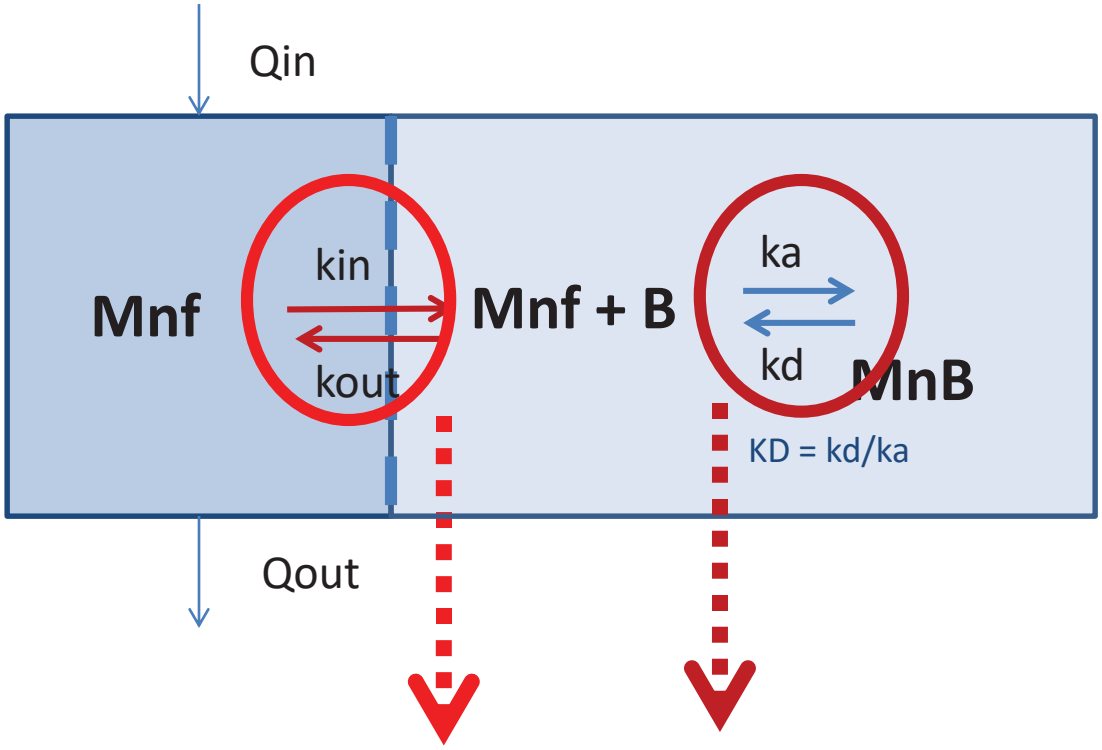
$$Mn_{total} = Mn_{free} + Mn_{bound}$$

$$B_{max} = B_f + Mn_{bound}$$

Although the model reproduced the non-linear behavior of Mn kinetics in different tissues, species, dose routes and life stages….

- Relatively small tissue dissociation rate constants for Mn binding in tissues controlled dose-dependent changes in Mn kinetics
- These slower processes restrict availability of Mn for interacting with binding sites or movement among tissues
- Expectation is that Mn should be more accessible than allowed by the slow tissue dissociation.

Developed a model with more rapid tissue association – dissociation processes



Cellular transport remains rapid

Binding kinetics are more rapid in the updated model allowing membrane transport kinetics control of Mn kinetics

In this rapid association-dissociation model

- A common dissociation constant (KD ~ 0.5 μM), consistent with biological studies of cellular Mn concentrations, was used in almost all tissues
- Variability in tissue binding capacities (Bmax) still accounts for different background levels of Mn-containing components in various tissues
- Both entry of Mn into tissues and interactions with binding pools are rapid.
- With this model, there was no need to add a term for induction of biliary excretion with increased dose

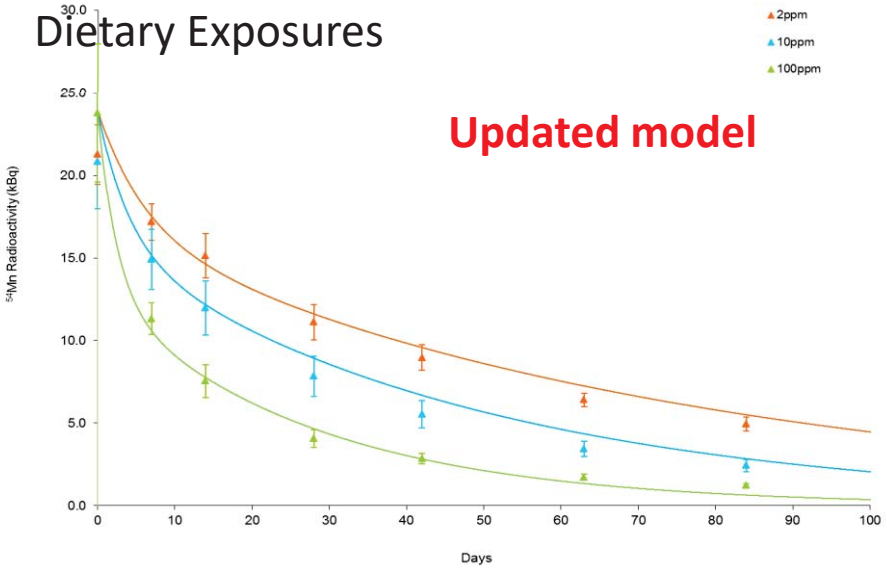
Comparison of the parameters: adult rat

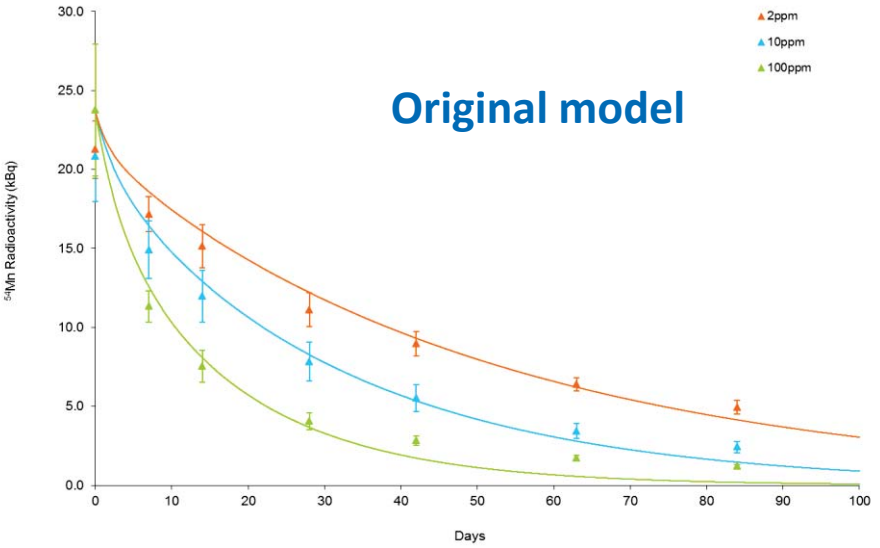
Nong et al., 2009 model	Bmax (μM)	KD (μM)	kass (/uM/hr)	kdiss (/hr)	Kin (ml/hr)	Kout (ml/hr)
Striatum	21	9.89	**1.3**	**0.001**	0.13	0.10
Cerebellum	19	0.01	**70.2**	**0.00013**	0.08	2.48
Olfactgory blub	35	0.16	**32.4**	**0.0003**	0.02	0.19
Liver	43	0.07	**14.6**	**0.0111**	na	na
Bone	11	4.04	**0.2**	**0.0238**	na	na
Lung	270	43.17	**5.4**	**0.38**	na	na
Rest of Body	4	0.03	**0.5**	**0.00454**	na	na

Updated model	Bmax (μM)	KD (μM)	kass (/uM/hr)	kdiss (/hr)	Kin (ml/hr)	Kout (ml/hr)
Striatum	10	0.46	**10**	**4.6**	0.08	0.06
Cerebellum	19	0.37	**10**	**3.7**	0.08	0.62
Olfactgory blub	15	0.46	**10**	**4.6**	0.08	0.03
Liver	46	0.46	**10**	**4.6**	22.49	26.46
Bone	7	0.46	**10**	**4.6**	0.09	0.38
Lung	37	0.46	**10**	**4.6**	0.65	7.10
	370	1.04	**5.4**	**5.6**	0.6	51.7
Rest of Body	4	0.37	**10**	**3.7**	0.85	1.69

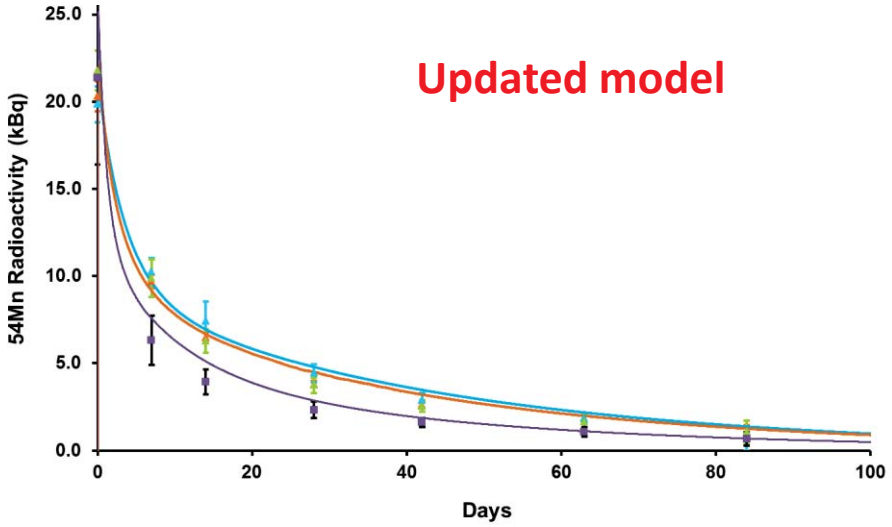
Most significant differences were noted with Mn Tracer kinetics

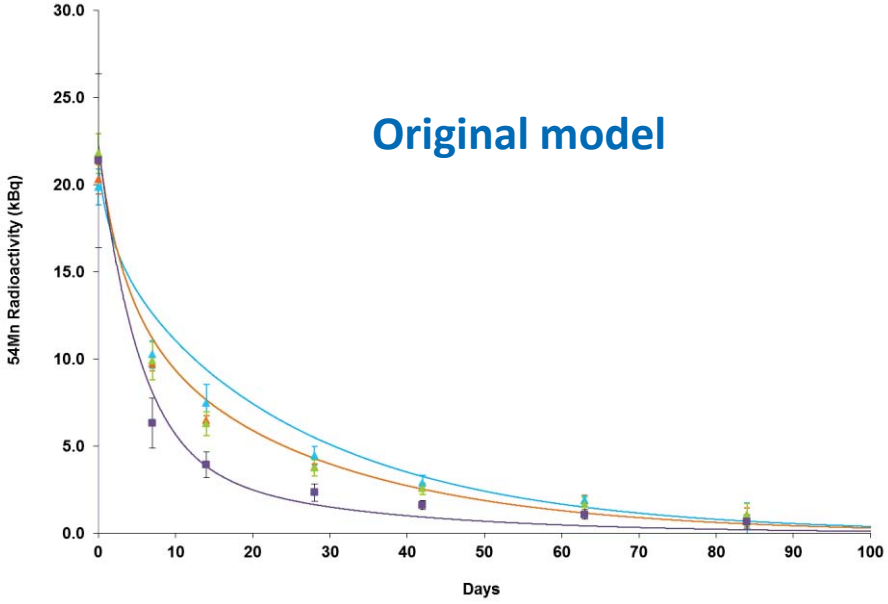
Dietary Exposures



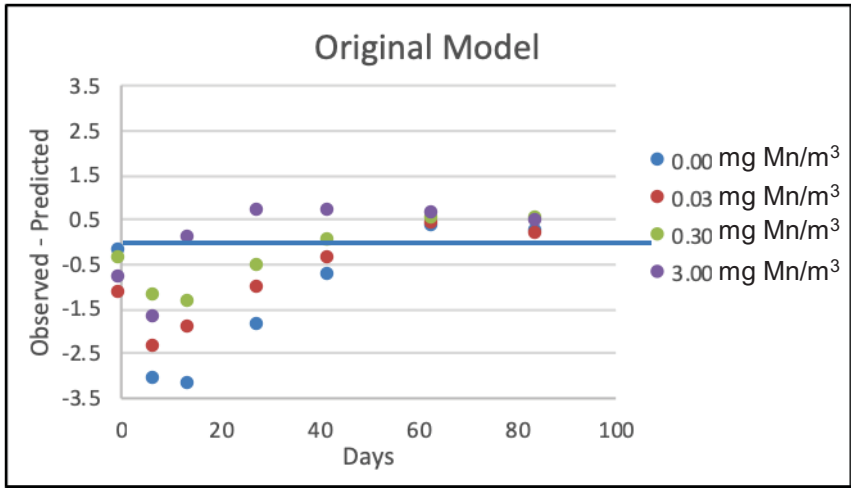


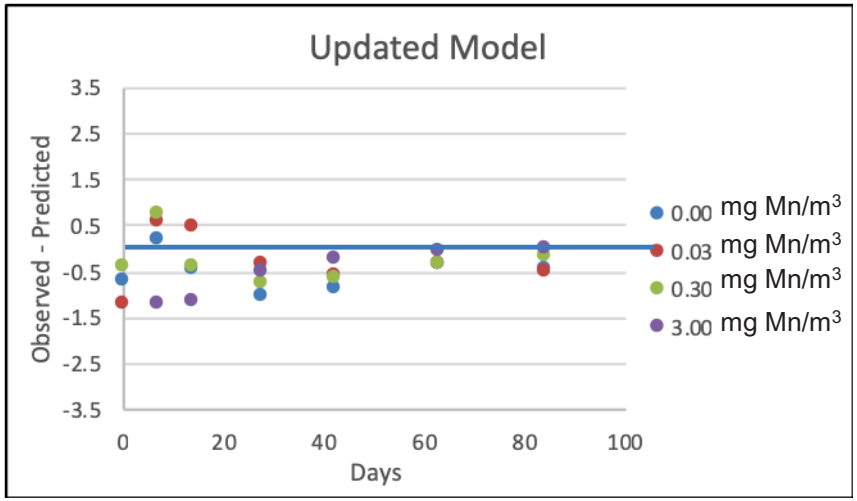
14 d inhalation

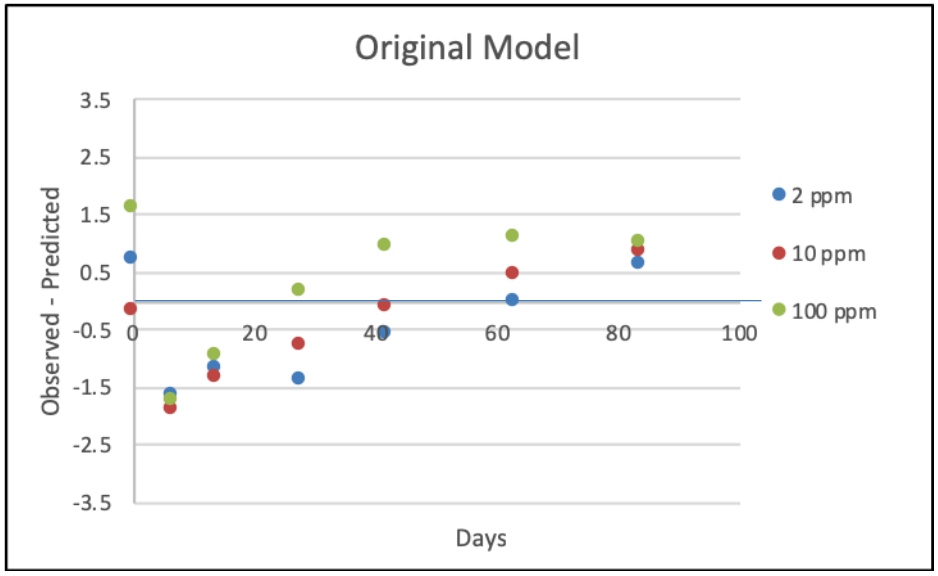


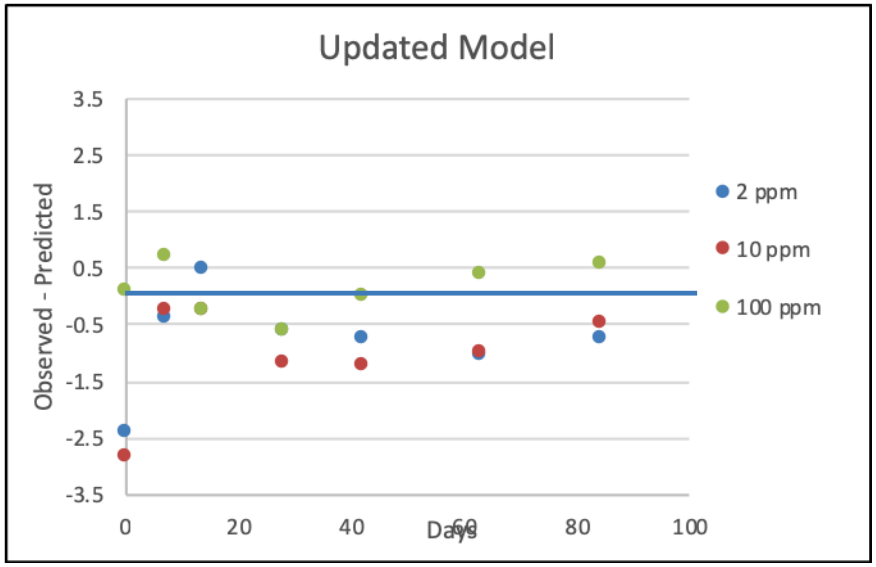


Residuals for tracer elimination studies using the two models





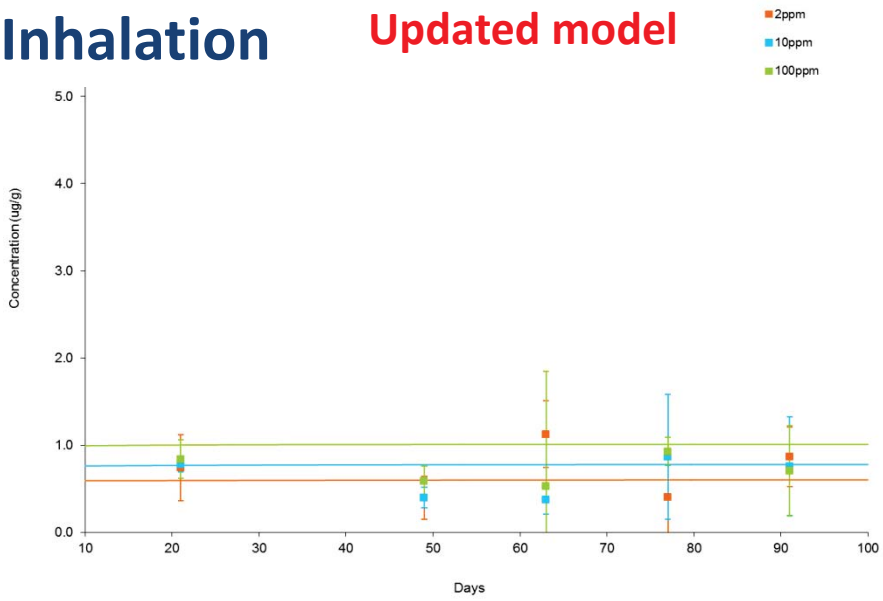




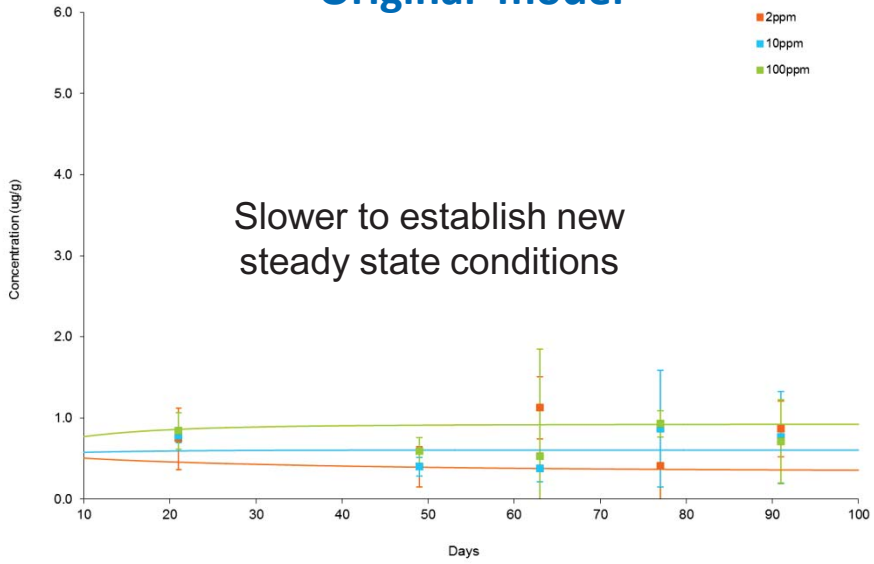
Brain Mn

Inhalation

Updated model



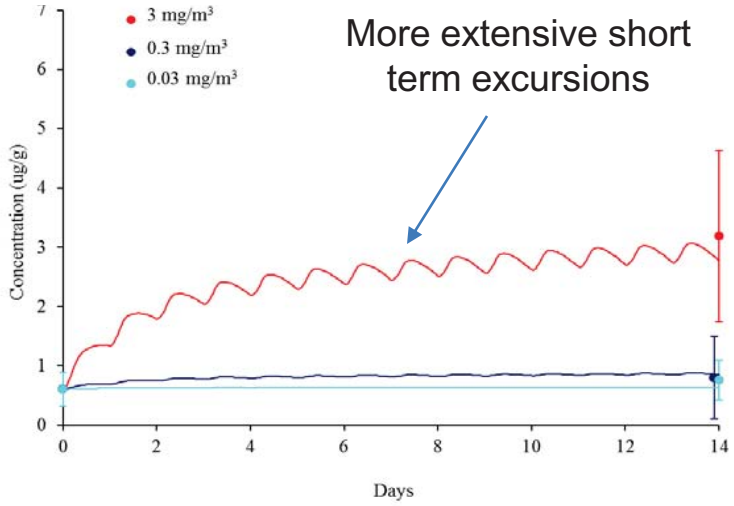
Original model



Slower to establish new steady state conditions

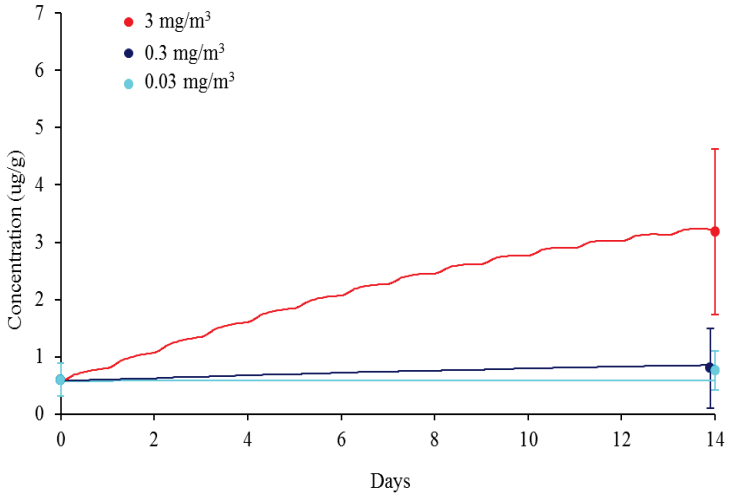
14 d inhalation

Udpdated model



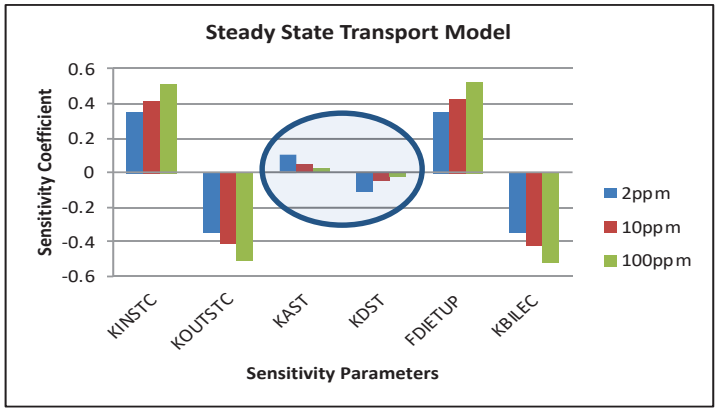
More extensive short term excursions

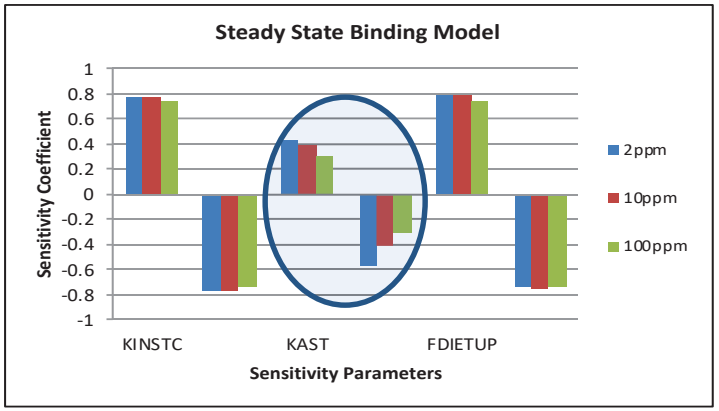
Original model



Sensitivity Analysis of Striatal Mn to various parameters

A.





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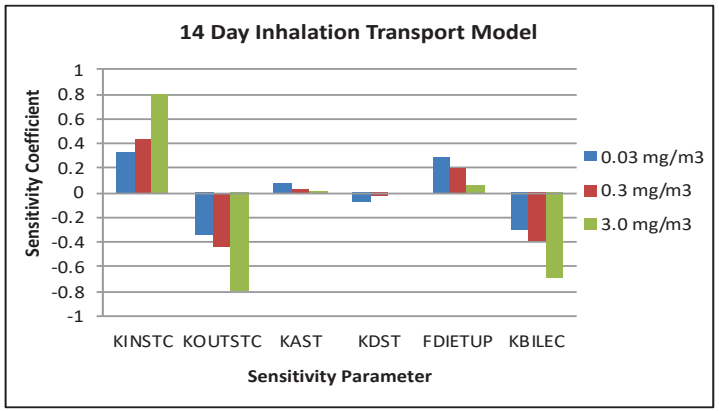
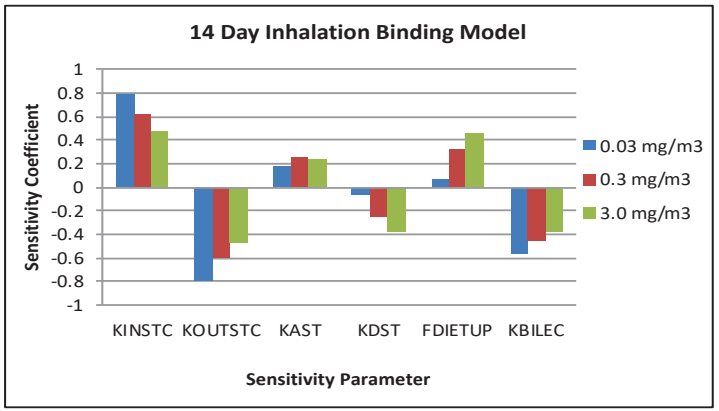
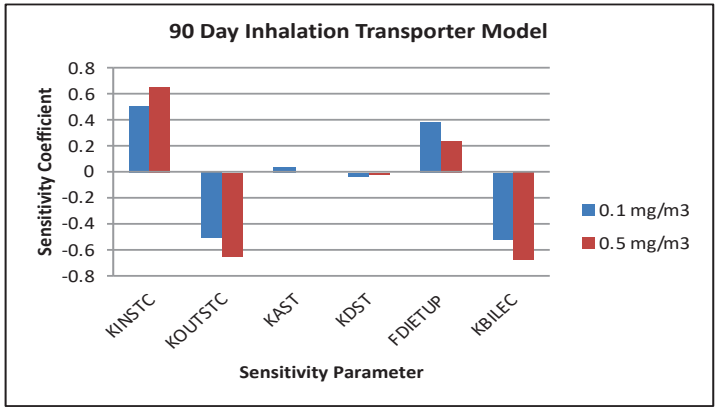
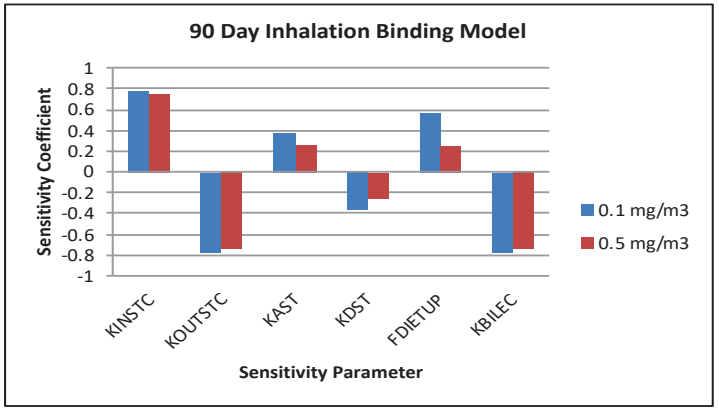




C.





Summary

- As with the earlier model using slower dissociation processes, the updated model describes Mn kinetics in tissues in adult rats exposed to Mn both in their diet and by inhalation

- Both model constructs capture the dose-dependent and tissue-specific kinetic behavior of Mn in adult rats

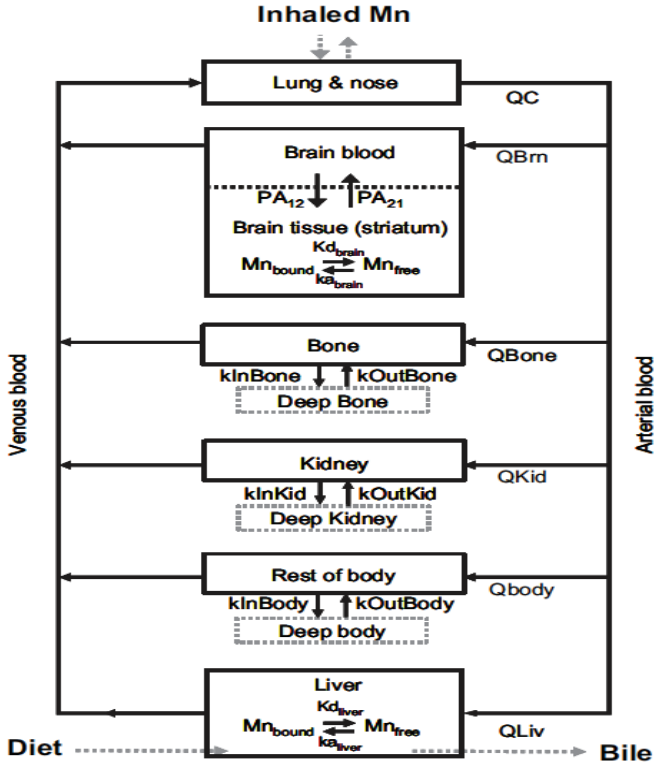
- The model with rapid binding was slightly better at capturing dose dependent tracer elimination, in describing periods in which there were changes in Mn-input rates and in accounting for more rapid elimination without increasing the biliary elimination rate constant.

The paper is now in final co-author review:

Updating PBPK Models for Manganese by incorporating rapid association/ dissociation processes in tissues

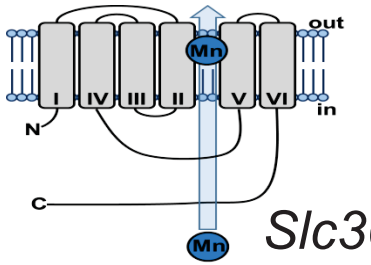
Yoon, M., Efremenko, A., Van Landingham, C., Gentry, R., Keene, A.M., Taylor, M.D., Clewell, H.J. and Andersen, M.E.

Intended journal: Toxicology and Applied Pharmacology



Essentially a reparameterization of Nong et al. models

Recent developments regarding of the biology Mn transporters



Slc30a10

- Human cohorts lacking a single active protein, the *Slc30a10c* transporter, develop high tissue concentrations of manganese and symptoms of manganism

- Tissue specific *Slc30a10* knock-outs in mice have been used to evaluate the role of the this transporter in controlling tissue Mn. Liver and intestinal *Slc3010a* work together to maintain normal tissue levels of Mn (Mercandante, C., *The Toxicologist*, 2018).

- Earlier decisions to use a parsimonious PK model for Mn (moving from Teeguarden et al. to the Nong et al.) are entirely consistent with the emerging data on the dominant role of this single efflux transporter in regulating tissue Mn.

2190 Studies of *Slc30a10*-Deficient Mice Reveal Novel Mechanisms of Manganese Homeostasis

C. J. Mercadante, M. E. Dash, C. Herrera, M. A. Pettiglio, M. Prajapati, and T. B. Bartinkas. *Brown University, Providence, RI.*

Manganese (Mn) is an essential metal and nutrient yet toxic in excess. Individuals at risk of Mn toxicity include patients receiving total parenteral nutrition supplemented with Mn, patients with liver cirrhosis, and miners and welders exposed to Mn rich fumes and particulates. In 2012, the first known inherited disease of Mn excess was reported in patients with mutations in SLC30A10, a Mn efflux transporter that is highly expressed in the liver, brain, and duodenum and hypothesized to be essential for Mn excretion. Characterized by increased blood Mn levels, dystonia, polycythemia (increased red blood cell counts), and liver cirrhosis, SLC30A10 deficiency is a novel disease that offers a unique opportunity to investigate systemic Mn regulation. The overall goal of this project is to establish the role of SLC30A10 in mammalian Mn homeostasis by generating and characterizing mouse models of Slc30a10 deficiency. Our studies indicate that mice globally deficient in Slc30a10 develop tissue Mn excess and polycythemia, similar to patients with SLC30A10 deficiency. As Mn is eliminated predominantly in the feces via hepatobiliary excretion, we hypothesized that hepatocyte-specific Slc30a10 deficiency would lead to a phenotype similar to that of global Slc30a10 deficiency. Surprisingly, mice with hepatocyte Slc30a10 deficiency have minimal tissue Mn excess. To explore the role of Slc30a10 in hepatobiliary Mn excretion, we employed a surgical approach in which we ligate the bile duct, cannulate the gallbladder, and inject 54Mn into the portal vein. From this surgery, we can determine the rate of 54Mn excretion into bile. Results indicate that global and hepatic Slc30a10 deficient mice have impaired hepatobiliary Mn excretion, suggesting that hepatic Slc30a10 is required for hepatobiliary Mn excretion. However, systemic 54Mn excretion studies show no impairment in fecal excretion. Instead, 54Mn absorption studies reveal increased Mn absorption in global Slc30a10 deficient mice, which may reflect a direct role for intestinal Slc30a10 in regulating Mn levels. Characterization of intestinal Slc30a10 deficient mice is underway. Understanding these mechanisms of Mn homeostasis is important for developing pharmacological treatment for both inherited and acquired Mn toxicity.

CONCLUSIONS

- Murine Slc30a10-deficiency leads to increased tissue Mn levels and polycythemia
- Hepatic Slc30a10-deficiency does not lead to severe Mn excess
- Hepatic Slc30a10 required for biliary Mn excretion, but not systemic Mn excretion
- Global Slc30a10 deficiency leads to increased Mn absorption
- Intestinal Slc30a10 KO mice have minimally increased Mn levels, normal ^{54}Mn excretion and absorption
- Intestinal- and hepatocyte-specific Slc30a10 deficient mice have greater Mn overload than either single Slc30a10-deficient tissue mouse model

Mn risk assessment concerns driving the development of a PBPK model and future work

- **Implications of essentiality of Mn / homeostatic control**
- **Potential differences between inhalation and oral exposure**
- **Different forms of Mn / rates of dissolution**
- **Possible early life sensitivity**
  - Gestation
  - Lactation
  - Childhood
- **Human interindividual variability**

Additional scenarios to evaluate uncertainties

- Age
  - Adults approximately 35 years of age
  - Aged Adults (Male and Female) > 60 years of age
  - Children – 8 to 10 years old
  - Teen ages 13-15 years old
  - Early life
    - Pregnant female
    - Neonate
    - Nursing infant
- Chemical Formulation
  - MnO_2 and $MnSO_4$ (and possibly $MnCl_2$, Mn_3O_4, Mn Phosphate)
- Route of Exposure
  - Inhalation versus diet and drinking water
  - Bolus vs continuous
    - Effect of using rate on intakes across day for diet and drinking water
      - Data from NHANES to get temporal changes over the day
- Low Iron scenario

Early life uncertainty scenarios

- Exclusive breast-feeding – 0-6 months, no DW or diet, with and without maternal Mn drinking water (DW) exposure
- Exclusive Formula milk-feeding, prepared in Mn containing DW or filtered water– 0-6 months, no diet
- Breast fed only no DW or diet Mn, no maternal Mn drinking water, after weaning switch to solid diet during 7-12 months and 1-3 years; repeat with Mn continuing DW exposure at each stage
- Formula fed with Mn DW, after weaning switch to solid diet and Mn containing DW during 7-12 months and 1-3 years; repeat scenario but with filtered water for formula and no DW Mn during 7-12 months and 1-3 years

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- Alina Efremenko

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  - U. Ottawa

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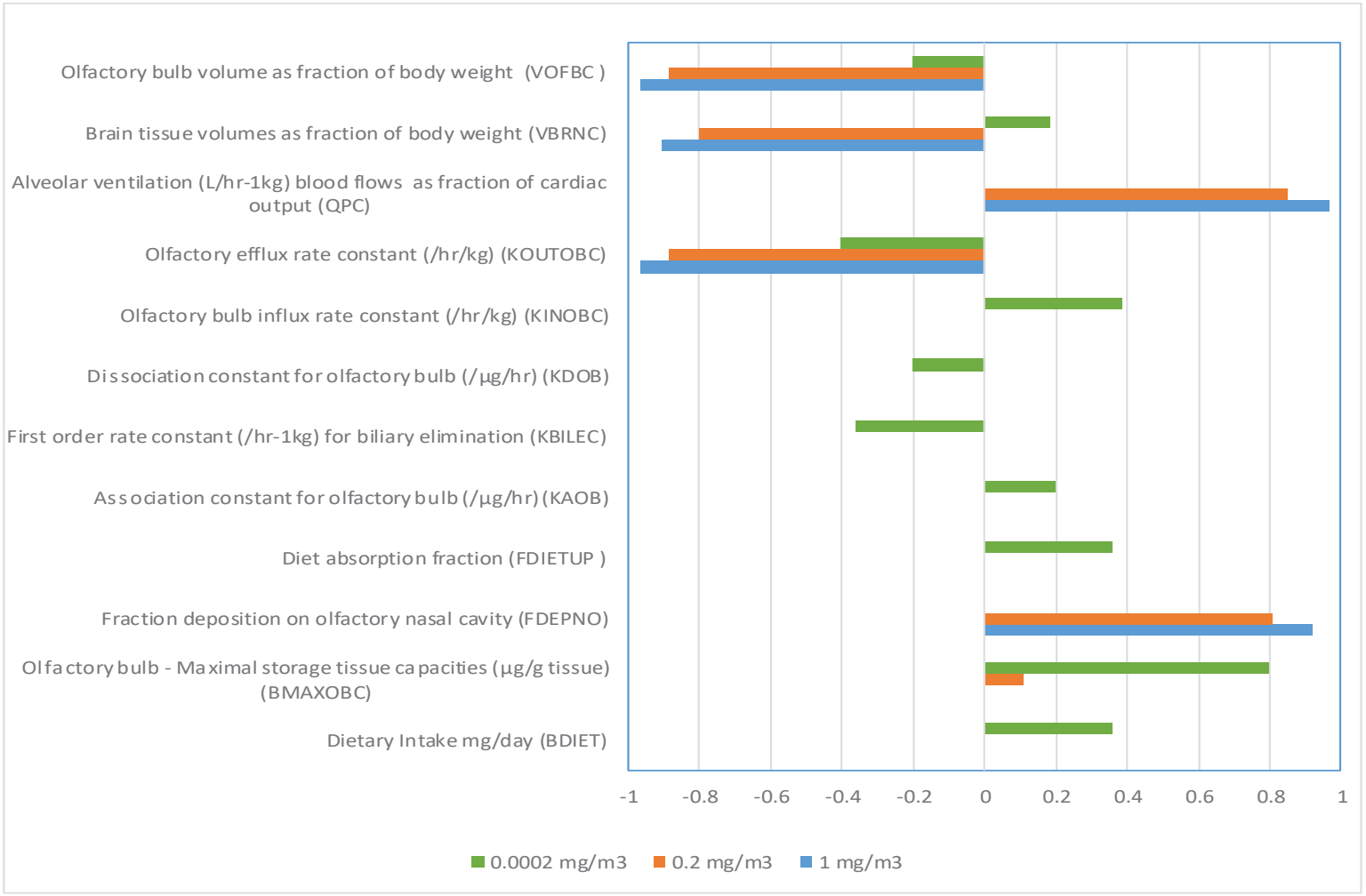
APPENDIX SLIDES

Sensitivity of the Predicted Concentration of Total Mn in the Olfactory Blub to Model Parameters at Multiple Air Concentrations Starting with $MnS0_4$ Deposition Fractions



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Sensitivity of the Predicted Concentration of Free Mn in the Olfactory Blub to Model Parameters at Multiple Air Concentrations

Sensitivity of the Predicted Concentration of Total Mn in the Globus Pallidus to Model Parameters at Multiple Air Concentrations

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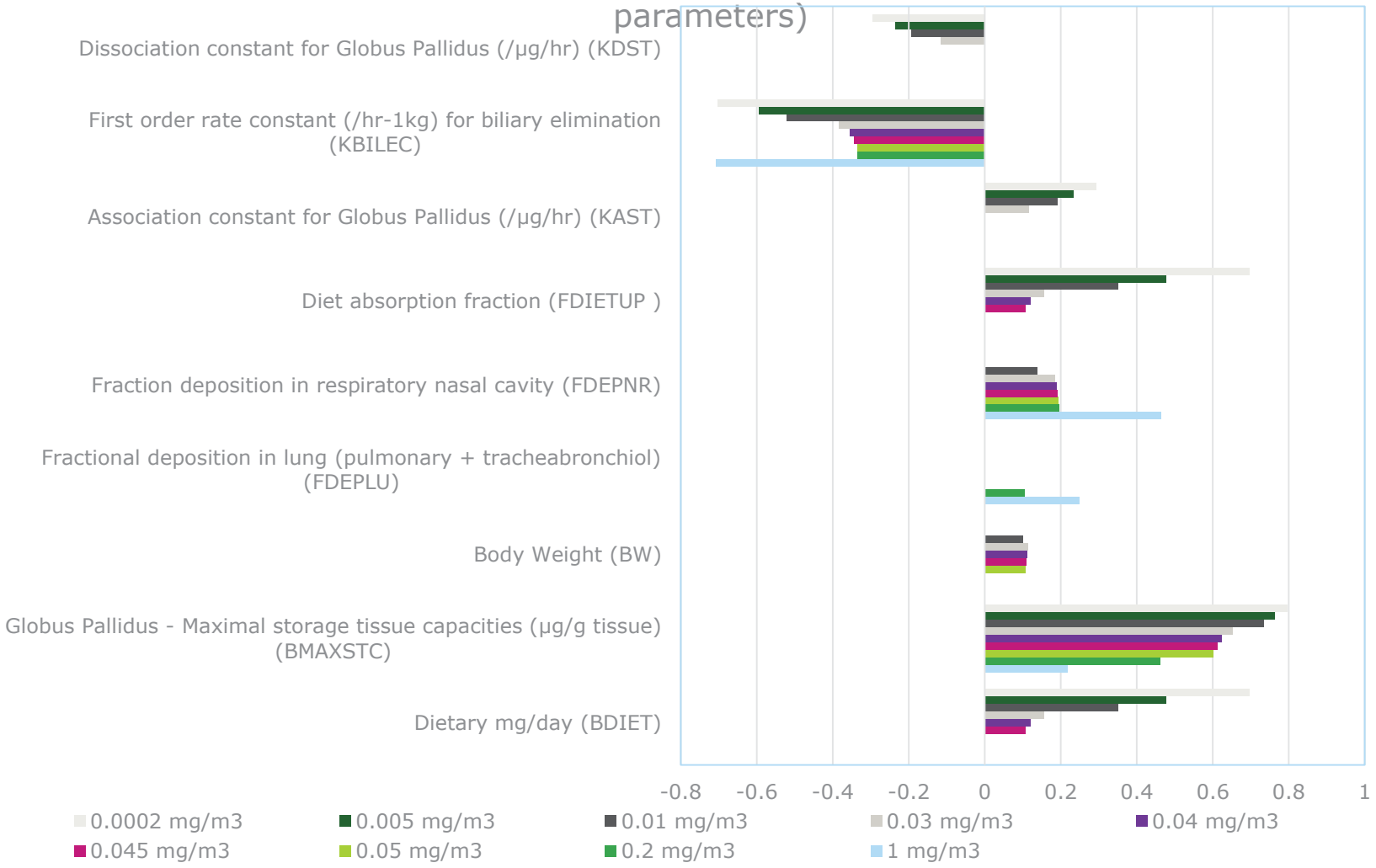
Sensitivity of the Predicted Concentration of Total Mn in the Globus Pallidus to Model Parameters at Multiple Air Concentrations (selected parameters)



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